Effect of Maternal Age on Offspring Quality in Tsetse (Diptera: Glossinidae)

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J. Med. Entomol. 35(3): 210–215 (1998)

ABSTRACT The effects of maternal age on offspring quality were studied in 1 line of *Glossina palpalis palpalis* Robineau-Desvoidy, 1 line of *G. p. gambiensis* Vanderplank, and 3 lines of *G. morsitans morsitans* Westwood by measuring offspring adult size and the duration of puparial period. *G. p. gambiensis* males also were examined for effects of maternal age on fluctuating asymmetry of wing veins. The puparial period was shorter in offspring of old females (late offspring) than in offspring of young females (early offspring). The difference was small but was greater for male than for female offspring. Early male offspring were larger than late males. Wing vein fluctuating asymmetry was slightly greater in early than in late offspring in *G. p. gambiensis*. The differences between early and late offspring were very small, and we conclude that old females produce offspring of marginally lower quality than those produced by young females and that these differences are not biologically significant.

KEY WORDS tsetse, maternal effects, fluctuating asymmetry, colony quality

THE OFFSPRING of young insects often differ from those of old insects in morphological, physiological, and life history traits that are important to fitness (Mousseau and Dingle 1991). Most studies that demonstrate negative correlations between maternal age and fitness traits have used autogenous Coleoptera or Lepidoptera that are typical r strategists (e.g., Fox 1993). How maternal age affects offspring fitness in K-selected insects, such as tsetse (*Glossina* spp.), is not known. For insects with low fecundity and long reproductive life, there may be considerable selective pressure to maintain offspring quality at advanced maternal ages, because late offspring contribute relatively more to female fitness than is the case in short-lived, highly fecund insects.

When rearing tsetse, ≈30% of the labor expended to maintain colonies involves manipulating young adult flies, therefore there is considerable pressure to maximize colony production by maintaining females for as long as possible. This results in a mixture of individuals whose mothers varied widely in age being used for a variety of physiological studies or for sterile-male release programs. If maternal age affects the traits under investigation and the maternal age distribution differs from that of natural populations, the results of these studies may not help in understanding and controlling natural populations.

Tsetse are haematophagous insects that reproduce through adenotrophic viviparity. During each reproductive cycle a female produces a single chorionated egg that is fertilized, develops and hatches in the uterus where the larva is nourished to maturity by secretions from "milk glands." The 1st larva is deposited when the female is 17–20 d old. Newly deposited larvae immediately burrow into the soil and pupariate. The puparial period lasts ≈30 d. Subsequent larviopositions occur at ≈9- to 10-d intervals, at 24°C during a reproductive period of several months (Buxton 1955). In a well managed colony, females have an average lifetime production of 7–10 offspring (Jordan and Curtis 1972). Tsetse differ from the subjects of most previous investigations of insect maternal age in 2 important respects: (1) They have a brief, nonfeeding, free-living larval stage, with teneral adults developing solely from nutrition provided by their mothers. (2) Tsetse flies are K strategists that produce very few offspring at evenly spaced intervals over a relatively long reproductive life. Accordingly, we expect females to maintain high offspring quality throughout their reproductive life, because late tsetse offspring are relatively important to fitness.

Investigations of maternal age effects ultimately are concerned with offspring fitness, but assessing fitness directly can be very difficult and therefore indirect estimates of quality are normally used. Although survival and development rates are employed, size is the most frequently used estimator of quality in investigations of insect maternal age effects. Size is measured easily and is generally thought to be correlated positively with fitness (Roff 1992), although this is not always the case (Zamudio et al. 1995).

