

Female promiscuity promotes the evolution of faster sperm in cichlid fishes

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Sperm competition, the contest among ejaculates from rival males to fertilize ova of a female, is a common and powerful evolutionary force influencing ejaculate traits. During competitive interactions between ejaculates, longer and faster spermatozoa are expected to have an edge; however, to date, there has been mixed support for this key prediction from sperm competition theory. Here, we use the spectacular radiation of cichlid fishes from Lake Tanganyika to examine sperm characteristics in 29 closely related species. We provide phylogenetically robust evidence that species experiencing greater levels of sperm competition have faster-swimming sperm. We also show that sperm competition selects for increases in the number, size, and longevity of spermatozoa in the ejaculate of a male, and, contrary to expectations from theory, we find no evidence of trade-offs among sperm traits in an interspecific analysis. Also, sperm swimming speed is positively correlated with sperm length among, but not within, species. These different responses to sperm competition at intra- and interspecific levels provide a simple, powerful explanation for equivocal results from previous studies. Using phylogenetic analyses, we also reconstructed the probable evolutionary route of trait evolution in this taxon, and show that, in response to increases in the magnitude of sperm competition, the evolution of sperm traits in this clade began with the evolution of faster (thus, more competitive) sperm.

sperm competition | sperm size | sperm swimming speed | sexual selection | correlated evolution

Postcopulatory sexual selection in the form of sperm competition occurs whenever access to ova of a female is contested by ejaculates from >1 male (1). Because fertilization success is thought to be influenced by characteristics of competing ejaculates, sperm competition theory predicts that the strength of sperm competition will be reflected in sperm traits. Specifically, theory predicts a positive relation between the size of spermatozoa and the strength of sperm competition (2), based on the assumptions that (i) longer spermatozoa swim faster, and (ii) faster-swimming sperm achieve higher fertilization success during competitive matings between multiple males (2, 3). Most comparative studies have shown that species experiencing greater levels of sperm competition have longer sperm (4, 5), although one widely-cited study demonstrated a negative relation between sperm competition and sperm length in bony fishes (6). Surprisingly, there is scant empirical evidence demonstrating that sperm competition promotes the evolution of faster-swimming sperm across species, and little evidence of a link between sperm morphology and swimming speed (4, 7, 8). For example, evidence of a positive relation between sperm length and swimming speed has been reported only from mammals, using data obtained by various methods (5, 9). Also, numerous intraspecific studies on diverse taxa provide almost no evidence for sperm size influencing sperm swimming speed (7). The only clear evidence within species that larger sperm move faster comes from a study of the nematode *Caenorhabditis elegans* (10),

a species with amoeboid sperm lacking a flagellum and, thus, unlike the sperm of most animals.

In the present study, we sought to examine the relation between sperm competition, sperm swimming speed, and sperm length in a methodologically rigorous and consistent fashion, both within and among species from a single family of fishes. We studied wild-caught, reproductively mature, male cichlids from Lake Tanganyika, Africa. Cichlids are ideal for the study of adaptations resulting from sperm competition, because there is a tremendous diversity in their mating behaviors (11) that influence the levels of sperm competition that males experience [Table 1 and supporting information (SI) Table S1]. Also, because Tanganyikan cichlid lineages have seeded explosive speciation events in Lake Malawi and Victoria, the evolutionary relationships among species have been extensively characterized (12, 13). In externally-fertilizing organisms, like most fishes, the natural fertilization environment is easily mimicked in vitro (14); hence, sperm swimming speed and duration can be studied experimentally in a biologically realistic fashion. Last, because all of the species we studied belong to a single family endemic to a single lake, we were able to minimize confounding variables, such as family-specific responses to sperm competition (15), which potentially confused the results of previous studies of sperm traits in other taxa.

Selection is unlikely to act on a sperm trait in isolation. Therefore, we examined multiple ejaculate traits in an effort to critically assess how sperm competition influences sperm traits in concert, while also assessing predicted trade-offs among sperm traits. We complemented these analyses by using modern phylogenetic techniques to assess the most probable evolutionary route from an ancestral to a derived sperm trait, so that we could identify how selection acts on sperm traits in the face of increasing levels of sperm competition.

