LETTERS

Intensity of sexual selection along the anisogamy–isogamy continuum

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Research into the evolution of giant sperm has uncovered a paradox within the foundations of sexual selection theory. Post-copulatory sexual selection on males (that is, sperm competition and cryptic female choice) can lead to decreased sperm numbers by favouring the production of larger sperm[1]. However, a decline in sperm numbers is predicted to weaken selection on males and increase selection on females[2,3]. As isogamy is approached (that is, as investment per gamete by males approaches that by females), sperm become less abundant, ova become relatively less rare, and competition between males for fertilization success is predicted to weaken. Sexual selection for longer sperm, therefore, is expected to be self limiting. Here we examine this paradox in *Drosophila* along the anisogamy–isogamy continuum using intraspecific experimental evolution techniques and interspecific comparative techniques. Our results confirm the big-sperm paradox by showing that the sex difference in sexual selection gradients[4] decreases as sperm size increases. However, a resolution to the paradox is provided when this finding is interpreted in concert with the 'opportunity for selection' and the 'opportunity for sexual selection'[5,6]. Furthermore, we show that most of the variation in measures of selection intensity is explained by sperm length and relative investment in sperm production.

Bateman's[2] quantitative description of sex differences in *Drosophila melanogaster* gave rise to the modern era of sexual selection theory[3,7–9] by showing that the slope of the line relating reproductive success to mating success (the sexual selection gradient) is nearly flat for females, whereas the slope of this line is much steeper for males. The magnitude of the sex difference in the strength of selection depends on the relationship between male and female sexual selection gradients[4,10,11]. Anisogamy generates the conditions for sexual selection, as numerically abundant male gametes compete to fertilize rare female gametes[12].

In contrast, in a truly 'isogamous' population, where males and females produce identical numbers of equal-sized gametes, it would be possible for every gamete to participate in a successful fertilization. Male and female sexual selection gradients would converge and have equivalent slopes, and the intensity of selection on each sex would be identical, assuming no parental care[13]. This theory has not been tested, however, because exceptionally high ratios of sperm number to egg number have been considered ubiquitous across taxa.

Selection generated by sperm competition is attributed with the evolutionary maintenance of anisogamy, or tiny sperm[14–16]. However, recent comparative analyses of some taxa[17,18], and experimental evolution studies in *D. melanogaster*[1] and *Caenorhabditis elegans*[19], indicate that postcopulatory sexual selection can also favour increased investment per sperm. The rise in costs associated with the production of longer sperm—including delayed reproductive maturity[20], decreased male fecundity[20,21] and increased energetic investment in testes (as measured by the gonadosomatic index, or GSI = (gonad mass/total body mass) × 100)—suggests that the strength of selection maintaining sperm length does not decline as isogamy is approached. Evidence suggesting intense sexual selection in species with longer sperm conflicts with the theoretical prediction that sexual selection should be weaker in species with longer sperm owing to the trade-off with sperm number.

Substantial variation in sperm size has been described for most taxa[22]; sperm length across *Drosophila* species is more variable than in the remainder of the animal kingdom[23]. Therefore, this genus serves as a useful system to examine the big-sperm paradox. In species with no parental care, as in most *Drosophila*[24], parental investment consists of the energy invested in sperm or eggs, and potential reproductive rates[8] can be determined by measuring gamete production rates. We quantified sex-specific gamete production rates in both a short- and long-sperm species: *D. melanogaster* (sperm length = 1.87 mm; GSI = 5.05; ref. 21) and *D. bifurca* (sperm length = 58.29 mm; GSI = 10.60; ref. 25). A sperm:egg production rate ratio of 29.3:1 for *D. melanogaster* versus 5.8:1 for *D. bifurca* was determined (Table 1). *D. bifurca* is nearly isogamous in terms of gamete size and gamete production rate (Fig. 1). Natural populations of *D. bifurca* probably further approach isogamy because males require 17 days posteclosion to produce fertile gametes, whereas females require only 7 days (ref. 25)—a factor not included in our rate calculations. GSI, which is widely reported and easily quantifiable, was used in the among-species analyses. Sperm length in *Drosophila* explains nearly all of the interspecific variation in GSI[21]. Although interspecific relationships are reported here using only GSI, qualitatively similar findings resulted from analyses using sperm length, and multiple regressions confirmed that testis mass—as opposed to body mass—is the component of GSI that explains the majority of the variation among species in all significant correlations (see below).

