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A Longitudinal Study of Changes in Fluctuating Asymmetry with Age in Jamaican Youth

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Abstract: Fluctuating asymmetry (FA), random deviation from perfect bilateral symmetry, is an indicator of developmental stability. Examining the ontogeny of FA can illustrate whether symmetry is actively maintained as the organism grows or breaks down as perturbations accumulate with age. Previous studies of changes in human FA with age have been cross-sectional studies and give conflicting results. We analyzed data from a longitudinal study of bodily FA in Jamaicans, using a composite index of seven paired traits. In addition, 288 children (ages 5–12) were first measured in 1996, and many were re-measured in 2002 and 2006 (maximum age = 22 years). Both within-individual longitudinal comparisons and between-individual comparisons across age groups demonstrate changes in FA with age. In males and females, FA increased until around age 13, but the pattern of change differed between the sexes. In males, FA increased rapidly approaching adolescence and then slightly declined into early adulthood. The increase in female FA was more gradual and then leveled off. The patterns observed likely reflect accumulation of developmental errors over time, rapid physical changes during puberty (especially in boys), and then regulation of symmetry when transitioning into adulthood. Although most changes in symmetry over time probably reflect random processes, the magnitude and direction of asymmetry in an individual at one point in time tended to be positively (though weakly) related to asymmetry in later years, pointing to underlying differences among individuals in developmental stability.

Keywords: symmetry; fluctuating asymmetry; developmental stability; human development; Jamaica

1. Introduction

Interest in fluctuating asymmetry (FA), small, non-directional deviation from perfect bilateral symmetry, arises from the relationship between FA and developmental stability [1–3]. High levels of FA may indicate high levels of stress during development or an inability to buffer the phenotype against stress, and therefore may be correlated with environmental or genetic quality (or both). However, despite the large literature on FA and developmental stability, relatively few authors have examined changes in FA as the organism develops.

The pattern of change in FA with age can help to determine how asymmetry arises during development and to interpret the underlying relationship between FA and developmental stability [4–7]. For example, as an individual grows, do random developmental accidents accumulate over time or does the organism correct deviations from symmetry via compensatory growth? Does an individual's current level of symmetry best reflect early developmental events or recent growth? Most previous studies of developmental changes in FA have been conducted on traits that are molted and re-grown, such as feathers [5,8–10] or components of an exoskeleton [4,11]. For traits like bones and stems that instead grow by adding to what is already present, developmental errors may accumulate because asymmetry at one point in time directly affects the degree of asymmetry at time t + 1 [3,12].

Measurement and analysis of FA is more difficult than it at first appears. Subtle differences between sides that are symmetrically distributed around a mean of zero must be separated from measurement error, which has a similar distribution [13]. FA also must be separated from other forms of asymmetry: directional asymmetry is a relatively consistent bias toward one side, while antisymmetry consists of large differences between sides in a random direction [1–3,13]. Another difficulty is that correlations between developmental stability and FA in any one trait are likely to be weak [14–16], and therefore composite indices of FA are often used that combine measurements across several traits [3,13–15].

Previous studies of changes in human FA with age have given conflicting results and compare individuals of different ages or age groups, rather than tracking within-individual changes in FA. Some studies show a decrease in FA with age. Wilson and Manning [17] found that English children became more symmetrical in a variety of traits from ages 2 through 18. This decrease in FA was interrupted by a temporary increase in FA at about ages 12–14, perhaps reflecting hormonal changes and rapid growth during adolescence. Hope et al. [18] also found a decrease in FA among Scottish children (fingers, ages 4 to 8), but a leveling off from about ages 8–13 and perhaps a further decrease from ages 13 to 15. In a rural Caribbean population (in Dominica), FA appears to decrease from ages 0–10 to ages 10–20 [19]. However, an early analysis of our sample of rural Jamaican children instead suggested a possible *increase* in bodily FA from ages 5 through 12 (see below) [20]. Hallgrímsson [6] also found evidence suggesting an increase in FA of skeletal traits with age in a heterogeneous sample, with cranial traits continuing to become more asymmetrical throughout the lifespan and post-cranial traits showing a non-significant increase up to about age 20. Others have found that FA is highest at the extremes of the age distribution. FA is higher among Israeli newborns than 26 to 42 year-old adults and is highest among the elderly (age 80–85), but otherwise varies little with age, showing no difference between age groups 5–13 and 14–18 [21,22]. A comparison of facial symmetry in young German adults with an elderly Scottish cohort also demonstrated elevated FA in the elderly [23].