Results

Using phylogenetically-controlled generalized least-squares (GLS) regressions (16), we assessed both the impact of sperm competition on sperm traits and the quantitative trade-offs among those traits that are predicted from theory (e.g., the size–number trade-off; see refs. 2 and 3). The level of sperm competition was ranked on a 4-point scale, with scores 1 and 2 for species in which sperm competition was absent or unlikely, respectively, and 3 and 4 for those in which it was moderate or

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Table 1. Levels of sperm competition for 29 cichlids ranked on a 4-point scale based on a composite score that combined behavioral, ecological, and within-brood paternity data

Sperm competition rank	Species characteristics		
	Mating system	Parental care	Fertilization location
1, none	Monogamous	Biparental	Mouth cavity
2, low	Monogamous	Biparental	Substrate
3, moderate	Polygynous	Female-only	Substrate
4, high	Polygynous with male alternative reproductive tactics or polygynandrous and lekking	Female-only	Substrate or mouth cavity

For additional information, see [Table S1](#).

high, respectively (Table 1). Species with higher sperm competition rank had relatively larger testes (controlling for body mass), and longer, faster-swimming, longer-lived spermatozoa compared with species experiencing lower sperm competition (Table 2). Across species, sperm swimming speed was positively related to sperm length, controlling for the influence of phylogeny (Fig. 1; [Table S2](#)). Interestingly, there was a significant positive relation between sperm swimming speed and sperm size in only 2 of the 29 species that we studied ([Table S3](#)), and a metaanalysis shows that sperm size was not related to sperm speed overall (Fisher's method of combining probabilities, $\chi^2 = 62.0$, $P = 0.34$). Also, sperm swimming duration (i.e., longevity) was positively related to sperm swimming speed at all but the earliest (30 s) postactivation time period that we sampled ([Table S2](#)). Most important, there was no evidence for trade-offs between sperm longevity and either sperm size or sperm swimming speed ([Table S2](#)). For example, the relations between sperm swimming speed and longevity at all times postactivation were positive, and almost all were significantly so. This result suggests that, across species, sperm swimming speed was not gained at the expense of longevity, as has been suggested in both theoretical analyses (2, 3) and empirical studies within species (4).

To explore further the coevolution of sperm traits and their evolutionary history, we performed a directional test of trait evolution (17). This analysis allows us to assess coevolution between 2 traits, and to determine whether transitions in the state of one trait are contingent on the state of a different trait. Directional tests of trait evolution are especially powerful if the probable ancestral traits are identified a priori, thereby offering insights into the most likely evolutionary pathways that lead from a reconstructed ancestral state to a derived state. To perform this analysis, the continuous sperm trait data and sperm competition ranks were coded as binary states [low (rank 1 and 2) vs. high (rank 3 and 4); for details, see [SI Methods](#)]. We used maximum likelihood methods for molecular-based phylogeny reconstruction to estimate the probable ancestral states (18). This analysis indicated that ancestral states in Tanganyikan cichlids were (i) low sperm competition, (ii) small spermatozoa,

and (iii) slow sperm swimming speeds (likelihood probability estimates, 0.76, 0.78, and 0.78, respectively).

The directional test of trait evolution shows that, within Tanganyikan cichlids, large and fast sperm evolved from small and slow sperm in response to increases in the strength of sperm competition ([Fig. S1](#)). We then assessed the evolutionary transition between sperm traits to ascertain whether sperm size or sperm swimming speed was first to respond evolutionarily to increased sperm competition. Clearly, there was an initial increase in sperm swimming speed ($q_{1,2} > 0$), followed by a subsequent increase in sperm size ($q_{2,4} > 0$; see [Fig. 2](#)). The alternative pathway of an initial increase in sperm size followed by an increase in sperm swimming speed was not supported by this analysis.

