

Sperm size of African cichlids in relation to sperm competition

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We compared pairs of closely related taxa of cichlid fishes from Lake Tanganyika to examine the relationship between sperm size and the presumed intensity of sperm competition. In contrast to previous reports of relatively short sperm in polygamous fishes across a variety of taxa, we found that polygamous cichlids had significantly longer sperm than their closest monogamous relatives. In addition, sperm length was significantly related to relative testis size (controlling for body size and phylogeny). The site of fertilization may also be correlated with sperm length, as species that fertilize in the female's buccal cavity had significantly shorter sperm than those that fertilized eggs on the substrate. Assuming that relatively large testes and polygamous mating are indicative of more intense sperm competition, these results indicate that sperm length is related to the intensity of sperm competition in this clade of cichlids, as has been found previously in insects, birds, and mammals. *Key words:* Cichlidae, Lake Tanganyika, mating systems, sperm competition, sperm morphology, testes. [*Behav Ecol* 12:726–731 (2001)]

Ever since Parker (1970) recognized the importance of sperm competition as an evolutionary force, many models that predict the effects of sperm competition on male anatomy, physiology, and behavior (for a review, see Parker, 1998) have received convincing empirical support (for a review, see Birkhead and Møller, 1998). For example, males in polygamous species are expected to suffer more sperm competition than their monogamous counterparts, so polygamous males are predicted to have larger testes relative to their body size (Parker, 1982, 1990a,b, 1993; Parker and Begon, 1993), and empirical studies support this (Clutton-Brock and Harvey, 1977; Dybas and Dybas, 1981; Harcourt et al., 1981; Kenagy and Trombulak, 1986; Warner and Robertson, 1978). Parker (1990a,b) also predicted a positive relation between the intensity of sperm competition and relative sperm production, and this has been found in insects (Gage, 1994), fishes (Stockley et al., 1997), birds (Birkhead et al., 1993; Møller and Briskie, 1995), and mammals (Gomendio and Roldan, 1991; Hosken, 1997; Møller, 1989).

In contrast, the relationship between sperm size and the intensity of sperm competition remains less clear (both theoretically and empirically). Across taxa, sperm vary enormously in size, more than 10 orders of magnitude (Chao et al., 1975; Pitnick et al., 1995). The theoretical models make different predictions about whether sperm competition explains this variation. Two models have been explored: the instantaneous fertilization model for internal fertilizers (internal fertilization occurs at one particular instant usually some time after mating) and the continuous fertilization model for external fertilizers (fertilization occurs in a continuous fashion immediately after mating). Longer sperm are thought to be faster swimming (but motile for a shorter period; Gomendio and Roldan, 1991). In the simplest form of the instantaneous fertilization model, sperm size is not predicted to be affected by the intensity of sperm competition (Parker, 1993), whereas in the continuous fertilization model (appropriate for most externally fertilizing fish species), sperm size is pre-

dicted to increase with sperm competition intensity (Ball and Parker, 1996). The different predictions are partly due to the fact that continuous fertilizers are likely to experience a relatively higher degree of sperm competition because males release sperm simultaneously, and sperm will race for the available eggs. Therefore, males will want to increase both sperm number and sperm swimming speed to maximize the number of collisions with eggs. Longevity will not be important when there is intense sperm competition because most of the eggs will be fertilized extremely quickly. In contrast, in internal fertilizers, ejaculates are released sequentially, and most sperm die before any competition can occur. Thus, the best strategy under high sperm competition in internal fertilizers is to maximize sperm number and only under particular circumstances increase longevity (Parker, 1998).

In contrast to theory, empirical studies of internal fertilizers have found an increase in sperm size with sperm competition (birds: Briskie et al., 1997, Johnson and Briskie, 1999; primates and rodents: Gomendio and Roldan, 1991; butterflies: Gage, 1994), and a comparative study of externally fertilizing fish found a decrease in sperm size with the intensity of sperm competition (Stockley et al., 1997). However, Stockley et al. (1997) used data from numerous literature sources of variable data quality, which may have confounded their analyses. As a result, their apparently anomalous findings about the relation between sperm size and the intensity of sperm competition should be interpreted with caution.

