Delayed Male Maturity is a Cost of Producing Large Sperm in Drosophila

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Evolution

Delayed male maturity is a cost of producing large sperm in *Drosophila*

(life history/age at maturity/body size/testis size)

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ABSTRACT **Among fruit-fly species of the genus *Drosophila* there is remarkable variation in sperm length, with some species producing gigantic sperm (e.g., >10 times total male body length). These flies are also unusual in that males of some species exhibit a prolonged adult nonreproductive phase. We document sperm length, body size, and sex-specific ages of reproductive maturity for 42 species of *Drosophila* and, after controlling for phylogeny, test hypotheses to explain the variation in rates of sexual maturation. Results suggest that delayed male maturity is a cost of producing long sperm. A possible physiological mechanism to explain the observed relationship is discussed.**

Extraordinary variation in sperm length is exhibited among *Drosophila* species (1–4); in fact, sperm length within this genus is more variable than in the remainder of the animal kingdom (2). Although various hypotheses have been offered, the selection pressures responsible for sperm length evolution in *Drosophila* have not been identified (4, 5).

Equally impressive, although previously undocumented, is the variation among *Drosophila* species in sex-specific ages of reproductive maturity (Fig. 1). The timing of specific developmental periods can be easily examined since *Drosophila* exhibits a pattern of discontinuous growth characterized by molting of the exoskeleton between larval instars, pupal, and adult stages. Whereas the egg-to-adult interval varies among *Drosophila* species (range: 10–16 days for species in Fig. 1), most of the variation in maturation time is attributable to the period delineated by the final molt (eclosion) and the onset of sexual reproduction (ranges: males, 0–19 days; females, 1–8 days; see Fig. 1). Although considerable variation exists in the duration of the nonreproductive adult phase across insect species, its causes remain obscure (12).

Males mature more rapidly than females in most insect species (12), indicating that *Drosophila* species are unusual in this respect. Because life history theory contends that the advantages of rapid reproduction and short generation time must be balanced by trade-offs with other fitness components to explain the evolution of delayed sexual maturity (see refs. 13 and 14 and references therein), we assume that delayed maturity is costly and examine two hypotheses to explain the highly variable and frequently protracted male sexual maturation time in *Drosophila*. The "sperm production" hypothesis contends that longer sperm—or, more likely, the machinery necessary to manufacture longer sperm—require more limiting resources and/or time to produce than do shorter sperm, thereby resulting in a trade-off between sperm length and male age at maturity (9, 10). The "allometry" hypothesis predicts that maturation time is a function of body size. For example, although flies have ceased growing by the time of eclosion, duration of the posteclosion maturation period could covary with larval development time (15–17), which is known to correlate positively with adult body size (18, 19). Moreover, the relationship between body size and maturation time was examined in detail because there is a positive relationship between body size and sperm length among *Drosophila* species (6). Consequently, a positive relationship between body size and maturation time, regardless of the mechanism, could cause spurious support for the sperm production hypothesis.

MATERIALS AND METHODS

Experimental Animals. Measurements were made on flies from laboratory cultures derived from multifemale collections. Species collection information and/or National *Drosophila* Species Resource Center (Bowling Green State University, Bowling Green, Ohio) strain numbers are available from the authors upon request. Every effort was made to rear all species under standardized conditions. All flies were reared under uncrowded conditions on medium in 200-ml bottles with live yeast at 24 ± 1°C at an approximately 12-hr light/12-hr dark photoperiodic cycle and an approximate 1:1 sex ratio. However, some species had unique culturing requirements: *D. busckii* were reared on instant *Drosophila* medium (formula 4-24; Carolina Biological Supply); *D. recens, D. subpalustris*, and *D. guttifera* were reared on instant medium to which dry powdered mushroom was added, and a quarter of a mushroom cap was placed in each culture; *D. affinis, D. pseudoobscura*, and *D. persimilis* were reared on standard cornmeal/agar/molasses medium; all remaining species were reared on standard banana medium; autoclaved, necrotic tissue of senita cactus, *Lophocereus schottii* (Englemann) Britton and Rose, was added to medium of *D. pachea*.