Discussion

Our findings provide phylogenetically-controlled evidence that species experiencing elevated levels of sperm competition have faster swimming sperm than closely-related monogamous species. These results support theoretical predictions (3), and match a recent study (that did not control for the effects of phylogeny), showing that sperm swimming speed is related to mating system in primates (19). Similarly, we showed that sperm competition also promotes the evolution of greater sperm numbers, size, and longevity; thus, demonstrating that selection acts on multiple ejaculate traits. However, contrary to theoretical assumptions (2, 3), we did not detect trade-offs among ejaculate traits, suggesting that either sperm quality is maximized at the cost of a trait not measured in this study, or that ejaculates from these externally fertilizing fish are not subject to the same expected constraints as ejaculates from internal fertilizers. We also document an underlying relation between sperm size and sperm swimming speed that is predicted from theory (3), but to date lacked compelling supportive evidence from taxa other than mammals (5, 9). Last, phylogenetic reconstructions of sperm trait evolution demonstrate that the selective force of sperm competition drove the evolution of larger and faster swimming sperm in this clade of fishes. Thus, our results support the causal order implicit

Table 2. Results of simple and multiple regression analyses of the relations between testes mass, sperm length, sperm longevity, and sperm swimming speed (at 0.5 min postactivation), and sperm competition rank, while controlling for phylogeny (by using GLS)

Trait	λ	Predictor	Estimate	t	P	r	df	C.I.
Testes mass	<0.001 ^{ns,*}	Body mass	0.90	4.92	<0.001	0.69	26	0.44–0.82
—	—	Sperm competition rank	0.44	3.37	<0.01	0.55	26	0.23–0.73
Sperm length	0.51 ^{*,ns}	Sperm competition rank	0.06	2.48	0.02	0.43	27	0.07–0.66
Sperm longevity	0.71 ^{ns,ns}	Sperm competition rank	0.09	2.30	0.03	0.41	27	0.04–0.64
Sperm speed	0.89 ^{*,ns}	Sperm competition rank	0.04	2.10	0.04	0.37	27	0.009–0.62

Body mass did not influence sperm length, longevity, or speed; therefore, it was removed from these models. Phylogenetic dependence was assessed by using the scaling parameter λ . Superscripts on λ represent significance levels of likelihood ratio tests with λ compared with 0 (first position) and 1 (second position). Significance levels are denoted as following: ns, not significant; *, $P \leq 0.05$. See [Table 1](#) for sperm competition ranks. A t test was used to compare the observed slope against a slope of 0. Effect sizes (r) were calculated from t values, and noncentral 95% C.I.s are presented (see ref. 29), with the degrees of freedom (df) used in each calculation. C.I.s that do not overlap with zero represent significant relations.

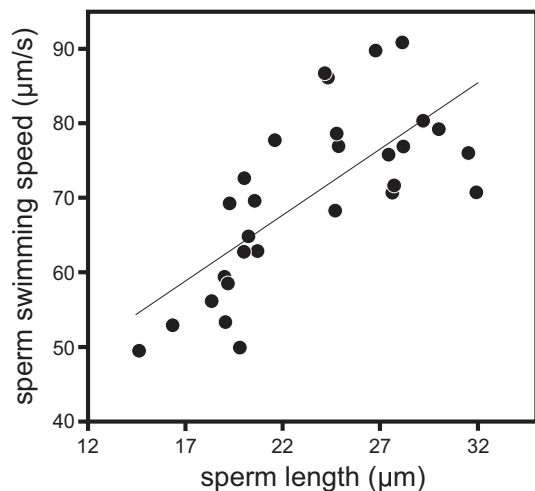


Fig. 1. Relation between sperm swimming speed at 0.5 min postactivation and sperm length [sperm length, estimate = 0.29; $t = 2.53$; $P = 0.02$; $r = 0.44$; degrees of freedom (df) = 27; C.I. = 0.08–0.66; $\lambda = 0.93$. See Table S2 for details of regressions between sperm size and speed at all other time periods postactivation and additional details on phylogenetic regressions. Data in this figure are not controlled for phylogeny.

in sperm competition theory by demonstrating that increases in sperm competition preceded increases in ejaculate traits.

