Genetica **89**: 235–244, 1993.

Parental age and developmental stability in *Drosophila melanogaster*

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Received 19 May 1993 Accepted 24 May 1993

Abstract

Two strains of *Drosophila melanogaster*, one outbred, recently derived from nature, and the other created by intensive directional selection on phototactic behavior for 19 years, were used to test the hypothesis that developmental stability is influenced by parental age. Three characters were examined: sternopleural bristle number, wing length, and wing area. The results do not support any relationship between parental age, either young or old, and developmental stability in offspring.

Introduction

Developmental stability is defined as the ability of an organism to buffer itself from environmental disturbances and develop according to its ontogenetic plan (Waddington, 1957). Quantification of developmental stability is typically measured via assessments of fluctuating asymmetry (FA), or the random differences between the right and left sides for any of a variety of characters in bilaterally symmetrical organisms.

The last 15 years have seen a resurgence of interest in developmental stability and fluctuating asymmetry across a wide range of approaches to the study of biology. For example, decreases in developmental homeostasis have been linked to homozygosity, to heterozygosity associated with introgression and its breakup of coadapted gene complexes, and to a variety of nongenetic stressors, leading conservation biologists to use measures such as FA as indicators of endangerment (Leary & Allendorf, 1989).

Parental age is one nongenetic stressor reported to influence developmental homeostasis in a number of organisms including fruit flies (Parsons, 1962) and humans (Livshits *et al.*, 1988). Parsons (1962) observed that in *Drosophila*, offspring often exhibit increased fluctuating asymmetry for sternopleural bristles when parents were very young or very old; he attributed this relationship to differences in the quality of eggs produced by age-extreme females, producing a maternal effect on developmental homeostasis of offspring. Advanced maternal age was found to be associated with increased asymmetry in human infants, possibly as a secondary effect conditioned by changes in length of gestation (Livshits *et al.*, 1988), but an increase in developmental instability has not been seen in the offspring of extremely young mothers.

In many natural populations of organisms, reproduction is occurring simultaneously by individuals of a variety of ages. In others, population age structure may be such that the mean reproductive age may vary as a function of the time at which a population is sampled. If developmental stability can vary with parental age, and parental age can vary temporally or spatially, studies seeking to evaluate the presence of various stressors may be easily confounded by parental age effects. With the increased employment of developmental stability as an indicator of population vulnerability by conservation biologists, it is important that all sources of developmental instability be carefully documented.

In order to ensure that such confounding effects have been sufficiently monitored, we repeated the earlier experiments of Parsons (1962) on parental age and fluctuating asymmetry in *Drosophila melanogaster*, but with an expanded design. In addition to measuring developmental stability for

sternopleural bristle number, we added two wing variables: a standard measure of wing length (Markow & Ricker, 1991) and a measure of wing area. We also employed comparison of a pair of strains of greatly contrasting genotype. One was a mass-reared wild-type strain, derived three generations earlier from a mass collection (n > 100) in the wild. The other strain had been intensely selected for photopositive behavior in a maze since 1972, had been through several bottlenecks, and contained little genetic variation (Markow, 1975). We also extended the assessment of progeny through a maternal age of 35 (photoselected) and 39 (wild-type) days, respectively, rather than 30 days, in order to increase the likelihood of detecting effects associated with greatly advanced age.

Materials and methods

Two strains of *Drosophila melanogaster* were utilized in this procedure: ASU390, a strain collected from a natural population breeding in citrus in Tempe, Arizona in March, 1990 and PHOTO+, a laboratory strain artificially selected for positive phototaxis for 20 years, hence relatively homozygous (Markow, 1975).

Ten male and ten female virgins from each strain were removed from uncrowded cultures and placed into collection vials containing a banana medium and yeast. Two such vials were prepared for each strain, each containing ten female/male pairs. These parent flies were transferred to fresh vials on a daily basis (between 20-28 hours from time of last transfer) for a total of 35 days (PHOTO+) and 39 days (ASU390), at which time breeding capacity was minimal.

The eggs laid in these vials were closely monitored and during the first instar larvae stage, 40 larvae per vial were removed from the substrate with a small spatula and transferred to a fresh vial labeled by maternal age (in days). In this fashion, even though female productivity declined with age, density during larvae development was kept constant. Larvae were collected daily for days 1-9, and every other day thereafter until eggs were no longer viable.