Fluctuating asymmetry (FA), random side to side deviations in bilaterally symmetrical traits, has become popular as an indicator of quality in animals (Leary and Allendorf 1989). Fluctuating asymmetry is thought to indicate developmental stability: the ability of an organism to express its developmentally programmed phenotype, in the face of epigenetic stresses

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(Van Valen 1962). Such stresses may be from genetic or environmental sources or from genotype-environment interactions (Markow 1995). Fluctuating asymmetry should vary among offspring produced at different maternal ages in species where maternal age affects developmental stability either through genetic stress (e.g., changes in aneuploidy) or through environmental stress (e.g., changing ability to provision eggs).

In the current article we examine the effects of maternal age on offspring quality, estimated through changes in adult size, development rate, and fluctuating asymmetry, in 3 species of tsetse. We present evidence from laboratory colonies showing that, although maternal age may have statistically significant effects upon bionomic parameters in the offspring, the magnitude of these changes are unlikely to be biologically significant.

Materials and Methods

Experimental Procedure. To determine the effect of maternal age on offspring quality in tsetse flies, we examined 3 lines (134, 165, TRL) of *Glossina morsitans morsitans* Westwood, 1 line (PALP-O) of *Glossina palpalis palpalis* Robineau-Desvoidy, and 1 line (GAMB-K) of *Glossina palpalis gambiensis* Vanderplank. Flies were from small colonies, each consisting of from 45 to 100 breeding females, that were maintained at 24°C by feeding on rabbits every 2nd d, using a protocol that conformed to the guidelines of the Canadian Council on Animal Care (University of Alberta Biosciences Animal Care Committee, SOP RP5109). Each line, except TRL, had been selected for one or more biochemical or visible marker genes. Lines TRL, 134, and 165 were derived from *G. m. morsitans* collected as puparia in Zimbabwe (see Gooding and Jordan [1986] for the early history of this colony). The TRL line was obtained from the Tsetse Research Laboratory, University of Bristol in 1985, and the other lines were derived from puparia obtained from the Tsetse Research Laboratory in 1973 and subjected to genetic selection since 1975. Line 134 was outcrossed to flies carrying the body color mutant gene *ocra* (described by Bolland et al. 1974) before being selected for several marker genes. GAMB-K originated, in the early 1980s, from a colony established at the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux laboratory at Maisons-Alfort, France, using material collected near Bobo-Dioulasso, Burkina Faso, in 1972. *G. p. palpalis* originating from Nigeria were obtained from Rijksuniversitair Centrum Antwerpen and subsequently subjected to selection for biochemical marker genes to establish the line PALP-O. All flies used were from longstanding colonies. However, there is little or no evidence for laboratoy adaptation during establishment of tsetse colonies (Gooding and Jordan 1986, Gooding 1990). Additionally, flies from a colony that had been in the laboratory for ≈48 generations were not significantly different from field flies with respect to survival, dispersal, mating success, or behavior (Vale et al. 1976).

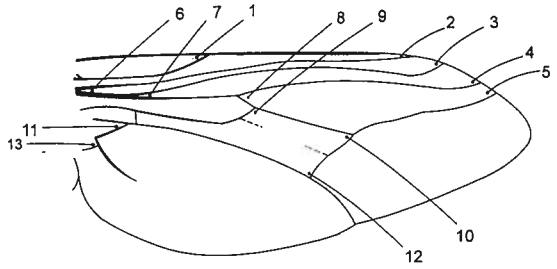

Fig. 1. Landmarks measured on the wings of *G. p. gambiensis* males. For all landmarks, the inner edge of the vein intersection was measured.

Two cages containing ≈15 females each were established from each line. One cage contained young females (≈20 d old) and the other cage contained old females (≈80 d old). Age of females at the beginning of each replicate varied by ±10 d for young flies and ±15 d for old flies, depending on availability. Newly deposited puparia were collected daily from each cage over a period of 20–24 d and placed individually in labeled tubes until emergence. Sex and date of emergence were recorded for each fly. At 30–54 h after emergence, teneral males were killed by freezing and the head width measured using an ocular micrometer in a dissecting microscope. Four replicates were run over a period of 4 mo. This is not a longitudinal study (i.e., the offspring of young and old females had different mothers).