By examining evolutionary changes in sperm size and sperm swimming speed, we were able to assess how selection probably acted to increase the competitiveness of ejaculates of a male. Our results demonstrate that increases in sperm size were contingent on evolutionary increases in sperm swimming speed, suggesting that sperm competition first acted on sperm energetics to increase sperm motility. Importantly, this scenario suggests that the relationship between sperm size and speed depends not only on the propulsive force generated by the flagellum, but may also be mediated by a third factor, namely, sperm energetics. ATP produced by mitochondria in the sperm midpiece powers sperm movement (20). Because spermatozoan ATP levels are positively related to sperm swimming speed (e.g., in bluegill, *Lepomis macrochirus*; see ref. 21), any evolutionary increase in ATP per spermatozoon (e.g., by increasing either mitochondrial efficiency or mitochondrial content in the midpiece, or both) could

increase sperm swimming speed. Therefore, in the face of higher levels of sperm competition, males able to make more energy (ATP) available in an individual spermatozoon would have been favored by selection. By enlarging midpiece size, ATP content per spermatozoon could, for example, be increased without affecting overall sperm length (longer midpieces house more ATP; see ref. 22). Thus, we suggest that sperm energetics were the initial target of selection due to sperm competition. Indeed, comparative studies demonstrate a positive relation between midpiece size and sperm competition intensity in birds and mammals (refs. 15 and 22–24, but see refs. 15 and 25), although experimental support of this hypothesis is lacking. Initial evolutionary responses of sperm energetics (speed rather than size) to elevated levels of sperm competition may explain why studies on male alternative reproductive tactics have generally found that sneaker spermatozoa of males have greater ATP content than those of conventional males, without detecting any morphological differences between the spermatozoa of male adopting different reproductive tactics (7).

In external fertilizers, selection on sperm swimming speed may be particularly intense, because sperm must race to the micropyle canal opening on the surface of the ovum (27), placing a premium on the production of faster sperm (28). However, because sperm swimming speed is a major determinant of fertilization success in various taxa in both noncompetitive and competitive contexts (5, 28–32), the findings of our study should be broadly applicable to species with external or internal fertilization and even those with sperm storage organs. Our use of directional tests of trait evolution provides a useful roadmap to show how selection in the form of female mating behavior may have acted in evolutionary time to shape various sperm traits and to produce superior ejaculates. Further use of this methodology in other taxa can illuminate probable evolutionary pathways and highlight the common selective pressures that have driven the evolution of ejaculate traits in animals in general.

Materials and Methods

In February–April 2004 and 2005, sexually mature males of 29 species from 5 tribes (see Fig. S2) were collected by using SCUBA at depths of 1–15 m near the southern shores of Lake Tanganyika (8° 46' S; 31° 46' E). Males were captured by using both handnets and a 5 × 1 m fence net, and then taken to the surface for dissection. Fish were exposed to a lethal dose of anesthetic (benzocaine; ethyl *p*-aminobenzoate; Sigma), and their body and testes masses were measured to the nearest 0.001 g. Spawning in these tropical cichlids occurs year round, so any