At three days post-eclosion, 15 male and 15 female offspring (per maternal age day) were collected at random and anesthetized. Counts for left and right sternopleural bristles were obtained using a dissecting microscope. Both wings were then removed, mounted on projection slides and labeled for 1), sex, 2) maternal age of eggs, and 3) wing side, R or L.

Upon completion of wing mounting procedure, two wing measures (Fig. 1) were obtaining using an IBM PC and BIOSCAN image analysis system.

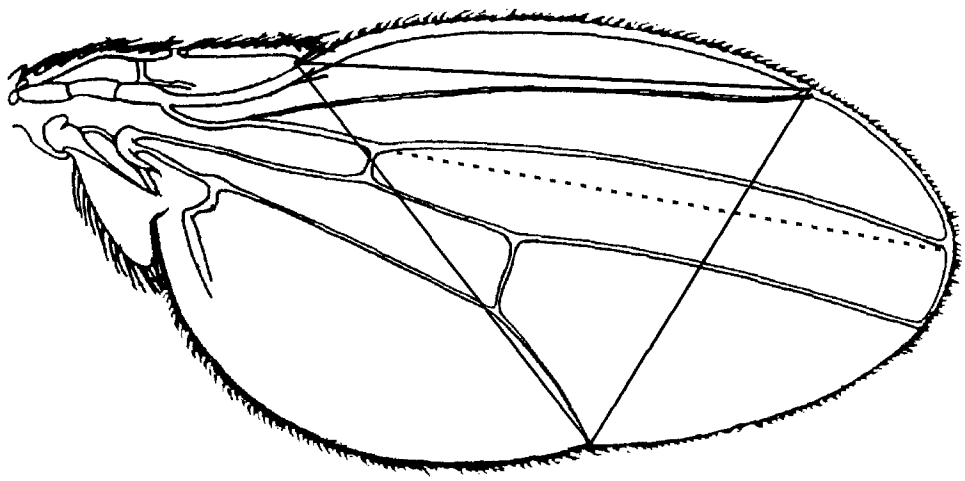

Fig. 1. The length and area measures made on Drosophila wings.

Slides were placed under a light source and camera connected to the computer. Wings were magnified 25× and the images viewed on the computer screen. Images were consistently oriented with the long axis parallel to the horizontal in order to minimize distortion as a function of rotation. Linear measure in mm at 25× was calibrated using a micrometer; all wing data were measured against this calibration. End points were manually placed at the appropriate wing junctures using an electronic mouse.

Both the linear and area measures were calculated and recorded in mm (and mm^2 respectively), as per calculation standard. Twenty random wings were remeasured at the end of the procedure to ensure consistent endpoint placement and calibration invariance and differences were found to be less than 0.5%.

A total of three bilateral characters was thus measured for each animal: sternopleural bristle number, wing length, and wing area. Each character was quantified by the formula R+L / 2, or an average of both sides, and it was these measures that were used to assess differences among strains and sexes for the characters themselves. Developmental stability was measured by calculating fluctuating asymmetry measures, R-L. Scale effects were tested for by seeking correlations between the R-L values and trait magnitude (R+L / 2). As none were found, subsequent analyses were performed on untransformed fluctuating asymmetry values (Palmer & Strobeck, 1986).

Results

Strain means for the three traits, wing length, wing area, and sternopleural bristle number, are shown in Table 1. Before evaluating the impact of parental age in the two strains, we first asked whether the strains and sexes differed in their mean wing and bristle measures. Data were analysed using the General Linear Models (ANOVA) procedure from SAS (SAS, 1985) on the ASU mainframe computer. The F values were significant at $p < 0.0001$ for each trait: wing length ($F = 837.92$, df 3, 1370), wing area ($F = 212.45$, df 3, 1361) and bristle number ($F = 91.43$, df 3, 1362). Both strain and sex were significant factors. Males of both strains have significantly smaller wings, in both the length and the area measures, than females, but males did not always show the smallest values. Bristle number was significantly lower in PHOTO+ males compared to females, while in ASU 390, females had lower mean bristle number. Greater values for all traits were observed in the PHOTO+ strain.