In the present study, we analyze data from the Jamaican Symmetry Project, a long-term study of FA [20] and digit ratios [24] in Southfield, Jamaica. The project began in 1996 with a sample of 288 children, aged 5 to 12 years old. Bodily FA of many of the same individuals was measured again in 2002 and 2006. Although designed as a longitudinal study, longitudinal changes in FA have not been analyzed before, as previous studies from the Jamaican Symmetry Project have focused on FA in a particular year or an average across years [25,26]. Trivers et al. [20], using the 1996 sample, reported an increase in FA from age 5 through age 12, but the age-FA relationship disappeared when height and weight were included as covariates in a multiple regression model. Relative composite FA (sum of FA corrected for trait size) was correlated with age, height and weight, and height and weight also increased with age. This and the prior studies of changes in human FA with age reviewed above have all been cross-sectional studies (although Flinn et al. [19] measured FA longitudinally in a subsample of their subjects, they averaged measures across years). We know of no previous longitudinal analyses of human FA.

2. Results

Fluctuating asymmetry varied with age (Figure 1). Composite standardized FA in males increases gradually through age 9 and levels off briefly. Male FA then increases markedly with adolescence, peaking around ages 12–15, and then slightly declines but remains above the values recorded during childhood. For females, FA increases gradually to about 13 years of age, and then levels off. Note that before about age 11, males tend to be more symmetrical than females (as reported in [20]), but because of the sharp increase in male FA around age 12, the direction of the sex difference reverses for a few years. By adulthood, no sex difference is apparent. The pattern of change in males best fits a steep linear increase until age 13 (r^2 = 0.41, b = 0.85; one selected measurement year per individual, see Materials and Methods) and then a shallower linear decline thereafter (r^2 = 0.12, b = −0.44), while, for females, there is a more gradual linear increase until age 13 (r^2 = 0.11, b = 0.30) and no increase or decrease thereafter (r^2 = 0.001, b = 0.036). Height in females also increases linearly to age 13 (r^2 = 0.75, b = 0.87) and then levels off or increases slightly thereafter (r^2 = 0.02, b = 0.13). Males continue to grow to age 16 (r^2 = 0.87, b = 0.93), and height then levels off (r^2 ~0, b = −0.02).