Data Collection. Virgin flies used in maturation experiments were collected on the day of eclosion, sorted by sex without anesthetization, and maintained in 8-dram vials containing medium, live yeast, and no more than 10 other same-sex individuals. Flies used for measurement of sperm and thorax length were first anesthetized with ether.

Sperm length (n = 3–5 males per species) and thorax length (n = the first 20 males and 20 females of each species to eclose from low-density population bottles) were determined as described by Pitnick and Markow (3). Thorax length reliably indicates total dry body mass (9). Age of reproductive maturity was defined by a behavioral criterion as the earliest age (days posteclosion, with day of eclosion = 0) at which 80% of sexually naive individuals (n = 30 per sex per species) were observed to copulate when placed with two reproductively mature individuals of the opposite sex for 2 hr in the morning. A physiological criterion of maturation was additionally determined for males (including the three species for which earlier reports are cited in Fig. 1), defined as the earliest age

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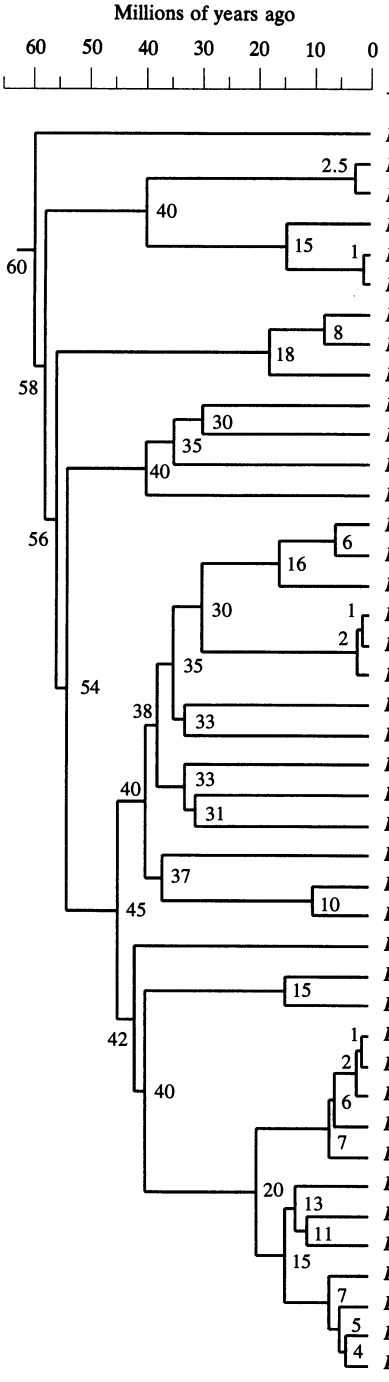