Fluctuating Asymmetry Measurements. GAMB-K was selected for study because this line provided the best combination of large sample size and wide separation of maternal age classes. Right (R) and left (L) wings of male offspring from young and old females were mounted in Euparal. Three sets of replicate measurements were made of 13 landmarks on each wing (Fig. 1) using a dissecting microscope and camera lucida with a Summasketch FX data tablet (Summagraphics, Seymour, CT). To estimate the accuracy of this apparatus, 10 measurements were made of a 1.0-mm stage micrometer. The mean of these measurements was 0.999 mm with a standard deviation of 0.0047. After culling flies with damaged wings, 27 offspring of old GAMB-K mothers (Gpg-LATE) and 43 offspring of young GAMB-K mothers (Gpg-EARLY) were measured by GSM. Gpg-EARLY wings were measured with 10 wk between 1st and 3rd replicates and Gpg-LATE wings were measured with 1 wk between 1st and 3rd replicates. Distances were calculated for all possible line segments between landmarks; designated by landmark numbers of the end points (i.e., line segment connecting landmarks 1 and 2 is designated L1-2).

Statistical Analyses. Sex ratio was compared between maternal age classes using chi-square. Distributions of puparial duration and head width were non-normal in most samples and no suitable transformation was found. Accordingly, differences between

Table 1. Mean age (days) of mothers at larviposition

Line	Offspring of young mothers		Offspring of old mothers	
	n	Mean ± SE	*n*	Mean ± SE
134	73	38.1 ± 1.12	60	86.6 ± 1.21
165	76	38.6 ± 1.14	32	91.4 ± 1.58
GAMB-K	123	27.4 ± 0.61	68	85.3 ± 1.26
PALP-O	89	30.6 ± 0.74	76	95.4 ± 1.01
TRL	113	33.1 ± 0.95	74	88.3 ± 1.05

maternal age classes were analyzed using the Student–Newman–Keuls test, which is insensitive to departures from normality, in a randomized block analysis of variance (ANOVA) with replicates as blocks. Size differences between Gpg-LATE and Gpg-EARLY flies also were analyzed using the landmark data collected for fluctuating asymmetry analysis. Centroid size, the square root of the sum of squared distances of landmarks from their centroid (Marcus et al. 1996), was calculated and compared between Gpg-LATE and Gpg-EARLY *G. p. gambiensis* progeny.

Only line segments with R/L variation significantly greater than measurement error, (R-L) distributed normally with a mean of zero, and no size dependence of |R-L| w]ere compared for differences in fluctuating asymmetry between Gpg-EARLY and Gpg-LATE offspring. Tests for these criteria followed Palmer (1994). To test for significance of differences in fluctuating asymmetry between age groups, a 2-way ANOVA of age × line segment was conducted for |R-L| (Palmer 1994). *F* tests were then used to compare the variances of (R-L) and the sum of $(R\text{-}L)^2$ divided by sample size (FA4 and FA5 $[\Sigma(R\text{-}L)^2/n]$ of Palmer [1994], respectively) between Gpg-LATE and Gpg-EARLY flies for individual line segments. The sequential Bonferroni correction was applied to the results to control for type I error using multiple tests (Rice 1989).

Results

Puparial duration, head width, sex ratio, fluctuating asymmetry and wing size did not differ among flies from the 4 replicates in this experiment (data not shown). The test for fluctuating asymmetry was very weak because of small sample sizes within each replicate. Young and old maternal age classes were well separated; mean age at larviposition differed by 48.5–64.8 d, depending on line (Table 1). Young mothers were in the most productive period of the life cycle in which the first 2–4 offspring are produced, whereas old mothers probably had produced 6–8 offspring before the experiment began. True maternal age was known to within ±2 d, so all analyses considered maternal age as a class variable with values "young" and "old." Within each line, sex ratio did not differ from 1:1 (data in Table 2, analyses not shown).