Fluctuating asymmetry values for each measure are reported in Table 2. In no case was any correlation observed between trait magnitude and degree of fluctuating asymmetry. Thus as mentioned above, subsequent statistical analyses were conducted using untransformed data. Wing length asymmetry (strain $F = 7.79$, df 1, 1370) and sternopleural bristle number asymmetry (strain $F = 7.72$, df 1, 1362) were significantly greater in flies from the PHOTO+ strain. PHOTO+ males showed even greater bristle asymmetry than females (sex $F = 7.36$, df 1, 1362) no other significant strain or sex differences emerged.

As expected for a strain having undergone prolonged directional selection, the PHOTO+ strain was characterized by slower development rate and an earlier decline of reproductive capacity (35 days) than the wild type strain (39 days). After day 31, a decrease in the number of eggs laid per day and a decrease in viability of eggs made it difficult to obtain 40 first instar larvae/vial.

The impact of parental age on character magnitude is seen in plots (Figs. 2, 3) of the mean of each character against the age of the mothers at the time of oviposition. Both linear and non-linear equations were used in attempting to find the best fitting

Table 1. Wing length and area (in mm and mm^2) and sternopleural bristle numbers by strain and sex. Standard errors are in parentheses.

Variable	N	Photo + females	Photo + males	390 females	390 males
wing length	330	1.4128 (0.0024)	1.2740 (0.0021)	1.3801 (0.0032)	1.2616 (0.0025)
wing area	326	0.5374 (0.0018)	0.4347 (0.0015)	0.5013 (0.0025)	0.4188 (0.0019)
bristle number	325	11.6385 (0.0699)	11.1167 (0.0689)	10.3062 (0.0605)	10.4592 (0.0578)

Table 2. Wing length and area (in mm and mm^2) and sternopleural bristle numbers by strain and sex. Standard errors are in parentheses. WL = wing length, WA = wing area, SP = sternopleural bristle number.

Variable	N	Photo + females	Photo + males	390 females	390 males
WL FA	330	0.0117 (0.0005)	0.0107 (0.0005)	0.0099 (0.0004)	0.0099 (0.0005)
WA FA	326	0.0100 (0.0004)	0.0081 (0.0004)	0.0088 (0.0004)	0.0079 (0.0004)
SP FA	325	1.1661 (0.0559)	1.3545 (0.0597)	1.0112 (0.0445)	1.1268 (0.0490)

Table 3. Linear and non linear regression ANOVAs for average character size on parental age. Plots and equations for best fitting lines are presented in Figures 2 and 3.

				Linear			Quadratic		
Trait	Strain	Sex	DF	Mean square	F	P	Mean square	F	P
WL	Photo +	F	1	0.00000286	0.005	0.9460	0.00000107	0.002	0.9670
		M	1	0.00036174	0.510	0.4833	0.00088102	1.285	0.2705
WA	Photo +	F	1	0.00002311	0.060	0.8096	0.00000320	0.008	0.9286
		M	1	0.00018238	0.521	0.4790	0.00034737	1.015	0.3257
SPB	Photo +	F	1	0.10356899	0.294	0.5935	0.12506723	0.356	0.5572
		M	1	0.65281122	1.502	0.2345	0.22032737	0.483	0.4951
WL	390	F	1	0.00257279	2.384	0.1369	0.00199543	1.805	0.1928
		M	1	0.00058442	0.875	0.3597	0.00018946	0.276	0.6044
WA	390	F	1	0.00217405	3.153	0.0896	0.00170974	2.406	0.1351
		M	1	0.00389986	0.970	0.3354	0.00011091	0.263	0.6132
SPB	390	F	1	0.02521376	0.211	0.6504	0.01336028	0.111	0.7418
		M	1	0.02735592	0.241	0.6283	0.04879880	0.434	0.5170

Table 4. Linear and non linear regression ANOVAs for fluctuating asymmetry on parental age. Plots and equations for best fitting lines are presented in Figures 4 and 5.