Species	Sperm length, mm	Age of reproductive maturity, days		Thorax length, mm	
		Females	Males	Females	Males
D. busckii	1.18 ± 0.01	2	0	0.90 ± 0.01	0.82 ± 0.01
D. simulans	1.14 ± 0.01	3	1	0.97 ± 0.01	0.87 ± 0.00
D. melanogaster	1.91 ± 0.01(6)	4	2	0.99 ± 0.01	0.88 ± 0.01
D. affinis	0.51 ± 0.00(7)	4	2	1.08 ± 0.01	0.92 ± 0.00
D. pseudoobscura	0.36 ± 0.00(8)	3	1	1.09 ± 0.01	1.01 ± 0.01
D. persimilis	0.32 ± 0.00(7)	4	0	1.06 ± 0.01	0.93 ± 0.01
D. recens	7.55 ± 0.21	5	4	1.09 ± 0.01	0.98 ± 0.01
D. subpalustris	5.96 ± 0.15	4	3	1.29 ± 0.02	1.23 ± 0.02
D. guttifera	10.29 ± 0.14	5	5	0.99 ± 0.01	0.94 ± 0.01
D. wassermani	4.52 ± 0.03(3)	4	12	1.16 ± 0.02	1.07 ± 0.01
D. nannoptera	15.74 ± 0.30(3)	4	8	1.08 ± 0.01	0.99 ± 0.01
D. pachea	16.53 ± 0.29(3)	3(10)	14(10)	1.12 ± 0.01	1.02 ± 0.01
D. acanthoptera	5.83 ± 0.09(3)	6	6	1.21 ± 0.01	1.13 ± 0.00
D. mojavensis	1.90 ± 0.04(6)	3	5	0.96 ± 0.01	0.89 ± 0.01
D. arizonae	1.52 ± 0.01	3	4	1.03 ± 0.00	0.95 ± 0.00
D. navojoa	1.88 ± 0.06	4	6	1.09 ± 0.00	0.99 ± 0.01
D. parisiena	2.10 ± 0.01	5	6	1.12 ± 0.01	1.06 ± 0.01
D. straubae	2.46 ± 0.00	6	7	1.06 ± 0.00	1.00 ± 0.01
D. mayaguana	1.90 ± 0.00	8	6	0.98 ± 0.01	0.94 ± 0.00
D. anceps	1.53 ± 0.01	5	3	1.28 ± 0.01	1.16 ± 0.01
D. nigrospiracula	6.30 ± 0.06	4	5	1.42 ± 0.01	1.27 ± 0.01
D. mettleri	2.79 ± 0.01	2	2	1.27 ± 0.01	1.17 ± 0.01
D. micromettleri	2.22 ± 0.01	3	1	1.06 ± 0.00	0.95 ± 0.00
D. eremophila	2.81 ± 0.04	3	0	1.01 ± 0.01	0.87 ± 0.00
D. bifurca	58.29 ± 0.67(4)	7(4)	17(4)	1.65 ± 0.00	1.60 ± 0.00
D. eohydei	18.11 ± 0.27(6)	3	7	1.50 ± 0.00	1.39 ± 0.00
D. hydei	23.32 ± 0.51(9)	3(11)	9(11)	1.44 ± 0.01	1.33 ± 0.01
D. robusta	6.63 ± 0.09	6	10	1.52 ± 0.01	1.44 ± 0.01
D. micromelanica	1.41 ± 0.01(6)	1	0	1.11 ± 0.01	0.98 ± 0.01
D. melanica	4.93 ± 0.09(6)	4	4	1.23 ± 0.01	1.12 ± 0.01
D. americana	5.22 ± 0.02	4	6	1.40 ± 0.00	1.38 ± 0.00
D. texana	5.08 ± 0.04	4	6	1.36 ± 0.01	1.29 ± 0.01
D. novamexicana	6.72 ± 0.15	4	6	1.30 ± 0.01	1.28 ± 0.01
D. lummei	7.79 ± 0.02	6	7	1.40 ± 0.00	1.34 ± 0.00
D. virilis	5.70 ± 0.16	3	6	1.33 ± 0.00	1.27 ± 0.01
D. kanekoi	24.29 ± 0.18	4	19	1.62 ± 0.01	1.59 ± 0.00
D. littoralis	7.72 ± 0.08	4	10	1.44 ± 0.01	1.38 ± 0.01
D. ezoana	15.33 ± 0.19	7	14	1.41 ± 0.01	1.37 ± 0.00
D. flavomontana	5.53 ± 0.01	5	9	1.38 ± 0.01	1.35 ± 0.01
D. borealis	7.54 ± 0.05	4	9	1.31 ± 0.01	1.28 ± 0.00
D. lacicola	2.52 ± 0.00	3	3	1.27 ± 0.01	1.21 ± 0.01
D. montana	3.34 ± 0.02	4	8	1.48 ± 0.01	1.41 ± 0.01

FIG. 1. The phylogeny, sperm lengths, sex-specific ages of reproductive maturity measured in days posteclosion, and sex-specific thorax lengths of 42 species of *Drosophila*. Scale bar and numbers at nodes represent times since divergence. Numbers in parentheses indicate references for previous reports of data.

at which 80% (n = 30 per species) of sexually naive males were found to have mature sperm in their seminal vesicles. The different criteria of male maturity produced highly congruent results (Pearson correlation of standardized independent contrasts computed through the origin: r = 0.813, df = 36, P = 0.0001). Physiologically determined ages of male maturity are reported in Fig. 1 and were used in all statistical analyses.