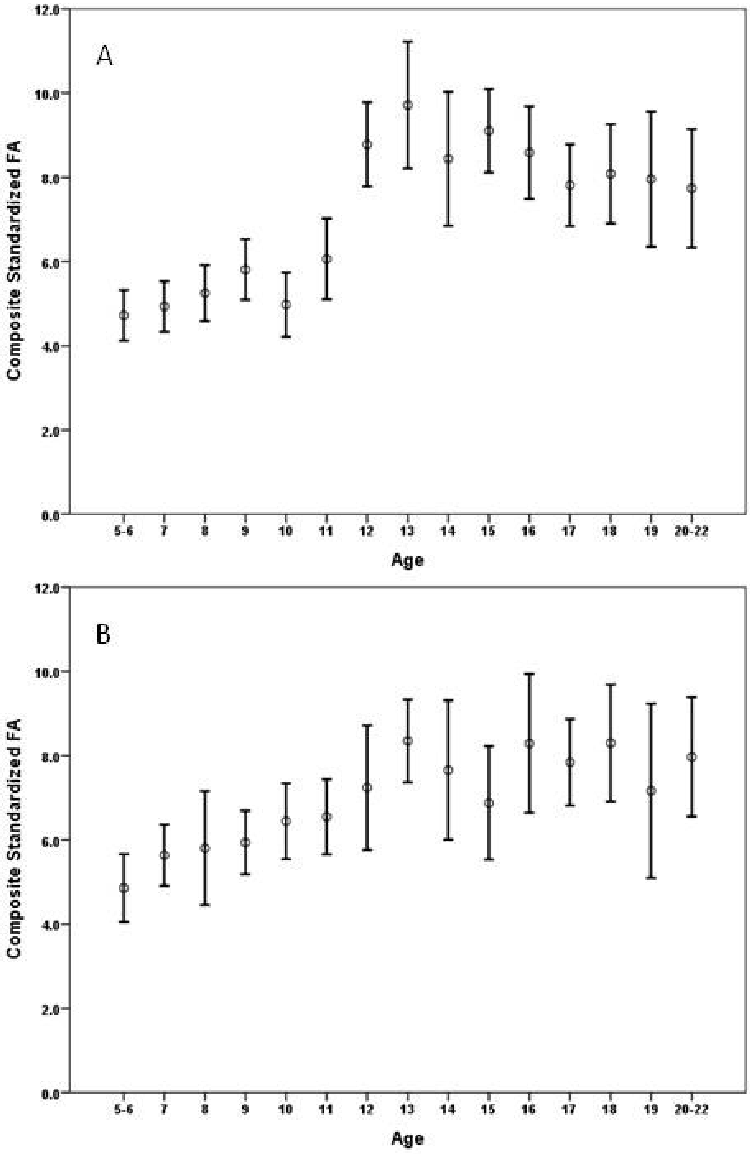

Figure 1. Mean (±95% Confidence Invervals) composite standardized FA across ages is shown for males (**A**) and females (**B**). Because of smaller numbers of subjects at the extremes of the age distribution, ages 5 and 6 are combined, as are ages 20, 21 and 22 years.

Tables 1 and 2 summarize means of both composite standardized and composite relative FA for both sexes across three age groups: 5–10, 11–15, 16–22. Results for relative FA are similar to those for standardized FA when averaged across the sexes. When split by sex, there is a more noticeable decline in male relative FA after age 15, while female relative FA changes little with age. Formal statistical tests for changes in FA with age were performed in two kinds of analyses, using composite standardized FA values: (1) a within-subjects (repeated measures) longitudinal approach; and (2) between-subjects comparisons across age groups.

Table 1. Means (standard deviations in parentheses) of composite standardized FA (Fluctuating asymmetry) and composite relative FA are shown for all subjects, divided into age groups and by sex.

Age	Sex	Composite Standardized FA	Composite Relative FA	*n*
5–10	Male	5.15 (1.65)	0.121 (0.039)	126
	Female	5.68 (1.95)	0.137 (0.046)	98
	Total	5.38 (1.80)	0.128 (0.043)	224
11–15	Male	8.49 (2.76)	0.154 (0.048)	89
	Female	7.39 (2.45)	0.141 (0.044)	84
	Total	7.96 (2.67)	0.148 (0.047)	173
16–22	Male	8.07 (2.82)	0.132 (0.047)	116
	Female	7.97 (2.81)	0.145 (0.049)	87
	Total	8.03 (2.81)	0.137 (0.048)	203

Table 2. Means (standard deviations in parentheses) of composite standardized FA and composite relative FA are shown for selected subjects, divided into age groups and by sex.

Age	Sex	Composite Standardized FA	Composite Relative FA	*n*
5–10	Male	5.28 (1.78)	0.123 (0.041)	43
	Female	5.93 (2.03)	0.141 (0.050)	36
	Total	5.57 (1.91)	0.131 (0.046)	79
11–15	Male	8.99 (3.00)	0.162 (0.052)	46
	Female	7.19 (2.29)	0.137 (0.042)	45
	Total	8.10 (2.81)	0.150 (0.048)	91
16–22	Male	7.88 (2.96)	0.129 (0.049)	58
	Female	7.78 (3.18)	0.142 (0.052)	41
	Total	7.84 (3.04)	0.135 (0.050)	99