Puparial Period. Females completed the puparial period more rapidly than did males (Table 2), and *G. p. palpalis* and *G. p. gambiensis* had longer puparial periods than *G. m. morsitans*. Within *G. m. morsitans*,

Table 2. Mean puparial period (days) of female and male offspring and head width (mm) of male offspring of young and old mothers from five lines of tsetse

Sex	Line	Offspring of young mothers		Offspring of old mothers	
		n	Mean ± SE	*n*	Mean ± SE
		Puparial period			
Females	134	36	28.4 ± 0.09	31	28.5 ± 0.12
	165[a]	39	28.7 ± 0.09	19	28.4 ± 0.16
	TRL	50	28.5 ± 0.07	37	28.3 ± 0.25
	GAMB-K	67	31.3 ± 0.06	26	31.1 ± 0.14
	PALP-O	51	31.2 ± 0.06	46	31.2 ± 0.07
Males	134[a]	37	30.9 ± 0.08	29	30.6 ± 0.12
	165[a]	37	30.7 ± 0.07	13	30.3 ± 0.24
	TRL	50	31 ± 0.09	37	30.7 ± 0.10
	GAMB-K	56	33.6 ± 0.07	42	33.3 ± 0.07
	PALP-O[a]	38	33.7 ± 0.09	30	33.3 ± 0.12
		Head width			
Males	134[a]	37	2.26 ± 0.015	29	2.20 ± 0.023
	165	37	2.29 ± 0.010	13	2.24 ± 0.036
	TRL	50	2.28 ± 0.011	37	2.27 ± 0.015
	GAMB-K	56	2.27 ± 0.017	42	2.23 ± 0.020
	PALP-O	38	2.30 ± 0.012	30	2.28 ± 0.015

[a] Means significantly different using Student–Newmann–Keuls procedure, $P < 0.05$.

there were no significant differences in puparial duration between lines. Sex and line accounted for 79.8% of the variation in puparial period. Early offspring had longer puparial periods than late offspring ($F = 30.51$; df = 1, 761; $P < 0.0001$) but maternal age accounted for only 0.4% of the variation in the duration of the puparial period. Differences between early and late male offspring were significant at the 0.05 level for lines 134, 165, and PALP-O. The effect of maternal age on puparial period was smaller in females than in males. Only in line 165 did female puparial duration correlate positively with maternal age.

Size. Male offspring of old mothers tended to be smaller than offspring of young mothers in all 5 lines (Table 2), but this difference was significant only for line 134. In the entire data set, maternal age accounted for 2.6% of the variation in head width ($F = 9.9$; df = 1, 356; $P < 0.01$). A Student–Newman–Keuls test revealed significant differences among lines at the 0.05 significance level. Males from line 134 were smaller than those from lines 165, TRL and GAMB-K, but PALP-O flies did not differ significantly from flies of other lines. Wing centroid size did not differ significantly between Gpg-EARLY and Gpg-LATE offspring (Gpg-EARLY 6.845 ± 0.0404; Gpg-LATE: 6.797 ± 0.0320; $F = 0.88$; df = 1, 68; $P > 0.05$).

Fluctuating Asymmetry. Of the 78 line segments calculated, 73 had R/L variation that was significantly greater than measurement error. Of these, 42 had significant directional asymmetry. Because directional asymmetry is not a measure of developmental stability (Van Valen 1962), was distributed throughout the wing without apparent pattern, and was not influenced by maternal age (data not shown), it was excluded from further consideration. Three line segments had significantly leptokurtic distributions and were not considered further. The remaining 31 line