Statistical Analyses. To examine evolutionary relationships between characters by the comparative method, it was first necessary to control for phylogenetic effects (20), which can confound interpretation of life history patterns (21–24). We therefore used Felsenstein's method of phylogenetically independent contrasts (25), which provide statistical independence of data points. Independent contrasts were computed (by using the phylogenetic topology and branch lengths presented in Fig. 1) with the Phenotypic Diversity Analysis Program of Garland *et al.* (26) and the CMSINGLE program of Martins and Garland (27). Each variable was $\log_{10}$-transformed prior to computation of contrasts. Standardization was accomplished by dividing each contrast by its standard deviation (the square root of the sum of its branch lengths) (28). The adequacy of this procedure was verified by a lack of significant linear or nonlinear trends in plots of the absolute value of each standardized independent contrast versus its standard deviation (26, 28). The analyses presented employ a model that assumes gradual evolutionary change in variables, with branch lengths

equal to estimated times of divergence (25, 27). Conclusions did not change qualitatively when a punctuational model of evolutionary change was assumed (i.e., all branch lengths equal) (27), when "minimum evolution" methods were used (27), or when raw character values for species were analyzed. All relationships among characters referred to in the text are derived from least-squares (model I) linear regressions through the origin, using standardized independent contrasts of characters.

When independent contrasts between two nodes are calculated, the values of each variable must be subtracted. Because the direction of subtraction is arbitrary, there is ambiguity in assigning signs to the contrasts. As a consequence, in all bivariate scatterplots, figures were "positivized" by giving a positive sign to the independent contrast graphed on the horizontal axis and simultaneously switching the sign of the other independent contrast as required (28, 29).

The phylogeny was compiled from a number of sources. The higher-level relationships were inferred from several morphological (30, 31) and molecular (32–38) data sets, some of which were reanalyzed to construct the figure. The lower-level relationships were determined both by published sources and by sequencing the mitochondrial cytochrome oxidase I (407-bp segment), II (688-bp, entire gene), and III (408-bp segment) genes for each species (G.S.S., unpublished work). Relationships for some species groups were inferred either entirely from the literature [*D. melanogaster* (39) and *D. obscura* (40)], or by using a combination of published phylogenies and our sequencing studies [*D. virilis* (41) and *D. repleta* (42)], or entirely from our sequencing studies (*D. quinaria, D. nannoptera*, and *D. melanica*). Details of the phylogeny will be published elsewhere.

RESULTS

Among *Drosophila* species, the sexual maturation rate of males was found to be far more variable than that of females: males matured faster than females in 13 species, at the same rate in 5 species, and required more time in 24 of 42 species (Fig. 1).

The sperm production hypothesis to explain variation in male maturation time was supported, as a highly significant positive relationship was found between male age at maturity and sperm length ($F = 72.61$, df = 1,40, $r^2 = 0.64$, $P < 0.0001$). This relationship was unaffected by statistical removal of body size effects from both variables by generating for analysis residuals computed from sperm length–body size and male age–body size regressions (Fig. 2*a*; $F = 54.48$, df = 1,40, $r^2 = 0.58$, $P < 0.0001$). Also, as predicted by the sperm production hypothesis, no statistically significant relationship was found between female age at maturity and sperm length, after statistical removal of effects of male maturation time ($F = 0.14$, df = 1,40, $r^2 = 0.004$, $P = 0.71$).