We repeated Bateman's[2] competitive mating experiment along the anisogamy–isogamy continuum with experimental evolution lines of *D. melanogaster* selected for longer (mean ± s.e.m. = 2.03 ± 0.01 mm) or shorter (mean ± s.e.m. = 1.67 ± 0.01 mm; ref. 1) sperm lengths. The resulting sexual selection gradients confirm the big-sperm paradox: as sperm length increases, the magnitude of sex differences declines (Fig. 3a). This decline results from the combined effect of a decrease in male slope (analysis of covariance (ANCOVA), $F_{1,58} = 4.689$, $P = 0.0345$) and, although not significant, an increase in female slope (ANCOVA, $F_{1,244} = 0.340$, $P = 0.5605$) as sperm size increases (Fig. 2a, b).

We continued our investigation interspecifically by conducting the 'Bateman experiment' with a separate, non-experimentally evolved *D. melanogaster* population, *D. bifurca*, and two species (*D. virilis* and *D. lummei*) with intermediate sperm lengths (5.70 mm and 7.79 mm, respectively[20]) and intermediate GSI (5.79 and 8.04, respectively; S.P., unpublished data). These experiments (Fig. 2c–f) showed that the sex differences in sexual selection gradients share the same negative relationship with investment in sperm production at the

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Table 1 | Sperm and egg production rates of *D. melanogaster* and *D. bifurca*

Species	Sperm length (mm)	GSI*	Gamete production rate†				Sperm:egg production rate
			Female	Male			
			No. eggs per day	No. sperm ($t = 0$ h)	No. sperm ($t = 6$ h)	No. sperm per day	
D. melanogaster	1.87	5.05	59.46 ± 1.80 ($n = 45$)	806.00 ± 99.72 ($n = 8$)	1,242.25 ± 144.59 ($n = 8$)	1,745	29.3:1
D. bifurca	58.29	10.60	38.13 ± 2.43 ($n = 39$)	78.36 ± 8.39 ($n = 11$)	133.64 ± 8.63 ($n = 11$)	221	5.8:1

*GSI, gonadosomatic index.
†Values are mean ± s.e.m.

macroevolutionary level as they do intraspecifically (Fig. 3b). The slope for males is significantly steeper than the slope for females in *D. melanogaster* (Fig. 2c) and *D. virilis* (Fig. 2d). In contrast, sex-specific slopes of *D. lummei* (Fig. 2e) and *D. bifurca* (Fig. 2f) are not statistically different. When comparing across all species, we discovered that male GSI explains most of the variation (that is, 93.5%) in sex difference in selection gradients (Fig. 3b).

The 'opportunity for sexual selection' (I_s; ref. 5) is a standardized index—based on variances in reproductive success—of sexual selection intensity on males and the sex difference in the strength of selection[9]. I_s is determined by subtracting the 'opportunity for selection' (I = variance in reproductive success/(mean reproductive success)^2; ref. 5) for females ($I_{females}$) from that for males (I_{males}). I_s and sexual selection gradients were expected to complement each other because the greater the male selection gradient slope, relative to the female slope, the greater the expected intensity of intramale competition for mates. However, I_s did not decrease across species—and, in fact, it increased significantly within species—as sperm size increased (Fig. 3d and c, respectively). Moreover, this positive relationship with sperm length was detected within species for both I_{males} and $I_{females}$ (Fig. 3e, g) and among species for $I_{females}$ (GSI explained 99.4% of the variation; Fig. 3h).

Why do the patterns revealed from analyses of sexual selection gradients and I_s differ? Although both approaches measure selection, they measure different aspects of the process. I_s estimates the overall intensity of sexual selection. Sex differences in selection gradients, however, measure the degree to which that selection may operate differentially on the sexes.