In this study, we used the comparative approach to study how cichlid sperm size is related to the intensity of sperm competition. Our results are based on samples we collected in the field. Cichlids are well suited to the investigation of sperm competition because they have diverse social mating systems (monogamy, polygyny and polygynandry; Keenleyside, 1991; Kuwamura, 1997), among which the intensity of sperm competition is expected to vary (Stockley et al., 1997). Extensive phylogenetic information is also available for this group (for a review, see Goodwin et al., 1998). In cichlids, relative male investment in gonads varies among species, and this, too, has been shown to correlate with the intensity of sperm competition in a variety of animal taxa (Harcourt et al., 1981; Møller, 1989; Møller and Briskie, 1995; Stockley et al., 1997).

Female cichlids typically lay eggs on the ground, either in

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Table 1
Taxon pairs used in this study

	Polygamous species	Monogamous species
1	<i>Altolamprologus compressiceps</i> (23.8, 15) ^a	<i>Altolamprologus calvus</i> (24.1, 3) ^a
2	<i>Neolamprologus tetracanthus</i> (27.2, 3) ^a	<i>Lepidiolamprologus elongatus</i> (18.2, 7) ^a
3	<i>Neolamprologus savoyi</i> (25.9, 1) ^a	<i>Neolamprologus caudopunctatus</i> (22.4, 10) ^a
4	<i>Telmatochromis vittatus</i> (33.3, 12) ^a	<i>Julidochromis ornatus</i> (31.7, 14) ^a
5	<i>Enantiopus melanogenys</i> (18.2, 5)	<i>Asprotilapia leptura</i> (15.5, 10)
6	<i>Cyathopharynx furcifer</i> (20.4, 6)	<i>Xenotilapia spilopterus</i> (19.4, 13)
	<i>Ectodus descampsi</i> (22.1, 5)	<i>Xenotilapia flavipinnis</i> (19.9, 5)
7	<i>Benthochromis tricoli</i> (18.7, 4)	<i>Gnathochromis permaxillaris</i> (19.9, 3)
8	<i>Tropheus moorii</i> (21.0, 10)	<i>Perissodus microlepis</i> (17.7, 2)
	<i>Gnathochromis pfefferi</i> (19.5, 12)	
9	<i>Bathybates fasciatus</i> (24.2, 2)	<i>Eretmodus cyanostictus</i> (20.6, 12)

Values in brackets after species names are mean sperm length (μm) and number of individuals sampled.

^a Species in which fertilization occurs exclusively on the substrate.

open nests or in cavities (caves, burrows, or snail shells), and males fertilize the eggs on the substrate as they are being laid or shortly thereafter. In many mouth-brooding cichlids, however, the female picks up her eggs in her buccal cavity before the male fertilizes them. In these species, the male presents the female with his anal or pelvic fins, which are covered with small spots similar in color and size to the female's eggs (Hert, 1989). The female attempts to pick up these false eggs, and, as she nips the male's fins, he ejects sperm into her buccal cavity (Mrowka, 1987). In previous comparative studies it has been assumed that mouth-brooding cichlids experience little or no sperm competition (Stockley et al., 1997). However, some female mouth-brooding cichlids move from one male's territory to another, spawning a few eggs with each male and thus collecting several males' sperm in their buccal cavity within a few minutes (Hulata et al., 1981; Kellogg et al., 1998; Parker and Kornfield, 1996; Rossiter and Yamagishi, 1997). Because cichlid sperm remains active for up to 15 min (Chao et al., 1987), sperm competition may be intense in these buccal-fertilizing species.

In this study, our aim was to determine whether sperm size is related to the intensity of sperm competition in a clade of cichlid fishes, as has been found in other taxa. By restricting our attention to pairs of close relatives within a single taxon of fishes living in one lake, we attempted to control for phylogenetic, ecological, and life-history variation that might influence sperm size, independent of the intensity of sperm competition. Sperm size has been shown previously to positively correlate with the number of ova in externally fertilizing species (Stockley et al., 1996), so we also examined how sperm length is related to both the number and size of ova produced at spawning. It is not clear why such correlations might be expected in fishes (see Stockley et al., 1996).