The allometry hypothesis was also supported, inasmuch as a highly significant positive relationship was found between male age at maturity and male body size ($F = 14.16$, df = 1,40, $r^2 = 0.26$, $P < 0.0005$). However, this relationship became only marginally significant when the effects of sperm length were statistically removed from both variables by generating for analysis residuals computed from thorax length–sperm length and male age–sperm length regressions (Fig. 2*b*; $F = 5.44$, df = 1,40, $r^2 = 0.12$, $P < 0.05$). The allometry hypothesis was further weakened, as it predicts a positive relationship between female age at maturity and female body size, and no statistically significant relationship was found (Fig. 2*c*; $F = 0.54$, df = 1,40, $r^2 = 0.01$, $P = 0.47$) despite a strong positive relationship between male and female size ($F = 395.40$, df = 1,40, $r^2 = 0.91$, $P < 0.0001$); females were slightly larger than males in all species (Fig. 1). We therefore suspect that the relationship between male maturation time and male size (Fig. 2*b*) was to a large extent the combined consequence of the positive

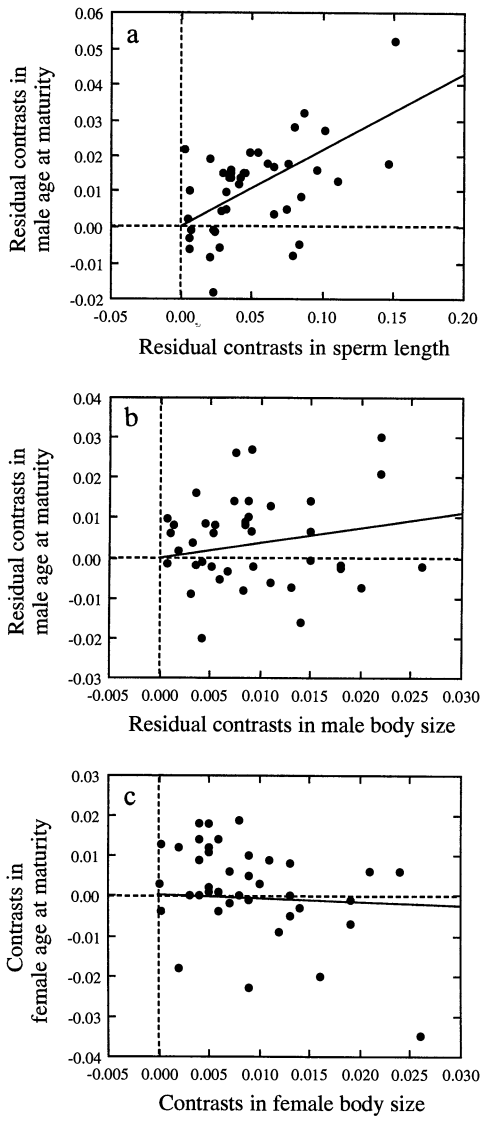

FIG. 2. (*a*) Interspecific relationship between residual male age of reproductive maturity and residual sperm length, after removal of body size effects from both variables. (*b*) Interspecific relationship between residual male age of reproductive maturity and residual male body size, after removal of sperm length effects from both variables. (*c*) Interspecific relationship between female age of reproductive maturity and female body size. Each bivariate scatterplot illustrates relationships among 41 standardized independent contrasts (42 species) of $\log_{10}$-transformed variables and has been "positivized."

relationship between sperm length and male body size (Fig. 3; $F = 7.69$, df = 1,40, $r^2 = 0.16$, $P = 0.0084$) and the relationship between male maturation time and sperm length (Fig. 2*a*). The unusual positive relationship between sperm length and male body size observed among the 42 *Drosophila* species studied here confirms a similar report from a study of 11 species (see ref. 6 for a detailed discussion of this relationship).