The resolution of the big-sperm paradox is achieved when sexual selection gradients and I_s are interpreted in tandem. In the most anisogamous species examined, *D. melanogaster*, I_s is relatively small. However, the disparity in slope between the male and female selection gradients demonstrates that selection on male, but not female, mating competition is likely to be a strong force in this species because increased male mating success leads to markedly improved reproductive success[26]. High I_s in *D. bifurca* and *D. lummei* exists despite there being no significant difference between the male and female sexual selection gradients within these species. This bolsters the claims of recent empirical work that sperm gigantism in *Drosophila* is a product of intense sexual selection[1,21]. Historically, models of sex differences have considered the evolution of sperm size strictly from the perspective of initial parental investment[3,15]. We contend that exaggerated sperm tails should not be considered as a form of parental investment in offspring or as a material gift to females. For example, in *D. bifurca* only a tiny portion of the sperm enters the egg; the vast majority is used neither by the egg nor the female[27]. These long sperm flagella are best thought of as ornaments or armaments—the result of directional postcopulatory sexual selection for traits that enhance competitive fertilization success[1].

The trade-off between sperm size and sperm number probably contributes to the significant intra- and inter-specific increases reported for $I_{females}$ as sperm size increases (Fig. 3g, h). Higher variance in female reproductive success is expected if fewer sperm are available and the sperm storage organs of all females are not filled to capacity. This, in turn, will lead to selection for increased female re-mating to ensure fertilization in systems with longer sperm. This aspect of our study will be examined in detail in subsequent work, with attention given to the fitness costs associated with multiple mating in females, optimal female re-mating rate, and the inter-relationship of female re-mating rate and sperm size with the strength of sexual selection at the precopulatory versus postcopulatory stage.

The solving of the big-sperm paradox provides a fresh perspective on sexual selection theory by focusing attention on the dual function

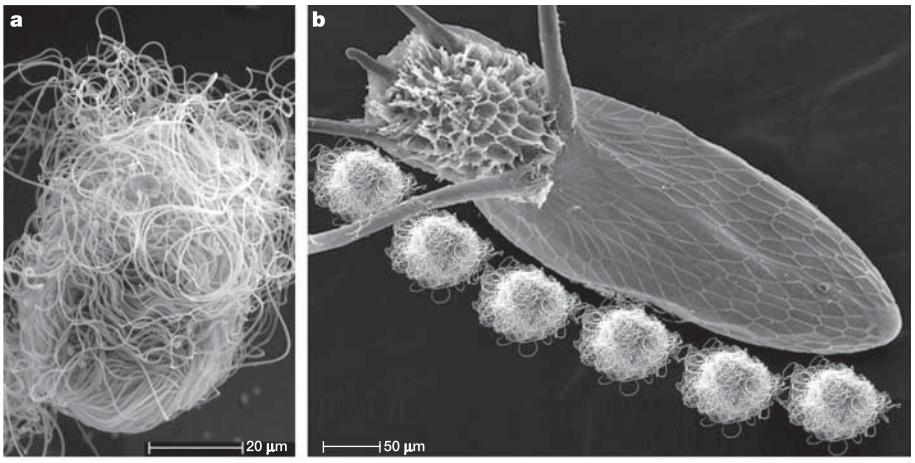

Figure 1 | *Drosophila bifurca* sperm and egg. Sperm are produced at a rate that is approximately six times faster than eggs in *D. bifurca* (see Table 1). **a**, Scanning electron micrograph (SEM) showing a single, 6-cm *D. bifurca* spermatozoon dissected from the seminal vesicle, where sperm are individually rolled into compact balls. **b**, SEM of a single *D. bifurca* sperm (copied six times) next to an SEM of a *D. bifurca* ovum at the same magnification. Micrographs by R. Dallai.