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ɔring of mothers		Offspring of old mothers
Mean ± SE	*n*	Mean ± SE
period		
8.4 ± 0.09	31	28.5 ± 0.12
8.7 ± 0.09	19	28.4 ± 0.16
8.5 ± 0.07	37	28.3 ± 0.25
1.3 ± 0.06	26	31.1 ± 0.14
1.2 ± 0.06	46	31.2 ± 0.07
0.9 ± 0.08	29	30.6 ± 0.12
0.7 ± 0.07	13	30.3 ± 0.24
31 ± 0.09	37	30.7 ± 0.10
3.6 ± 0.07	42	33.3 ± 0.07
3.7 ± 0.09	30	33.3 ± 0.12
width		
.26 ± 0.015	29	2.20 ± 0.023
.29 ± 0.010	13	2.24 ± 0.036
.28 ± 0.011	37	2.27 ± 0.015
.27 ± 0.017	42	2.23 ± 0.020
.30 ± 0.012	30	2.28 ± 0.015

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segments were distributed normally for (R-L) and regressions of |R-L| on head width and on line segment length [(R+L)/2] were nonsignificant. For these line segments mean and standard error for (R+L)/2, (R-L), and |R-L| and *F* statistics and probabilities of fluctuating asymmetry (variance [R-L]) differences between groups then were computed, as were FA5 values ($[(R\text{-}L)^2/n]$, Palmer [1994]) and the associated statistics (Table 3).

A 2-way ANOVA of age by line segment on |R-L| revealed that Gpg-LATE progeny had higher fluctuating asymmetry than Gpg-EARLY progeny (mean |R-L| ± SE: Gpg-LATE = 0.0298 ± 0.00062 mm, Gpg-EARLY = 0.0325 ± 0.00049 mm, $F = 11.72$; df = 1, 2,108; $P < 0.001$), but maternal age effects explained less than 1% of the total variation. Because many of the 31 line segments used in the age by line segment ANOVA are not truly independent observations, several subsets of line segments were tested separately. These subsets consisted of 7–9 non-intersecting line segments with no more than 2 line segments sharing a single landmark. No subset tested produced a significant effect of maternal age on fluctuating asymmetry. No individual line segment exhibited an effect of maternal age on variance of (R-L), but FA5 was significantly higher in Gpg-EARLY than in Gpg-LATE offspring for L4-7 (Table 3).

Discussion

Old female tsetse produced offspring that were smaller, developed faster, and had marginally lower levels of wing vein fluctuating asymmetry than did the offspring of young females. These changes could indicate a decrease in quality or fitness of offspring from old females. The small decrease in size observed among male offspring from old females may indicate a small decrease in the quality of late offspring. During the hot dry months, male tsetse flies in the smallest quartile (6–7% smaller than the largest quartile) have lower survival in the field than do other males (Phelps and Clarke 1974). Late male offspring were 1.4% smaller than early offspring in the current study. It is conceivable that late progeny of field insects fall into the smallest quartile more often and experience a selective disadvantage.

The effect of maternal age on the duration of the puparial period was smaller in female offspring than in male offspring. Given the positive correlation of development rate and size in males (*G. morsitans* $r = 0.45$, $P < 0.001$; *G. palpalis*: $r = 0.21$, $P < 0.01$), a similar correlation is likely in females. Therefore, female size probably is less affected by maternal age than is male size, because of the tighter constraints on body size of females. Small females are incapable of producing normal sized offspring (Žděrek and Denlinger 1993) and are at a definite fitness disadvantage. Although small males can mate normally under laboratory conditions (unpublished data), they may be at a reproductive disadvantage in the field because of their reduced longevity (Phelps and Clarke 1974) and possibly because small size may limit their ability to pursue and capture mates.