METHODS

Data collection

Between January and May 1998 we collected sexually mature males of 21 cichlid species from various locations on the southern tip of Lake Tanganyika, from the Kalambo River to Ndole Bay in Zambia. We focused particularly on species pairs of close relatives with different mating systems (Table 1). Reproductive information was taken from Brichard (1989), Konings (1998), Kuwamura (1997), and Loiselle (1985).

To capture male fish, we used handnets and a 10 \times 1 m fence net. Females were released immediately, and males were placed in individual collection bags and brought to the sur-

face within 1 h. Sample sizes per species are listed in Table 1. At the surface we measured each specimen's standard length (mm) and body mass (g). Then we anesthetized each male (using MS222), killed them by quickly severing the spinal cord with a scalpel, and carefully removed the testes. All of the species used in this study were abundant in the lake, and no threatened species were killed as part of the study. Testes were placed immediately in 1.5 ml microcentrifuge Eppendorf tubes, fixed in formalin (4%), and brought back to the laboratory. Because the species in our study breed throughout the year, we assumed that all of the males sampled were sexually active.

In the laboratory, we measured testis lengths to the nearest 0.5 mm and wet masses to the nearest μg with a Cahn C-31 microbalance. Testes were then slit open, and free milt was distributed on a slide. If free milt was not apparent, testes were squeezed or scraped and the liquid spread finely with formalin. The slides were then allowed to air dry before viewing at 400 \times . Using a video link to a computer with a flat-screen monitor, we recorded and digitized images of 10 sperm from each slide using NIH Image (version 1.59 of the public domain NIH Image program developed at the U.S. National Institutes of Health, available at <http://rsb.info.nih.gov/nih-image>). We analyzed 10 sperm from each of the males in each of the 21 species. Sperm length was measured from the center of the head to the end of the tail to the nearest 0.01 μm by tracing a freehand line using NIH Image. We used the center of the head as a point of reference because the junction between the sperm head and tail was not always easy to locate. All of the species we studied had small, spherical sperm heads whose centers were estimated by eye. To prevent observer bias, all measurements (testes and sperm) were taken without knowledge of the species identification. Repeatability (see Sokal and Rohlf, 1995) was high in an analysis of 20 sperm images, each measured 3 times independently ($r_1 = 0.97$).

Data analyses

Our study species were chosen so that we could analyze sperm differences between pairs of the closest possible extant relatives (Table 1), thus minimizing the effect of phylogeny. We took phylogenetic information from a consensus supertree (Sanderson et al., 1998) that we compiled for the species used in this study (see Figure 1). We chose to accept Kocher et al.'s (1995) placement of *Eretmodini* basal to *Lamprologini*, which yielded nine taxon pairs for our comparisons (Table 1). If we accept instead Nishida's (1997) placement of *Eretmo-*