A Repeated Measures General Linear Model (GLM) analysis was conducted among all individuals who had complete measurements at all three sampling times (1996, 2002, 2006, $n = 135$). The repeated measure (year) effect was highly significant ($F_{2,\,266} = 81.61$, $p < 0.001$, partial eta$^2 = 0.38$), demonstrating within-individual changes in FA with age. There was no overall sex difference in FA ($F_{1\,,133} = 0.007$, $p = 0.93$, partial eta^2~0), but a significant interaction between year and sex ($F_{2,\,266} = 3.36$, $p = 0.036$, partial eta$^2 = 0.03$), confirming that the pattern of variation in FA across years varies with sex. If we include height and weight in the Repeated-Measures analysis, the year effect on composite standardized FA remains significant ($F_{2,\,240} = 77.05$, $p < 0.001$, partial eta$^2 = 0.39$), but the sex X year interaction does not ($F_{2,\,240} = 2.22$, $p = 0.11$, partial eta$^2 = 0.02$). Of course, height and weight also increase across years as children grow toward adulthood (both $p < 0.001$). Males tend to be taller than females ($p < 0.001$) and perhaps heavier ($p = 0.078$), but the sexes do not differ in FA ($p = 0.92$).

Between-subjects approach: subjects were divided into three age groups (5–10, 11–15, 16–22), and only one measurement year per individual was selected to avoid pseudoreplication (see Materials and Methods). A GLM with age group and sex as independent variables and composite standardized FA as the dependent variable demonstrated a significant effect of age group ($F_{2,\,263} = 22.38$, $p < 0.001$, partial eta$^2 = 0.15$) and a significant sex X age group interaction ($F_{2,\,263} = 4.96$, $p = 0.008$, partial eta$^2 = 0.04$), but no sex difference in FA ($F_{1,\,263} = 1.64$, $p = 0.20$, partial eta$^2 = 0.01$). Post hoc tests revealed that the 5 to 10 year-olds were significantly more symmetrical than subjects in each of the two older age groups (Tukey's Honest Significant Difference Test, both $p < 0.001$), which did not significantly differ from each other ($p = 0.78$; see Table 2 for descriptive statistics). If height and weight are included as covariates in the between-subjects GLM, both the age group and sex X age group interactions remain significant, although age group explains a smaller proportion of the variance in FA than previously (age group: $F_{2,\,248} = 3.14$, $p = 0.045$, partial eta$^2 = 0.03$; sex X age group: $F_{2,\,248} = 6.19$, $p = 0.002$, partial eta$^2 = 0.05$). There is also a significant positive relationship between height and FA ($F_{1,\,248} = 4.62$, $p = 0.033$, partial eta$^2 = 0.02$), but no relationship between FA and weight ($F_{1,\,248} = 0.86$, $p = 0.35$, partial eta$^2 = 0.003$) or sex ($F_{1,\,248} = 0.13$, $p = 0.72$, partial eta$^2 = 0.001$).

Most individual traits follow the trends shown above for composite FA. The most obvious increases in FA with age were for ankles and feet (Tables S1–S6). The one exception to the general pattern is the elbow, which became more symmetrical with age. In females, the decrease in elbow FA was apparent across all three age groups, while for males, FA changed little from ages 5–10 to ages 11–15, and then decreased in ages 16–22. Although most traits showed a similar pattern of increase in FA with age, correlations among traits within an individual were weak (as is typical for studies of FA [14,15]). Even without any correction for multiple comparisons, there would be few significant correlations in unsigned FA among traits (1996: knee–foot $r = 0.20$, ankle–foot $r = 0.18$, knee–ankle $r = 0.16$, digit 3–wrist $r = 0.13$; 2002: digit 3–digit 4 $r = 0.22$; 2006: ankle–foot $r = 0.17$, knee–foot $r = 0.16$, wrist–elbow $r = 0.15$).