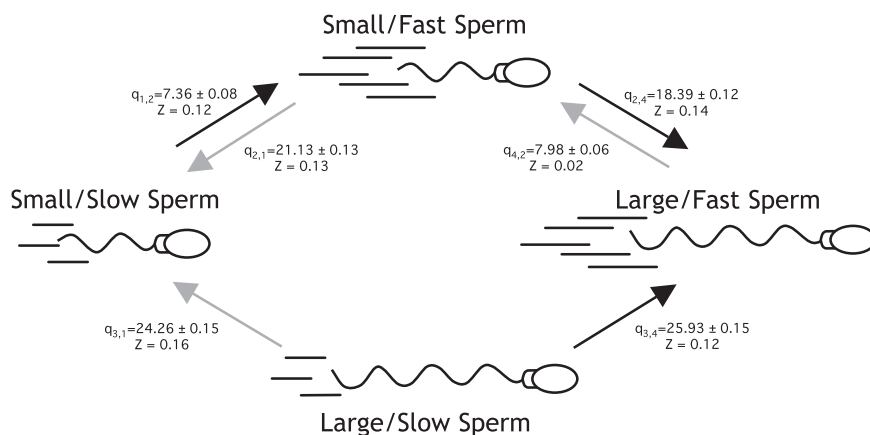


Fig. 2. Transition representation illustrating the evolutionary pathways from the ancestral state of slow, small sperm to the derived state of fast, and large sperm. Intermediate states are represented in the middle of the diagram. Forward transitions are depicted with black arrows; back transitions with gray arrows. Nonsignificant transitions were removed from the diagram. For each transition, we report both a q value (mean \pm SE transition parameter, an indicator of how likely a particular transition is to have occurred), and a Z value (proportion of iterations assigned to zero). For this analysis, sperm swimming speed was measured at 0.5 min postactivation. See SI Methods for additional details on these tests.

males whose testes contained motile sperm were considered reproductively active and used in the analyses presented here.

We assessed the level of sperm competition by using detailed behavioral information from the literature and, whenever possible, within-brood paternity data (Table 1 and Table S1). Although relative testes size (log-transformed, controlling for male mass) is a widely used index of the level of sperm competition (26), this trait may be influenced by factors other than sperm competition (7, 33), suggesting that it should be used with caution. Thus, in this study we used both relative testes mass and reproductive system ranks as independent indices of the level of sperm competition, and we find them to be significantly positively correlated (Table 2). Analyses using either index gave similar results, but we use sperm competition rank in our main analyses. We include analyses using relative testes size (Table S2) to facilitate comparisons with other studies.

Sperm Analyses. In the field, after being weighed, testes were slit with a scalpel and free-flowing sperm was collected, diluted with water, spread over a microscope slide, and allowed to air dry. Sperm lengths were measured from these microscope slides under phase-contrast at 400 \times magnification on our return to the laboratory. Sperm swimming speed, viewed on a Leica DME light microscope (Leica Microsystems), was also recorded in the field by using a PixelINK Megapixel PL-A662 digital video camera (PixelINK) and assessed in the laboratory by using a CEROS (v.12) computer-assisted sperm analysis system (Hamilton-Thorne), with the protocols described in ref. 34. We examined curvilinear (VCL) and smooth path velocities (VAP) for 1 s at 8 time periods (at 30 s and 60 s postactivation, then at 1-min intervals until 7 min postactivation) for each ejaculate sample. We present results for 30-s postactivation data here, because this is the likely period during which most fertilization occurs (35). Data from the remaining time periods are presented in Table S2. For the sake of brevity, only VCL data are presented here, but VCL and VAP were highly correlated (34), and they gave qualitatively similar results in all analyses. Sperm longevity was measured as the time from activation until 95% of spermatozoa were immotile (34). All sperm traits were measured blind to the identity of males.

Phylogenetic Analyses. Data were analyzed with regression and multiple regression by using a generalized least-squared (GLS) approach in a phylogenetic context (16, 18) to control for any statistical nonindependence that can arise from shared ancestry (36). We used a mtDNA-derived phylogeny for the species examined in this study (Fig. S2 and Table S4). By using likelihood ratio tests, we calculated the scaling parameter λ to assess the degree of phylogenetic dependence exhibited by our data (16, 18). Before analyses, all data were log-transformed. All analyses were performed by using R v 2.6.1 (R Foundation for Statistical Computing 2007).

Because the same variables were used repeatedly in different phylogenetically controlled regressions, we calculated effect size (r) from each test to determine the strength of the relations between the traits of interest (37), calculated from t values obtained from the GLS model (38). The noncentral 95% confidence interval (C.I.) for r was used to assess statistical significance (38).