				Linear			Quadratic		
Trait	Strain	Sex	DF	Mean square	F	P	Mean square	F	P
WL FA	Photo +	F	1	0.00000718	1.974	0.1753	0.00000586	1.583	0.2229
		M	1	0.00000446	0.850	0.3674	0.00000273	0.512	0.4820
WA FA	Photo +	F	1	4.613^{-10}	0.000	0.9916	1.369^{-7}	0.034	0.8562
		M	1	1.618^{-7}	0.033	0.8579	0.00000254	0.530	0.4750
SPB FA	Photo +	F	1	0.00489296	0.086	0.7722	0.01491442	0.265	0.6124
		M	1	0.18299524	2.277	0.1469	0.10527840	1.250	0.2769
WL FA	390	F	1	3.20975^{-7}	0.076	0.7859	0.00000281	0.681	0.4181
		M	1	0.00022931	3.991	0.0583	0.00001568	2.582	0.1223
WA FA	390	F	1	0.00000535	1.371	0.2541	0.00000411	1.038	0.3194
		M	1	0.00000177	0.491	0.4909	1.48772^{-8}	0.004	0.9500
SPB FA	390	F	1	0.00878989	0.139	0.7133	0.01159170	0.183	0.6290
		M	1	0.000536773	0.070	0.7145	0.01926944	0.252	0.6209