Because of within-individual changes in FA with age, FA of individuals measured in 1996 was only weakly positively correlated with their FA in later years. We calculated bivariate Pearson Correlation Coefficients between composite standardized FA of subjects at each of the three sampling periods (p-values adjusted for three comparisons using the Sequential Bonferroni Correction). Correlations between 1996 and 2002 and between 2002 and 2006 were marginal, while that between 1996 and 2006 was highly significant (1996 and 2002, $r = 0.17$, $n = 167$, $p = 0.056$; 2002 and 2006, $r = 0.16$, $n = 138$, $p = 0.070$; 1996 and 2006, $r = 0.24$, $n = 163$, $p = 0.006$).

The traits studied show fluctuating, rather than directional, asymmetry with no consistent bias in the population toward one side [20,25]. Within individuals, as the degree of asymmetry in a trait changes with age, does the direction of asymmetry also change, or is it consistent? With only one exception (elbows from 1996 to 2002), the direction of asymmetry stayed the same from one sampling period to the next in >50% of subjects (Table 3). It is very unlikely that the pattern observed in Table 3 could have occurred by chance. Because 18 comparisons were made, one would expect nine to be above 50% and nine below. Instead 17 of 18 are above 50%, or 16 of 17 if those that round to 50% are excluded (kneecaps, 50.3%). In a simple two-tailed Sign Test, the observed results clearly differ from random expectation (17 vs. 1, $p = 0.0001$; 16 vs. 1, $p = 0.0003$). However, for several traits, the percentage was just slightly above 50% and therefore similar to chance when examining particular traits (Table 3). Traits that appeared to be the most consistent in the direction of asymmetry were the three digits (3rd, 4th, 5th) and perhaps ear height. Because of problems of multiple comparisons due to the large number of traits and non-independence of traits within individuals, separate significance tests for each trait were not performed.

Table 3. For each trait, the direction of asymmetry was compared within individuals across years (1996, 2002, 2006). The percentage of individuals in which the sign of right–left stayed the same is shown.

Trait	Year Interval	Same Direction (Percent)	n
3rd Digit	96–02	64.9	174
	02–06	72.5	138
4th Digit	96–02	65.3	176
	02–06	60.6	137
5th Digit	96–02	70.9	175
	02–06	68.1	138
Ankle	96–02	51.7	174
	02–06	53.4	146
Elbow	96–02	40.0	175
	02–06	52.4	145
Foot	96–02	54.7	172
	02–06	56.8	146
Wrist	96–02	61.4	176
	02–06	52.1	142
Knee	96–06	51.8	168
Kneecap	02–06	50.3	145
Ear height	96–06	65.5	171
Ear width	02–06	56.3	144

3. Discussion

In a longitudinal study of Jamaican youth, we demonstrated that bodily FA increases as children grow. Although FA of males and females in our sample did not differ overall, the pattern of change in FA differed between the sexes. FA of boys increased rapidly in early adolescence and then slightly declined into early adulthood. In females, there was a more continuous gradual increase in FA until about age 13 and then a leveling off. This sex X age interaction may reflect sex differences in life history tradeoffs [23,27], and is likely mediated by the increasing hormonal and physical sex differences that arise during puberty. We used a composite measure of FA combined across seven traits, which gives a better indication of developmental stability than analysis of a single trait [3,13–15]. This composite variable was designed so that no individual trait contributed more to the index than the others [15], and it is noteworthy that (other than elbows) the individual traits generally followed the same trends as composite FA. We also find similar results using both longitudinal and cross-sectional analyses.

The increase in FA with adolescence fits Wilson and Manning's [17] suggestion that humans may become less symmetrical during this period of rapid growth and changes in hormones and metabolic rate. The decrease in FA in males from adolescence to adulthood may also fit with their prediction that symmetry should be optimized when approaching adulthood. However, we found an increase in FA with age in young children, while they found a decrease. Other human studies of age and FA also give conflicting results (see the Introduction). Some authors lumped males and females together, which would obscure sex differences in patterns of change with age, but should not produce opposite patterns among studies. Why these studies of human FA give seemingly contradictory patterns is unclear, but it is probably a combination of many factors such as population differences, socioeconomic differences, different kinds of traits, changes in confounding variables with age, and methodological differences [3,20,27]. It is possible that some authors have transformed data inappropriately, which can give the appearance of a decrease in FA with body size [12,13]. Although not statistically significant, variation in measurement error with trait size and across years suggests that measurement error may have been higher for the youngest children in our sample (see Materials and Methods), but such a trend cannot explain the consistent patterns of change in FA that we observed.