DISCUSSION

Among 42 species within the genus *Drosophila*, striking variation was observed in sperm length, sex-specific ages of reproductive maturity, and body size. For example, sperm length (range: 0.32–58.3 mm; Fig. 1) commonly varied among sibling species by 50–100 standard deviations, with the greatest difference between species ≈600 standard deviations. Whereas some phylogenetic trends were evident, particularly with respect to body size, all traits varied considerably within

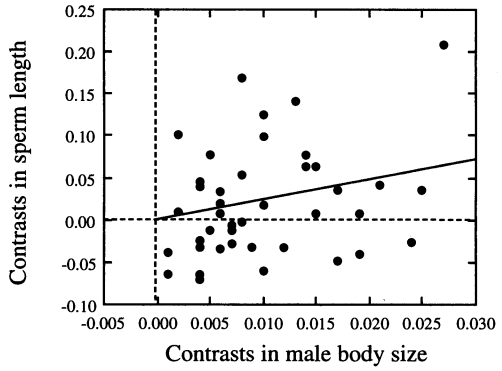

FIG. 3. Interspecific relationship between sperm length and male body size, using a set of 41 standardized, statistically independent contrasts (42 species) for each variable following $\log_{10}$ transformation. The plot has been positivized.

some monophyletic lineages. Giant sperm and delayed male reproductive maturity have independently evolved in numerous monophyletic lineages (Fig. 1).

Although this correlational study strongly supports the hypothesis that delayed male maturity is a cost of producing long sperm, verification of this hypothesis awaits the identification of the physiological mechanism responsible for generating the observed relationships. Presently, evidence most strongly implicates a constraint imposed by growth of the testes. To illustrate, the nature of spermatogenesis in *Drosophila* requires the testes to be longer than the sperm they manufacture (1, 6, 43). Despite extensive interspecific variation in sperm length, however, the ontogeny of spermatogenesis proceeds at a similar rate in all species. For example, at the start of pupation in *D. melanogaster, D. virilis*, and *D. hydei*, the germ cells have developed only to the primary-spermatocyte stage and testis elongation has not yet begun (43–45). Consequently, in species with long sperm, such as *D. pachea* (10) and *D. hydei* (S.P., unpublished data), the testes need to more than double in length between times of eclosion and sexual maturation.

Furthermore, longer sperm are more energetically costly to produce. After independent contrasts were used to control for phylogeny (6), a significant amount of the interspecific variation among 11 species of *Drosophila* in the amount of energy invested in sperm production, estimated by the relative dry mass of testes, was explained by sperm length but not by the number of sperm produced. Species producing relatively short sperm had testes constituting 1–5% of the total dry body mass, whereas testes of species with relatively long sperm represented 8–11% of the total dry mass. This was so despite the fact that males of species with longer sperm were larger-bodied (6). The posteclosion maturation time of males may thus represent time required to develop the large testes needed to manufacture sperm of a given length.

Whereas the relationship identified here between male gamete production and maturation time is unprecedented, there are analogous examples of male initiation of reproduction being delayed by the need to procure materials necessary for successful reproduction. For instance, males of the cockroach *Diploptera punctata* must feed for some time to obtain key nutrients contained in spermatophores transferred to females (46), and males of the dragonfly *Plathemis lydia* must feed for a long time to acquire energy reserves sufficient to control territories essential for mating (47).

Confirmation of whether variation in male age at maturity in *Drosophila* results from a physiological trade-off with sperm production awaits quantitative genetic analyses of the relationships among these characters within species (48) and the phylogenetic examination of additional reproductive and life history characters that may covary with sperm length and age at maturity. For example, age at maturity as measured from birth to reproductive competence has been demonstrated to positively correlate with life expectancy in birds (24) and in mammals (49, 50). However, there is no *a priori* expectation of a relationship between longevity and sperm length, and only the sperm production hypothesis presently predicts a relationship between the duration of the adult male nonreproductive phase in *Drosophila* and sperm length. The estimable life history cost of producing longer sperm identified here is illustrated by a study of longevity in *D. pachea* (10), which suggests that males of this species spend 30–50% of their adult life in a nonreproductive state. With the presumption that the benefits of producing longer sperm must outweigh the costs, the present study reinforces the need to elucidate the selection pressures responsible for sperm length evolution in *Drosophila.*

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