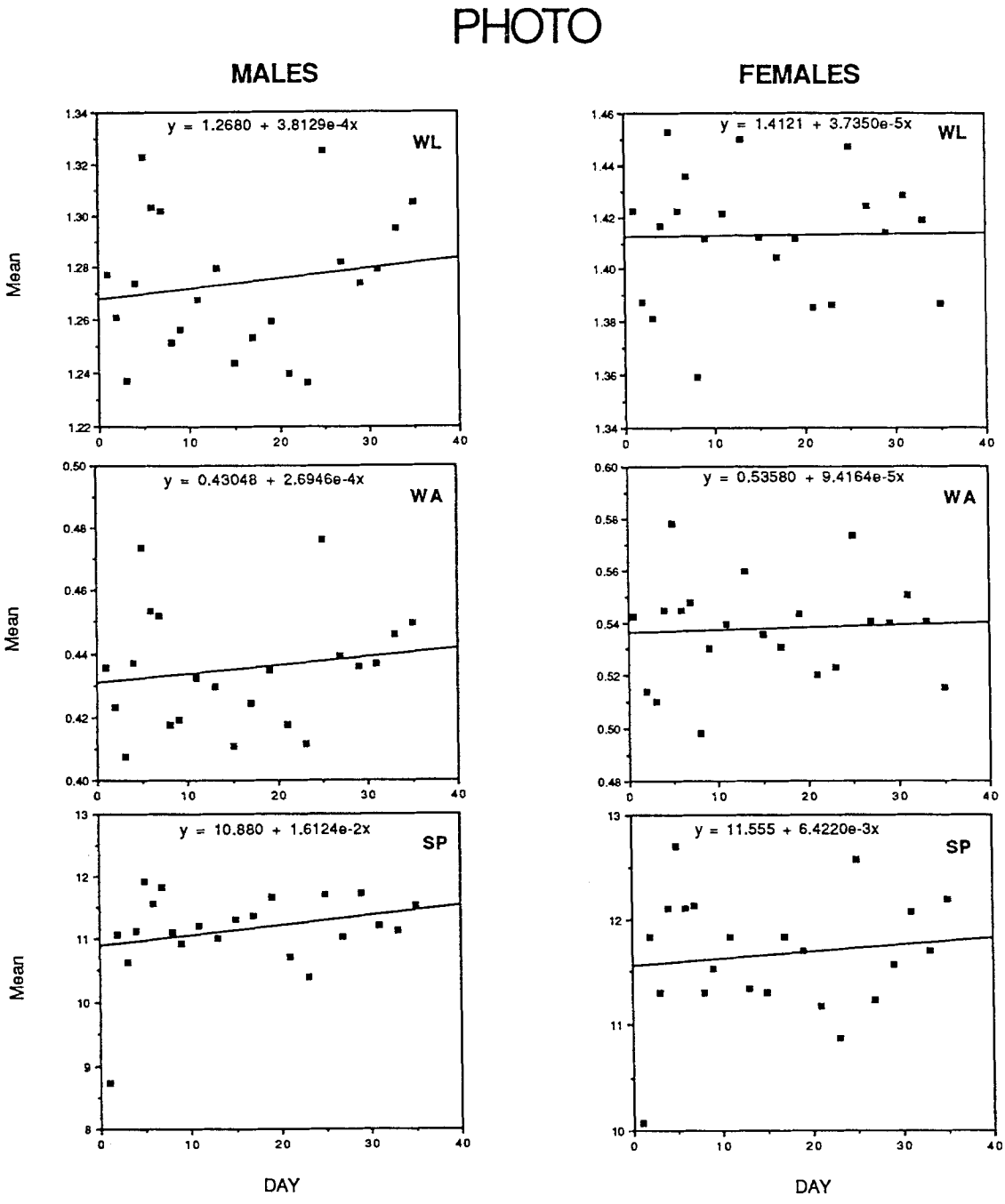

Fig. 2. Regression of trait magnitude on maternal age in the photo positive strain. WL = wing length, WA = wing area, and SP = sternopleural bristle number.