In an attempt to resolve the conflict with the results of Wilson and Manning [17], Trivers et al. [20], using our 1996 sample, showed that the increase in FA with age in young children disappears (perhaps even becoming a decrease in boys, though not significant) when height and weight are included as covariates in a multiple regression. Doing so is somewhat misleading, however, because it is expected that changes in FA with age in children would be due to growth and that body size would increase with age more strongly than would FA. This study also used relative FA, which had already been size-corrected before body size was included as a variable. We find that the increase in FA in Jamaican youth is real, not a statistical artefact. Our results, therefore, suggest that there may be underlying differences between human populations in growth patterns and the development of FA [20,26].

That FA increases during periods of rapid growth is expected because it is more difficult to maintain symmetry in structures that are changing in size, there can be trade-offs between growth rate and developmental stability, and rapid growth increases energetic demands which may increase stress [17,28–30]. Of the various models of changes in FA with growth [4–7], our data best fit with random accumulation of developmental accidents ("morphogenetic drift") up until about 13 years of age. After that, processes of compensatory growth or bone remodeling and changes in the distribution of soft tissue may make the individual more symmetrical or maintain the current level of symmetry. Such active maintenance of symmetry would be most important for males in our sample because rapid growth continued to age 16, past age 13 when FA stopped increasing, and began to slowly decrease. In females, FA leveled off at the same age at which the growth rate slowed down. It is possible that asymmetries are not corrected for until a threshold level of asymmetry is reached [4,7], and therefore FA continues to increase with age and growth until this point. Active processes for producing symmetry may also be an adaptive component of the maturation process, as individuals would transition to adulthood with the best possible phenotype given their developmental history [17,18]. Tradeoffs between growth and the maintenance of symmetry [29,30] would also disappear once an individual reaches adult body size. How FA changes throughout adulthood is not known from our data because the oldest individuals in our sample were 22 years old, although we predict an increase in FA with senescence [21,23]. Measurements taken at smaller time intervals than in our study are also needed to fully characterize the relationship between growth and FA.

Because FA changes with age, within-individual correlations in FA across years were weak, although it is notable that FA in 1996 is more strongly correlated with FA 10 years later in 2006 than six years later in 2002. The 2002 sample ranged in age from 11 to 18, and was therefore the year that overlapped the most with adolescence. Although the pattern of change we observed suggests that current FA reflects recent growth more closely than early developmental events [7,11], FA can indicate long-lasting aspects of developmental stability. For example, we previously demonstrated that lower-body (especially knee) FA in 1996 was related to sprinting speed in 2010 [26]. In other human populations, relationships have been demonstrated between socioeconomic status early in life and facial FA in the elderly [27] and between the growth rate of infants and bodily FA at age 9 [29]. In addition to within-individual correlations in the magnitude of asymmetry, there may also be consistency in the direction of asymmetry within individuals despite the lack of directionality in the population overall [4,8,9]. We found that the sign of right minus left stayed the same in more than 50% of subjects for nearly all traits studied. For those traits in which the percentage is only slightly above 50%, the direction of change is most likely random because the starting point is not zero. In other words, if an individual's right ankle is already larger than the left, then the right may remain larger than the left if there is a small shift in the magnitude of asymmetry in either direction. However, for traits where a more consistent within-individual side-bias was observed, it is likely that the direction of asymmetry is nonrandom because we observed an overall increasing trend in the magnitude of asymmetry over time.