We reconstructed probable ancestral states by using Mesquite (39) with likelihood reconstruction methods (18) in a Markov, k -status, 1-parameter model with the maximum state restricted to 1, to accommodate our binary data. The directionality of sperm trait evolution was assessed by using Bayes-Discrete (www.evolution.rdg.ac.uk; see ref. 17). This program examines the evolutionary pathway that gave rise to the observed traits by using reversible-jump Markov chain Monte Carlo (RJ MCMC) methods, by assessing the likelihood that changes in one trait preceded the evolution of another trait. Previous applications of this technique addressed the evolution of sexual size dimorphism, the relationship between parental care and sexual selection, and how mating systems influenced the evolution of estrus advertisement (17, 40–42). We created binary states from continuous trait data by classifying each trait as “low” (below the mean value for all species) or “high” (above the mean value for all species), and by collapsing our 4-point sperm competition scale into low (rank 1 and 2) and high (rank 3 and 4) levels. We controlled for phylogenetic uncertainty by assessing transitions among 500 trees generated from our MrBayes tree analysis (see *SI Methods*). Transitions between states (Z scores) that were frequently assigned to zero (approximating independent models of trait evolution) were considered unlikely, whereas those only rarely assigned to zero (approximating dependent models of trait evolution) were considered to be highly likely evolutionary transitions. We also determined the mean \pm SE transition parameter (q value), which indicates the strength of the each transition. Additional details on the logic underlying these analyses can be found in the *SI Methods*.

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- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567.
- Parker GA (1998) Sperm competition and the evolution of ejaculates: Towards a theory base. *Sperm Competition and Sexual Selection*, eds Birkhead TR, Møller AP (Academic, New York), pp 3–54.
- Ball MA, Parker GA (1996) Sperm competition games: External fertilization and “adaptive” infertility. *J Theor Biol* 180:141–150.
- Snook RR (2005) Sperm in competition: Not playing by the numbers. *Trends Ecol Evol* 20:46–53.
- Gomendio M, Roldan ERS (2008) Implications of diversity in sperm size and function for sperm competition and fertility. *Int J Dev Biol* 52:439–447.
- Stockley P, Gage MJG, Parker GA, Møller AP (1997) Sperm competition in fishes: The evolution of testis size and ejaculate characteristics. *Am Naturalist* 149:933–954.
- Montgomerie R, Fitzpatrick JL (2009) Testis size, sperm size and sperm competition. *Reproductive Biology and Phylogeny of Fishes*, ed Jamieson BGM (Science Publishers, Enfield, NH), pp 1–53.
- Humphries S, Evans JP, Simmons LW (2008) Sperm competition: Linking form to function. *BMC Evol Biol*, 8:319. doi:10.1186/1471-2148-8-319.
- Gomendio M, Roldan ERS (1991) Sperm competition influences sperm size in mammals. *Proc R Soc London Ser B* 243:181–185.
- LaMunyon CW, Ward S (1998) Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans*. *Proc R Soc London Ser B* 265:1997–2002.
- Kuwamura T (1997) Parental care. *Fish Communities in Lake Tanganyika*, eds Kawanabe H, Hori M, Nagoshi M (Kyoto Univ Press, Kyoto, Japan), pp 59–86.
- Koblmüller S, Salzburger W, Sturmbauer C (2004) Evolutionary relationships in the sand-dwelling cichlid lineage of lake Tanganyika suggest multiple colonization of rocky habitats and convergent origin of biparental mouthbrooding. *J Mol Evol* 58:79–96.
- Day JJ, Santini S, Garcia-Moreno J (2007) Phylogenetic relationships of the Lake Tanganyika cichlid tribe Lamprologini: The story from mitochondrial DNA. *Mol Phylogenet Evol* 45:629–642.
- Levitan DR (1998) Sperm limitation, gamete competition, and sexual selection in external fertilizers. *Sperm Competition and Sexual Selection*, eds Birkhead TR, Møller AP (Academic, San Diego), pp 175–217.
- Immler S, Birkhead TR (2007) Sperm competition and sperm midpiece size: No consistent pattern in passerine birds. *Proc R Soc London Ser B* 274:561–568.
- Freckleton RP, Harvey PH, Pagel M (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *Am Naturalist* 160:712–726.
- Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am Naturalist* 167:808–825.
- Pagel M (1999) Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
- Nascimento JM, et al. (2008) The use of optical tweezers to study sperm competition and motility in primates. *J R Soc Inter* 5:297–302.
- Christen R, Gatti JL, Billard R (1987) Trout sperm motility. The transient movement of trout sperm is related to changes in the concentration of ATP following the activation of the flagellar movement. *Eur J Biochem* 160:667–671.
- Burness GP, Moyes CD, Montgomerie R (2005) Motility, ATP levels and metabolic enzyme activity of sperm from bluegill (*Lepomis macrochirus*). *Comp Biochem Physiol A* 140:11–17.
- Vladić TV, Afzelius BA, Bronnikov GE (2002) Sperm quality as reflected through morphology in salmon alternative life histories. *Biol Reprod* 66:98–105.
- Johnson DDP, Briskie JV (1999) Sperm competition and sperm length in shorebirds. *Condor* 101:848–854.
- Anderson MJ, Nyholt J, Dixon AF (2005) Sperm competition and the evolution of sperm midpiece volume in mammals. *J Zool* 267:135–142.
- Anderson MJ, Dixon AF (2002) Sperm competition-motility and midpiece in primates. *Nature* 416:496.
- Gage MJG, Freckleton RP (2003) Relative testis size and sperm morphometry across mammals: No evidence for an association between sperm competition and sperm length. *Proc R Soc London Ser B* 270:625–632.
- Yanagimachi R, Cherr GN, Pillai MC, Baldwin JD (1992) Factors controlling sperm entry in the micropyles of salmonid and herring eggs. *Dev Growth Differ* 34:447–461.
- Gage MJG, et al. (2004) Spermatozoal traits and sperm competition in Atlantic salmon: Relative sperm velocity is the primary determinant of fertilization success. *Curr Biol* 14:44–47.