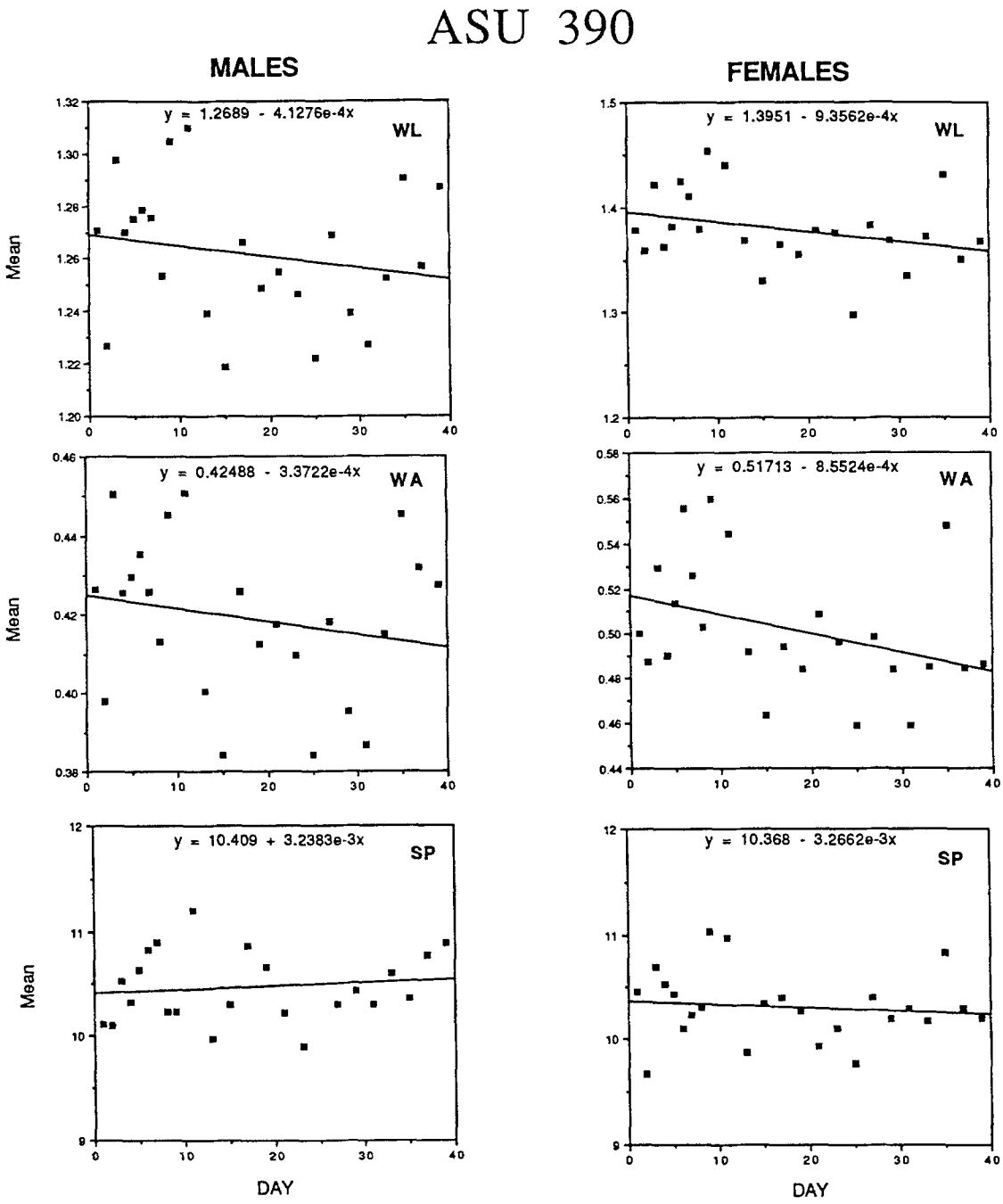

Fig. 3. Regression of trait magnitude on maternal age in the ASU 390 wild type strain. WL = wing length, WA = wing area, and SP = sternopleural bristle number.