Although most changes in symmetry over time probably reflect random processes, that the magnitude and direction of asymmetry in an individual at one point in time tends to be positively (though weakly) related to asymmetry in later years likely reflects differences among individuals in developmental stability. These individual differences may lead to differences in fitness. FA is often associated with important variables such as health status and attractiveness [2], although effect sizes tend to be small [31]. Relationships between symmetry and both sprinting speed and willingness to participate in sprints [26] point to differences in athletic ability and possibly health among rural Jamaican youth. Developmental stability also affects their social interactions. More symmetrical males tend to be less cooperative, as tested in an economic game, and may be more aggressive, as reported by their teachers [25].

This study of changes in human FA with age has implications for the links between developmental stability, growth and symmetry. There are also methodological implications. If FA changes as organisms grow, then it may be best for investigators to either conduct a longitudinal study (if practical) or to remove heterogeneity due to growth by selecting a very specific age, developmental stage, or size-range of individuals to study [4,32]. At the very least, age (or nonlinear effects of age) should be included as a covariate or otherwise controlled for in any study of FA that includes a heterogeneous sample [27], and the sexes should be analyzed separately. Some of the contradictory or weak results in the FA literature [16,31] may have resulted from different authors mixing together individuals of different ages.

4. Materials and Methods

4.1. Subjects and Measurements

The data analyzed here were collected as part of a longitudinal study of fluctuating asymmetry in Jamaica [20]. When FA was first measured in 1996, the subjects included 288 Afro-Caribbean children (155 males, 131 females and 2 of unrecorded gender) from Southfield, St. Elizabeth parish, mostly from the same elementary school (Top Hill Primary School, a nearly complete sample) In 1996, the children ranged in age from 5 to 12 years (mean age ±SD = 8.18 ± 1.73). The subjects were then measured again for FA in 2002 (n = 179, mean age = 14.12 ± 1.74) and 2006 (n = 174, mean age = 17.98 ± 1.72). Across the time course of the study, ages ranged from 5 to 22. Sample sizes for analysis are typically large, but smaller than those reported here (see Results) because not all subjects had complete measurements of all traits in all years.

Research protocols for the symmetry measurements and other projects involving this cohort were approved annually by the Institutional Review Board for the Protection of Human Subjects in Research of Rutgers, the State University of New Jersey (most recently Protocol #10-378M, approved 18 February 2010). Participation of subjects included written parental consent for minors and written informed consent for those who had reached age 18.

The measurement protocol is described in Trivers et al. [20] and included the following bilateral traits in 1996: length of the 3rd, 4th and 5th digits, ear height, elbow width, wrist width, knee width, ankle width, foot length, and hand width. As is standard in FA studies, we measured the traits twice per side for each individual to quantify measurement error and ensure that differences between sides are greater than measurement error. Furthermore, by averaging the replicate measurements, measurement error is reduced [3,13]. Other than hand width, which showed directional asymmetry, asymmetry in these traits reflects true FA and the between-sides variation was much greater than measurement error (20, 25 and below). Height and weight were also measured in each year and are included as covariates in some of the analyses. The same traits were measured in 2002 and 2006 following the same procedures as in 1996, with the following exceptions: (1) hand width was eliminated, because of the presence of directional asymmetry [20]; (2) in 2002, diameter of the kneecap was measured rather than width of the knee. In 2006, both kneecap and knee measurements were performed; (3) in 2002, width of the ear was measured rather than ear height. In 2006 both ear height and width were measured; and (4) digit lengths were measured directly in 1996, but from photocopies of the hands in 2002 [24] and electronic scans in 2006.

Repeatabilities of measurements for all traits were very high in both 1996 (all $r > 0.96$) and 2002 (all $r > 0.99$) and for most traits in 2006 (all $r > 0.93$ except ear width (0.86 left, 0.90 right) and kneecap (0.90 left, 0.93 right)). There was no significant relationship between trait size and measurement error (ME) in any year and the direction of the trend varied across years (1996, $r = -0.49$, $p = 0.18$; 2002, $r = 0.08$, $p = 0.84$; 2006, $r = 0.25$, $p = 0.46$). As the repeatabilities above suggest, there was also no clear pattern of variation in ME with age: ME was higher in 1996 than 2002, but varied among traits in 2006. FA was significantly greater than ME for all traits in all years (Sides X Individuals interaction in mixed-model ANOVA [13], all $p < 0.0001$).