29. Froman DP, Feltmann AJ, Rhoads ML, Kirby JD (1999) Sperm mobility: A primary determinant of fertility in the domestic fowl (*Gallus domesticus*). *Biol Reprod* 61:400–405.
30. Birkhead TR, Martinez JG, Burke T, Froman DP (1999) Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc R Soc London Ser B* 266:1759–1764.
31. Malo AF, et al. (2005) Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. *Biol Reprod* 72:822–829.
32. Levitan DR (2000) Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc R Soc London Ser B* 267:531–534.
33. Calhim S, Birkhead TR (2007) Testes size in birds - quality vs quantity: Assumptions, errors and estimates. *Behav Ecol* 18:271–275.
34. Fitzpatrick JL, Desjardins JK, Milligan N, Montgomerie R, Balshine S (2007) Reproductive-tactic-specific variation in sperm swimming speeds in a shell-brooding cichlid. *Biol Reprod* 77:280–284.
35. Hoysak DJ, Liley NR (2001) Fertilization dynamics in sockeye salmon and a comparison of sperm from alternative male phenotypes. *J Fish Biol* 58:1286–1300.
36. Harvey PH, Pagel MD (1991) *The Comparative Method in Evolutionary Biology* (Oxford Univ Press, Oxford).
37. Nakagawa S (2004) A farewell to Bonferroni: The problems of low statistical power and publication bias. *Behav Ecol* 15:1044–1045.
38. Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biol Rev* 82:591–605.
39. Midford PE, Garland T, Jr, Maddison W (2002) PDAP: PDTREE package for Mesquite, Version 1.01.1. Available at http://mesquiteproject.org/pdap_mesquite/index.html.
40. Pérez-Barbería FJ, Gordon IJ, Pagel M (2002) The origins of sexual dimorphism in body size in ungulates. *Evol* 56:1276–1285.
41. Thomas GH, Székely T (2005) Evolutionary pathways in shorebird breeding systems: Sexual conflict, parental care and chick development. *Evolution* 59:2222–2230.
42. Gonzalez-Voyer A, Fitzpatrick JL, Kolm N (2008) Sexual selection determines parental care patterns in cichlid fishes. *Evol* 62:2015–2026.