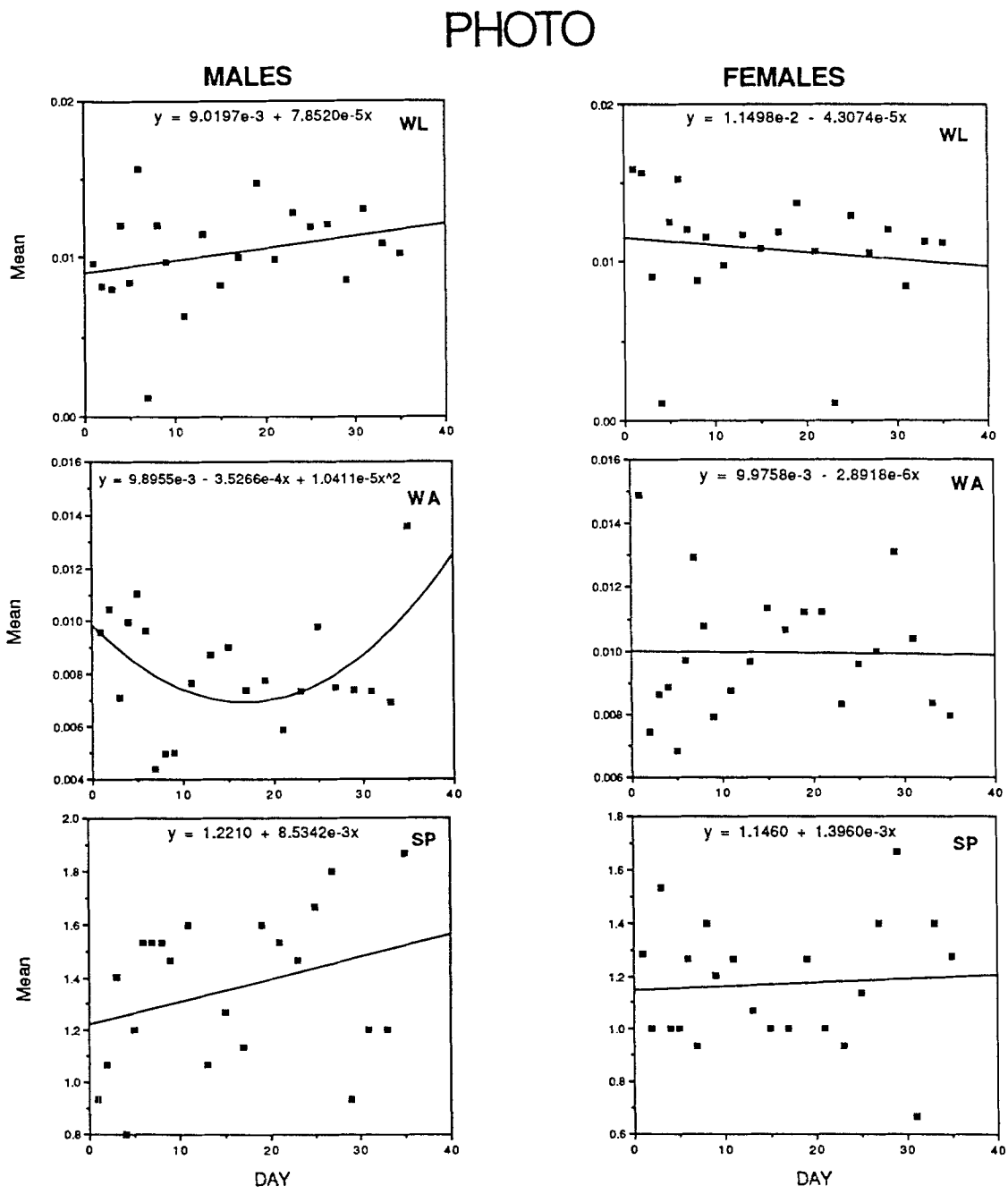

Fig. 4. Regression of trait fluctuating asymmetry on parental age in the photo positive strain. WL = wing length, WA = wing area, and SP = sternopleural bristle number.