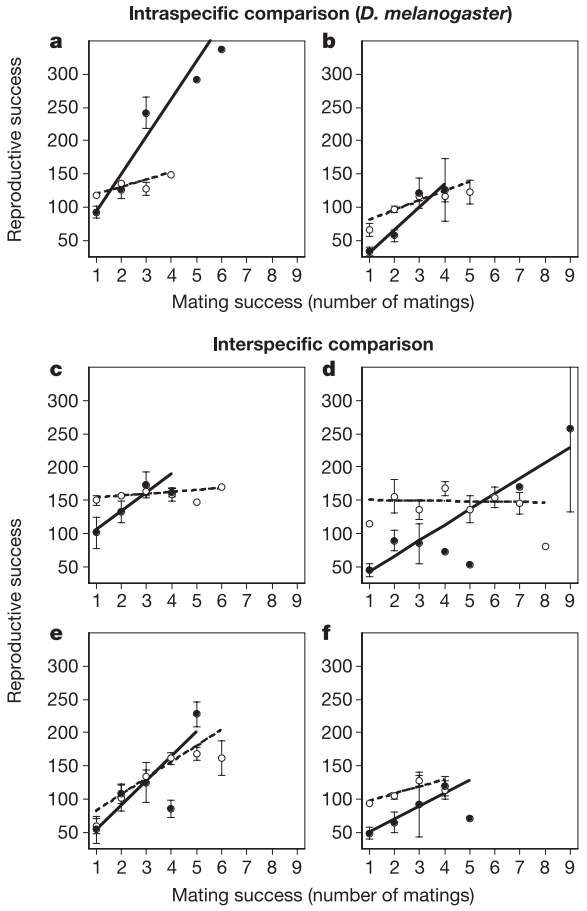

Figure 2 | Intraspecific and interspecific sexual selection gradients in *Drosophila*. Open circles (dashed lines) represent females; closed circles (solid lines) represent males. Each symbol represents a mean, though regressions (see Supplementary Table 1) are based on the complete raw data sets; error bars represent one s.e.m. Significance tests refer to analyses of covariance (ANCOVA) showing that male and female slopes are statistically different in all species/populations except *D. bifurca* and *D. lummei*, which manufacture the largest sperm. **a**, *D. melanogaster* short-sperm population, $F_{1,151} = 36.511$, $P < 0.0001$. **b**, *D. melanogaster* long-sperm population, $F_{1,151} = 6.119$, $P = 0.0145$. **c**, *D. melanogaster*, $F_{1,172} = 9.237$, $P = 0.0027$. **d**, *D. virilis*, $F_{1,52} = 13.972$, $P = 0.0005$. **e**, *D. lummei*, $F_{1,99} = 1.745$, $P = 0.1896$. **f**, *D. bifurca*, $F_{1,135} = 0.922$, $P = 0.3388$.