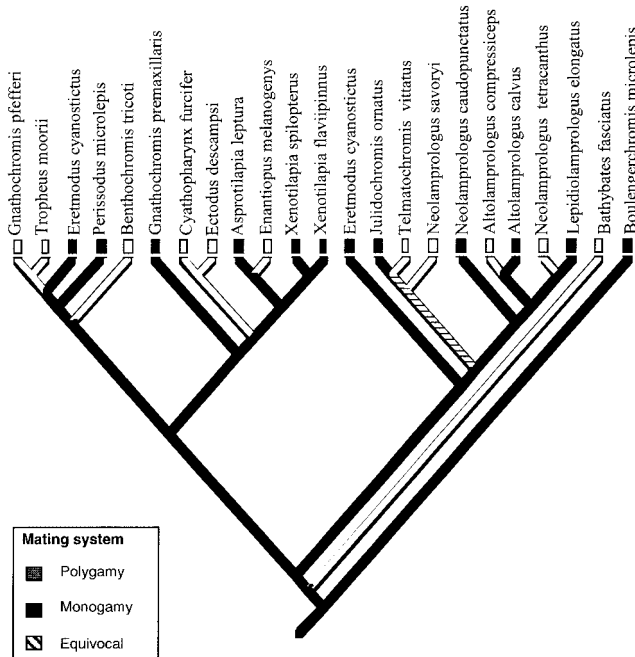


Figure 1
Supertree constructed for the 21 cichlid species used in this study, compiled according to methods described in Sanderson et al. (1998) from four previously published trees for Lake Tanganyikan cichlids (Kocher, 1995; Nishida, 1997; Sturmbauer and Meyer, 1993; Sturmbauer et al. 1994). No consensus was possible for placing *E. cyanostictus*, so we have placed it twice in this tree, once basal to the Lamprologini in accordance with Kocher (1995) and once basal to the Trophini as suggested by Nishida (1997).

dinii basal to Trophini (see Figure 1), then eight comparisons are possible, but we find the same patterns as described below.

We used Burt's (1989) method of paired comparisons to examine the relation between mating systems and reproductive traits. To do this, we paired each available species with its closest relative that differed in mating system. In three cases, two closely related species were grouped for comparison with other taxa (Table 1), and in those cases we took mean values for the two species in each taxon. Sample sizes are therefore the number of independent taxon pairs and not the number of species originally used in the analysis. In all cases, taxon pairs that we compared also had the same site of fertilization (buccal vs. substrate), so the analysis by mating system is not confounded by differences due to fertilization site.

To test relationships between (log-transformed) continuous variables, we used Comparative Analysis by Independent Contrasts (CAIC; version 2.6.5; Purvis and Rambaut, 1995). CAIC is based on methods for comparative analysis of continuous data, as described by Harvey and Pagel (1991). This method identifies contrasts for each node in the phylogeny that exhibits variation in the test variable. Linear regressions were forced through the origin (Harvey and Pagel, 1991).

For correlation analyses on raw data (rather than contrasts), measurements were log transformed to normalize distributions and residuals. None of the distributions of log-transformed variables (such as testis size or body size) was significantly different from normal, so we used parametric statistics throughout.

Because we were testing hypotheses based on theory and considerable empirical evidence, we used directed statistical tests (Rice and Gaines, 1994) to increase statistical power. Directed tests are a useful alternative to one-tailed tests and provide the safeguard that results in the opposite direction to

those predicted (e.g., Stockley et al., 1997) can be interpreted statistically. In our study, sample sizes were often small, so we particularly wanted to minimize the chance of Type I error by maximizing power.

For directed tests we followed the recommendations of Rice and Gaines (1994) and set $\alpha = 0.05$, $\gamma = 0.04$, and $\delta = 0.01$. The resulting directed p values for the tests we performed are thus 0.625 times the two-tailed p . We used directed tests when analyzing the relations between sperm length and both relative testis mass and mating system because both theory and a number of previous studies have suggested that sperm will be longer when sperm competition is more intense. Higher sperm competition is expected in species with relatively large testes and polygamous (vs. monogamous) mating systems. Thus, a positive relation is also expected between testes size and mating system.

RESULTS

There was no difference in body mass between polygamous and monogamous species (paired t test, $t = 0.58$, $p = .58$, $n = 9$ taxon pairs), so body mass did not confound any comparison between mating systems in this study. However, testis mass was significantly and positively correlated with body mass across species ($r = .72$, $p < .001$, $N = 21$ species). Thus, to measure testis mass independent of body size, we used the residuals from the regression of testis mass on body mass. This residual testis mass is functionally equivalent to the gonadosomatic index ($GSI = 100 \times \text{testes mass/body mass}$) used in many studies of fish reproductive biology (e.g., Stockley et al., 1997). As expected, cichlids with polygamous mating systems had significantly higher residual testis mass (paired $t = 3.12$, directed $p = .009$, $n = 9$ taxon pairs) than their closest monogamous relatives (Table 1).