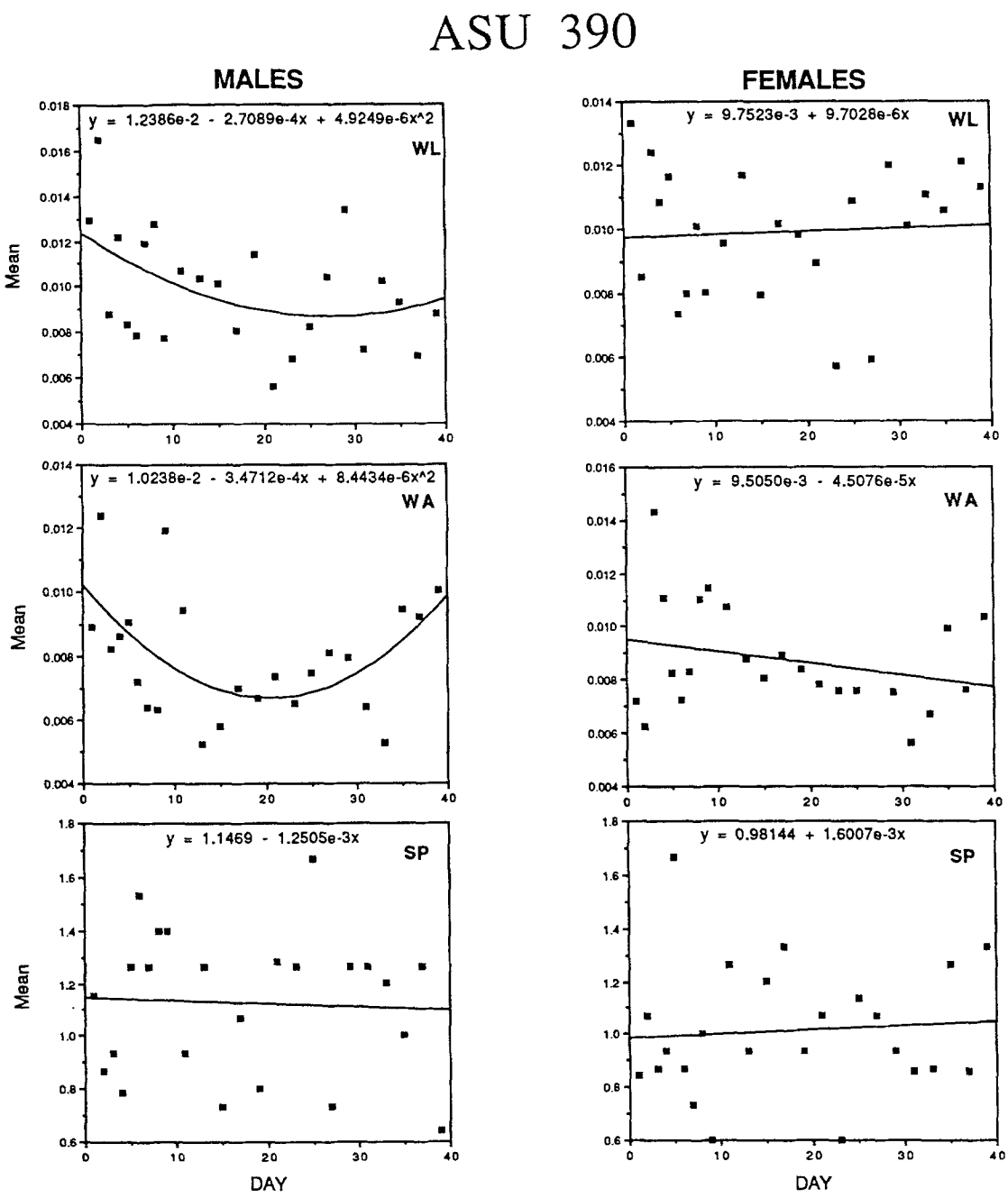

Fig. 5. Regression of trait asymmetry on parental age in the ASU 390 wild type strain. WL = wing length, WA = wing area, and SP = sternopleural bristle number.

relationships. Lines represent the best fit, not necessarily statistically significant ones. Sexes were examined separately to see if they differed in their potential sensitivity to maternal age effects. Figure 2 presents the data for the PHOTO+ and Figure 3 for ASU 390. Analysis of variance revealed that no significant relationships, either linear or quadratic, were present for any of the three traits and parental age (Table 3).

Changes in developmental stability with parental age are presented in Figure 4 for the PHOTO+ strain and Figure 5 for ASU 390. Again, lines are derived from the best fitting equations, regardless of statistical significance. None of the measures of fluctuating asymmetry showed a significant relationship to maternal age in any regression component (Table 4).

Discussion

In our study, parental age was not observed to influence the magnitude of any traits. Parsons (1962) found a significant parental age effect on egg size and argued that this effect was responsible for differences observed in the developmental stability of flies developing from those eggs.

The discussion offered by Parsons (1962) is intuitively satisfying and may well apply in many cases. By observing a different result with different *Drosophila* strains in a different laboratory, we feel that the relationship between parental age and developmental stability is likely to be less general than proposed earlier. For example, Parsons reported, in addition to the findings previously mentioned, that fluctuating asymmetry was even more enhanced in offspring that were incubated at 30 °C relative to those incubated at 25 °C. Temperature is therefore a clear stress factor. However, the temperature of minimal or negligible stress is unknown, and our laboratory temperature of 24 °C may have contributed to the lack of maternal age effect by eliminating a potential confounding interaction.

Unlike Parsons, we did not measure egg size in our study. We did however, investigate the additional FA indicator concerning the degree to which parental age influenced developmental stability of wings and bristles. Several factors in the present study should have enhanced our ability to detect a parental age effect on developmental stability if it existed. First, the upper age limit of parental flies in the present study was greater than in the earlier report. Second, we used a strain of flies, PHOTO+, which was higly inbred and thus should have been highly sensitive to parental age effects. However, no influence of parental age on developmental stability of any trait in any strain was observed. The PHOTO+ strain did yield the expected elevated incidence of developmental instability, but no parental age effect.

Flies from the two strains differed from each other in the magnitude of the three traits. Photostrain flies had significantly higher averages than flies of the wild-caught strain. We had expected that flies from ASU 390 would be larger, due to their recent derivation from nature and concomitant high levels of genetic variability and heterozygosity. The larger trait magnitudes attained by photostrain flies may reflect their longer developmental times. Photostrain adults emerged 1-3 days later than did imagoes from strain 390.

Our observations are not that surprising in view of the report by Livshits and Kobyliansky (1991) on nongenetic factors that influence developmental stability in humans. These investigators found only a minimal influence of maternal age on fluctuating asymmetry for dermatoglyphic traits. Interestingly, the most significant nongenetic predictor of fluctuating asymmetry in offspring was found to be maternal fluctuating asymmetry. Maternal condition, not necessarily maternal age, may be of more importance among factors capable of influencing offspring developmental and fitness. Further, as noted, the work of Livshits *et al.* (1988) suggests that increased FA in humans as a function of maternal age is secondary to complications of gestational age; exclusion of preterm infants would control for this complication. All of these factors should be more thoroughly investigated across taxa before they can be confidently discussed.

Acknowledgements

We thank Dr. Michael Douglas for his advice and use of his image analysis system, Dr. Gregory Livshits for comments on the manuscript, and Mrs. Ann Bradley for running a fine *Drosophila* kitchen. This work was supported in part of NSF grant *GR BSR8919362* to T. A. Markow.

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