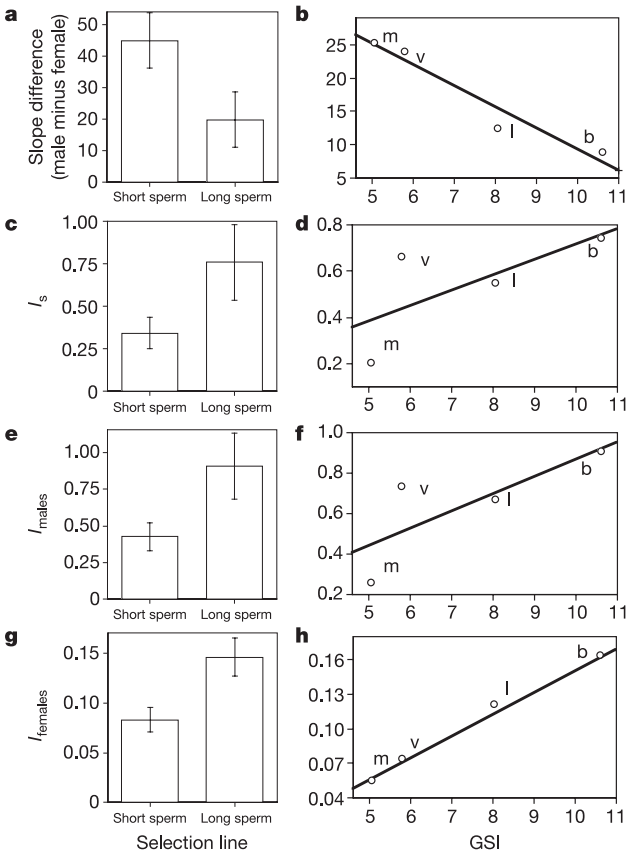

Figure 3 | Mating system measures in relation to investment in sperm production. Each interspecific regression point (open circles) represents a *Drosophila* species (m = *D. melanogaster*, v = *D. virilis*, l = *D. lummei*, b = *D. bifurca*). Error bars, which represent one s.e.m., and significance values for comparisons of the *D. melanogaster* selection lines were obtained using the bootstrap method with replacement (number of replications = 1,000). **a**, Intraspecifically, the sex difference in selection gradients is less in the long-sperm population than in the short-sperm population ($P = 0.018$). **b**, Sex difference in sexual selection gradients decreases significantly across species as investment in testes (GSI) increases ($y = -3.189x + 41.122$, $F_{1,2} = 28.807$, $R^2 = 0.935$, $P = 0.0330$, $n = 4$). **c**, In contrast, the opportunity for sexual selection, I_s, is greatest in the long-sperm *D. melanogaster* population ($P = 0.055$). **d**, I_s increases with GSI across species, though not significantly ($y = 0.067x + 0.084$, $F_{1,2} = 1.942$, $R^2 = 0.493$, $P = 0.2981$, $n = 4$). **e**, The opportunity for selection on males, I_{males}, is marginally greater for long-sperm males ($P = 0.068$). **f**, Interspecific changes in I_{males} do not correlate with GSI ($y = 0.086x + 0.008$, $F_{1,2} = 3.187$, $R^2 = 0.614$, $P = 0.2162$, $n = 4$). **g**, $I_{females}$ is greater in the long-sperm population ($P = 0.006$). **h**, $I_{females}$ rises significantly with GSI ($y = 0.019x - 0.04$, $F_{1,2} = 322.259$, $R^2 = 0.994$, $P = 0.0031$, $n = 4$).

of sperm as both primary and secondary sexual traits. Our results underscore the importance of considering sex-specific gamete investment strategies[22] and postcopulatory processes when exploring the nature of sex differences. The joint analysis of I_s and sexual selection gradients provides a resolution to the paradox. Previously, it has been widely recognized that female re-mating rate, parental investment, and operational sex ratio are critical descriptors of mating systems and sex differences[3,7,8,28]. This current study suggests that sperm length and relative investment in sperm production serve as additional indicators of the most widely accepted measures of sexual selection intensity. Thus, sperm size and spermatogenic investment may provide simple and accurate assays for comparative analyses of the strength of sexual selection in the vast number of species without postmating parental investment.

METHODS

Experimental animals. *D. virilis* and *D. lummei* were obtained from the Tucson Drosophila Species Stock Center. The *D. bifurca* strain was derived from individuals collected near San Luis Potosi (SLP), Mexico in June 2002. For the intraspecific experimental analyses, we used lines of *D. melanogaster* subjected to bidirectional artificial selection for sperm length (see ref. 1). For the interspecific comparative analyses, experiments with *D. melanogaster* used a large outbred population that had adapted to the laboratory for over 250 generations (LH_m; provided by A. Chippindale). Additional rearing conditions are described in Supplementary Information.

Gamete production rate. Methods used to quantify sperm and egg production rates are described in Supplementary Information.

Intensity of sexual selection. Copulations were observed in competitive mating vials containing four males and four females for 4 h each morning over four consecutive days. To identify individuals in copulating pairs, wings were uniquely clipped in a manner that does not affect mating success[29], defined as the copulation number. Flies were separated into individual vials for the remaining 20 h of each day. Female reproductive success was determined by counting all eggs laid in these vials. We used the sterile-male technique to determine male reproductive success. Within each mating vial, one of the four males (the 'focal male') was wing-clipped. The other three males were exposed to an X-ray dose (15 krad for *D. melanogaster*; 17.5 krad for other species) determined previously to sterilize sperm without disrupting fertilization (that

is, eggs were fertilized but did not hatch). Male mating success and reproductive success, determined by counting the number of hatched eggs laid by their mates, was quantified for focal males only. Individuals that failed to mate were included in the calculation of I_s. Because the most salient aspect of the sexual-selection-gradient approach addresses the sex-specific fecundity benefits of multiple mating, we chose not to include the zero-mating category in the analyses of sexual selection gradients. However, its inclusion produced qualitatively identical results. Supplementary Information provides a more detailed description of the analyses.

Scanning electron microscopy. The scanning electron microscopy (SEM) procedure is described in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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