Early male offspring of *G. p. gambiensis* females had marginally greater fluctuating asymmetry levels than did late male offspring. This effect was only detectable when the most powerful statistical tests were used. High fluctuating asymmetry is thought to indicate low developmental stability and low overall quality (Van Valen 1962). Our results indicate that, contrary to expectations, early offspring of tsetse females have higher fluctuating asymmetry and thus lower quality than late offspring. The causes for the elevated fluctuating asymmetry in early offspring have not been determined, but 2 possible explanations are as follows: (1) Only high quality females, that consistently produce offspring with low fluctuating asymmetry survive to advanced ages; therefore, old females are a nonrandom sample of the entire population. (2) The slightly longer puparial period experienced by early offspring increases the risk of elevated fluctuating asymmetry by increasing the time during which processes causing fluctuating asymmetry act.

Parsons (1962) found that fluctuating asymmetry was correlated positively with maternal age in *D. melanogaster*. However, Wakefield et al. (1994) found that fluctuating asymmetry was not correlated with maternal age in different strains of this species, and we have found a negative correlation between fluctuating asymmetry and maternal age in *G. p. gambiensis*. Such diverse results imply that the relationship between fluctuating asymmetry and maternal age varies among strains and among species. However, these conflicting results also may be a result of differences in stress experienced by the insects. Parsons (1962, 1994) argued that benign laboratory conditions may make normal effects of stressors, including those affecting fluctuating asymmetry, difficult to discern because in the absence of environmental challenge most insects have high levels of developmental stability. In our study, culture conditions were close to optimal. How increased environmental stress affects the relationship of fluctuating asymmetry to maternal age in tsetse flies is unknown.

In assessing if tsetse females produce lower quality offspring at later ages, we are forced to balance the elevated fluctuating asymmetry of early offspring with the more rapid development and smaller size of late offspring. If the differences in size and development rate are more important than differences in fluctuating asymmetry, then old tsetse females produced offspring of slightly lower quality than young females. The differences, especially in fluctuating asymmetry, between early and late offspring are very small, indicating that the life history of tsetse has constrained them to maintain constant offspring quality throughout female reproductive life. Overall our results indicate that investigators are justified in using tsetse flies from mothers of different ages in the same study.

This study raises several questions about the biology of maternal age effects in natural populations. Most important are the questions of whether wild tsetse females exhibit the same pattern of maternal age ef-

Table 3. Means and standard errors for length (R-L) and |R-L| and sum of $(R\text{-}L)^2/n$ (i.e., FA5 of Palmer 1994) for line segments from *C. P. gambiensis* wings with normally distributed (R-L) values, significant R/L variation, and nonsignificant DA