Because not all traits were measured in all years, when constructing a composite variable for overall bodily FA (see below), hand width, ear height and width, knee width and kneecap width were excluded. Except for hand width, we do consider these traits when presenting data for single traits rather than composite FA. Manning et al. [33] advise against using indirect finger measurements in studies of symmetry. However, several lines of evidence suggest that the reliability of finger measurements was not decreased by switching to indirect measurements in later years. Repeatabilities for all finger measurements were above 0.98 in 1996 and above 0.99 in 2002 and 2006, and measurement error as a percent of between-sides variation [13] was much lower for the indirect measurements in 2002 and 2006 (1.8% to 3.4%) than for the direct measurements in 1996 (14.9% to 19.9%). Correlations between 1996 FA and FA in other years were higher for digits (r values range from 0.23 to 0.54, all $p < 0.005$; $p < 0.05$ after adjustment for 18 comparisons) than for other traits (compare with between-year correlations for composite FA in the Results). In addition, the direction of finger asymmetry is relatively consistent within-individuals between years (Table 3), and we observed similar patterns of change with age among traits, including digits (see Tables S1–S6).

4.2. Statistical Analysis

Composite measures that combine FA from multiple traits typically better reflect overall developmental stability than any single trait [3,14,15]. Before creating a composite index, unsigned FA (absolute value of right−left) was calculated for each trait in each year for every subject. Simply adding these values across traits can create bias, as more asymmetrical traits would add more to the composite FA index [3,15]. The traits we studied differ in FA; for example, in our 1996 sample, lower-body traits were more symmetrical than upper-body traits [20,26]. We used composite FA 2 of Leung et al. [15]: unsigned FA was first divided by mean FA for that trait, and these standardized values were added across traits within an individual. This sum provides an unbiased and powerful composite index of FA, which we refer to as "composite standardized FA". Many previous authors (ourselves included) have analyzed relative FA (unsigned FA divided by trait size) and composite relative FA (relative FA summed across traits), but several authors have warned against "correcting" FA for trait size, which can lead to spurious relationships between variables especially if size-scaling is absent, weak, or non-isometric [3,13,15]. We found very weak evidence for size-scaling in our dataset. Of 29 possible unsigned FA-trait size correlations, with no correction for multiple comparisons, only 6 were significant positive correlations, 1 was a significant negative correlation, and none had an absolute value of $r > 0.20$. Our statistical analyses are based on composite standardized FA, but we also show summary data for composite relative FA to allow easier comparison with previous publications.

Statistical analysis was performed with IBM SPSS Statistics 21 (IBM Analytics, Armonk, NY, USA). Parametric tests are used, as recommended by Gangestad and Thornhill [34], all with a two-tailed α-level of 0.05. We used a combination of repeated measures tests (within-individual comparisons across years) and between-subjects tests (comparisons of different age groups). Age groups (5–10, 11–15, 16–22) were selected following Wilson and Manning [17] and to keep sample sizes among groups similar. To avoid pseudoreplication, a single observation year was selected for each individual in the between-subjects design [10]. We did so semi-randomly to achieve a more even spread of ages because more individuals were measured in 1996 than in 2002 or 2006, in the following manner: (1) if a subject was measured in only 1 year, we used that year; (2) if a subject was measured in only 2 years, a coin flip determined the year to use; and (3) if a subject was measured in all three years, 1996 was eliminated and a coin flip determined whether to use 2002 or 2006 data.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-8994/8/11/123/s1. Tables S1–S6 Standardized FA for individual traits across age groups: S1 All subjects, both sexes; S2 Selected subjects, both sexes; S3 All males; S4 Selected males; S5 All females; S6 Selected females.

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Author Contributions: Robert Trivers designed the study and oversaw data collection in each year. Brian G. Palestis analyzed the data and wrote the paper. Both authors edited and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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