Line segment	Early offspring				Late offspring			Variance of (R-L)		FA5	
	(R+L)/2	(R-L)	|R-L|	FA5	(R+L)/2	(R-L)	|R-L|	FA5	*F*	*P*	*F*	*P*
1-4	3.864 (0.0164)	0.022 (0.0044)	0.034 (0.0029)	0.0013	3.836 (0.0245)	0.016 (0.0061)	0.034 (0.0032)	0.0012	1.188	0.303	1.073	0.431
1-5	4.107 (0.0164)	0.021 (0.0046)	0.034 (0.0028)	0.0013	4.076 (0.0251)	0.015 (0.0063)	0.033 (0.0035)	0.0013	1.196	0.297	1.025	0.483
1-13	1.753 (0.0097)	0.025 (0.0031)	0.030 (0.0024)	0.0010	1.843 (0.0190)	0.014 (0.0051)	0.028 (0.0025)	0.0009	1.725	0.057	1.174	0.334
3-9	2.593 (0.0115)	0.017 (0.0046)	0.029 (0.0033)	0.0012	2.613 (0.0171)	0.025 (0.0067)	0.037 (0.0045)	0.0018	1.330	0.201	1.528	0.105
3-10	1.485 (0.0078)	0.021 (0.0040)	0.031 (0.0024)	0.0011	1.486 (0.0131)	0.029 (0.0057)	0.034 (0.0046)	0.0017	1.253	0.252	1.494	0.118
3-11	4.369 (0.0190)	0.016 (0.0055)	0.037 (0.0030)	0.0015	4.385 (0.0322)	0.019 (0.0073)	0.037 (0.0049)	0.0018	1.120	0.364	1.175	0.312
3-12	2.281 (0.0103)	0.023 (0.0035)	0.029 (0.0026)	0.0010	2.297 (0.0183)	0.030 (0.0049)	0.034 (0.0039)	0.0015	1.205	0.289	1.420	0.149
3-13	4.695 (0.0203)	0.033 (0.0055)	0.042 (0.0041)	0.0024	4.720 (0.0352)	0.033 (0.0068)	0.040 (0.0051)	0.0023	1.044	0.463	1.043	0.463
4-7	4.269 (0.0178)	0.021 (0.0052)	0.037 (0.0031)	0.0016	4.303 (0.0290)	0.005 (0.0041)	0.022 (0.0017)	0.0005	2.536	0.007	3.359	0.001*
4-8	3.281 (0.0122)	0.019 (0.0042)	0.031 (0.0023)	0.0011	3.306 (0.0192)	0.005 (0.0051)	0.025 (0.0026)	0.0007	1.068	0.438	1.569	0.109
4-9	3.100 (0.0117)	0.027 (0.0046)	0.035 (0.0034)	0.0016	3.124 (0.0182)	0.019 (0.0053)	0.030 (0.0032)	0.0011	1.202	0.313	1.471	0.145
4-10	1.880 (0.0083)	0.033 (0.0030)	0.036 (0.0025)	0.0015	1.886 (0.0147)	0.025 (0.0043)	0.031 (0.0030)	0.0011	1.256	0.250	1.321	0.224
4-11	4.874 (0.0198)	0.026 (0.0048)	0.038 (0.0033)	0.0017	4.894 (0.0335)	0.014 (0.0053)	0.027 (0.0033)	0.0009	1.303	0.240	1.777	0.058
4-12	2.627 (0.0110)	0.037 (0.0035)	0.040 (0.0029)	0.0019	2.647 (0.0192)	0.029 (0.0034)	0.032 (0.0029)	0.0012	1.635	0.093	1.615	0.094
4-13	5.186 (0.0209)	0.044 (0.0045)	0.048 (0.0037)	0.0027	5.214 (0.0363)	0.027 (0.0051)	0.034 (0.0039)	0.0014	1.227	0.294	1.926	0.037
5-7	4.496 (0.0177)	0.019 (0.0047)	0.033 (0.0029)	0.0013	4.525 (0.0294)	0.004 (0.0043)	0.025 (0.0023)	0.0005	1.891	0.044	2.561	0.006
5-8	3.508 (0.0123)	0.017 (0.0038)	0.028 (0.0025)	0.0009	3.529 (0.0197)	0.004 (0.0052)	0.026 (0.0022)	0.0007	1.176	0.314	1.267	0.260
5-9	3.318 (0.0118)	0.025 (0.0041)	0.032 (0.0031)	0.0013	3.337 (0.0185)	0.018 (0.0055)	0.030 (0.0031)	0.0011	1.123	0.361	1.199	0.313
5-10	2.059 (0.0087)	0.031 (0.0032)	0.035 (0.0026)	0.0014	2.061 (0.0148)	0.023 (0.0043)	0.031 (0.0029)	0.0010	1.093	0.391	1.371	0.194
5-11	5.087 (0.0197)	0.024 (0.0044)	0.035 (0.0027)	0.0014	5.102 (0.0338)	0.013 (0.0055)	0.029 (0.0035)	0.0009	1.005	0.506	1.462	0.149
5-13	5.390 (0.0206)	0.041 (0.0040)	0.045 (0.0033)	0.0024	5.414 (0.0365)	0.026 (0.0058)	0.033 (0.0043)	0.0015	1.297	0.222	1.564	0.110
6-12	3.088 (0.0138)	−0.022 (0.0053)	0.038 (0.0034)	0.0016	3.108 (0.0240)	−0.018 (0.0061)	0.030 (0.0043)	0.0013	1.175	0.337	1.264	0.262
7-10	2.539 (0.0115)	−0.010 (0.0043)	0.028 (0.0022)	0.0009	2.563 (0.0186)	−0.015 (0.0051)	0.028 (0.0033)	0.0009	1.108	0.397	1.057	0.426
7-12	2.200 (0.0095)	−0.016 (0.0040)	0.029 (0.0021)	0.0009	2.221 (0.0156)	−0.021 (0.0049)	0.032 (0.0030)	0.0011	1.048	0.459	1.159	0.326
7-13	1.118 (0.0062)	0.020 (0.0024)	0.025 (0.0016)	0.0006	1.112 (0.0112)	0.020 (0.0045)	0.028 (0.0026)	0.0009	2.242	0.010	1.455	0.133
8-10	1.590 (0.0070)	−0.009 (0.0032)	0.022 (0.0019)	0.0005	1.604 (0.0102)	−0.013 (0.0045)	0.025 (0.0021)	0.0007	1.255	0.251	1.356	0.183
8-12	1.440 (0.0069)	−0.010 (0.0029)	0.020 (0.0017)	0.0004	1.454 (0.0098)	−0.014 (0.0046)	0.025 (0.0027)	0.0007	1.620	0.080	1.653	0.069
8-13	1.986 (0.0117)	0.025 (0.0034)	0.031 (0.0026)	0.0011	1.988 (0.0205)	0.022 (0.0046)	0.029 (0.0030)	0.0010	1.149	0.337	1.051	0.454
9-12	1.166 (0.0061)	−0.011 (0.0032)	0.023 (0.0017)	0.0006	1.185 (0.0082)	−0.011 (0.0056)	0.027 (0.0031)	0.0009	1.992	0.023	1.702	0.058
9-13	2.105 (0.0117)	0.016 (0.0035)	0.027 (0.0020)	0.0008	2.111 (0.0207)	0.007 (0.0047)	0.023 (0.0028)	0.0006	1.153	0.333	1.196	0.315
11-12	2.573 (0.0106)	−0.012 (0.0035)	0.025 (0.0020)	0.0007	2.580 (0.0179)	−0.016 (0.0053)	0.029 (0.0031)	0.0010	1.405	0.160	1.431	0.144

F values are ratios of the variance of (R-L) and of FA5. *P* values are before sequential Bonferroni correction. $n = 43$ for Early offspring and $n = 27$ for Late offspring. *, $P = < 0.05$ after Bonferroni correction.

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We thank A. R. Palmer fc
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gave valuable help with the
in part by a grant from the
Research Council of Cana
from the University of Alb
52-01094) to R.H.G.

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8-13	1.986 (0.0117)	0.025 (0.0034)	0.031 (0.0026)	0.0011	1.988 (0.0205)	0.022 (0.0046)	0.029 (0.0030)	0.0010	1.149	0.337	1.051	0.454
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fects on offspring that our laboratory flies showed, and whether the differences in size, puparial duration, and fluctuating asymmetry have any relationship to offspring fitness in nature. Further work is needed to determine the physiological/behavioral mechanisms that allow old tsetse females to maintain high offspring quality when most insects previously examined do not (Mousseau and Dingle 1991). Similarly, the evolutionary pressures that have led to the patterns shown by tsetse need to be identified.

Acknowledgments

We thank A. R. Palmer for helpful suggestions on statistical analyses and for reviewing an earlier version of the manuscript, and G. Vale and J. Jargrove for blunt but useful comments on the penultimate draft of the work. James Hammond gave valuable help with the graphic. This work was financed in part by a grant from the Natural Sciences and Engineering Research Council of Canada (No. A-3900) and by a grant from the University of Alberta Central Research Fund (No. 52-01094) to R.H.G.

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Received for publication 24 January 1997; accepted 5 September 1997.