Variation in sperm length among species was greater than that within species (ANOVA, $F_{20, 133} = 40.3$, $p < .0001$, species nested within mating system), with 81% of the variation in sperm length occurring among species. *Telmatochromis vittatus* had longest average sperm lengths, on average more than twice as long as those of *Asprotilapia leptura*, which had the shortest (Table 1).

Substrate-fertilizing species had sperm significantly longer than buccal-fertilizing species ($t = 3.92$, $p = .001$, $n = 8$, 13 species; Figure 2a). Even after controlling for significant differences in both relative testis mass (ANCOVA, $F_{1,17} = 6.2$, $p = .02$) and the relation between sperm length and residual testis mass (ANCOVA, $F_{1,17} = 5.5$, $p = .03$) between buccal and substrate fertilizers, the sperm of substrate fertilizers is significantly and about 23% longer (comparing least squares means) than that of buccal fertilizers (ANCOVA, $F_{1,17} = 12.0$, $p = .003$). Unfortunately, neither of these two analyses could be controlled for phylogeny as all the substrate-fertilizing species in Lake Tanganyika come from the same subfamily (the Lamprologini).

Sperm lengths of polygamous cichlids were significantly longer than those of their closest monogamous relatives (paired t test, $t = 2.62$, directed $p = .02$, $n = 9$ taxon pairs; Figure 2b). Indeed, in seven of the nine paired comparisons, polygamous species had the longer sperm. The alternative phylogeny showed similar results; sperm lengths of polygamous cichlids were longer (paired $t = 2.23$, directed $p = .04$, $n = 8$ taxon pairs). On average, the sperm of polygamous cichlids (mean \pm SE = $23.1 \pm 1.35 \mu\text{m}$, $n = 11$ species) were about 10% longer than the sperm of monogamous species ($20.9 \pm 1.41 \mu\text{m}$, $n = 10$ species). This pattern is also seen within buccal and substrate fertilizers, where in three of four and four of five taxon pairs, respectively, polygamous taxa had the longer sperm (Table 1).

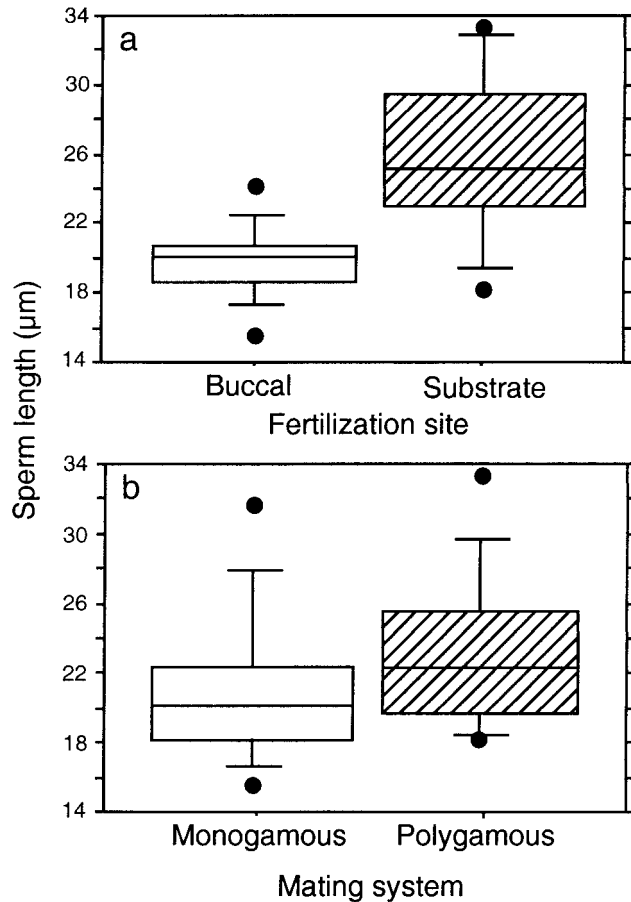


Figure 2
Sperm lengths (μm) of (a) buccal- and substrate-fertilizing cichlids and (b) polygamous and monogamous cichlids (nine taxon pairs; see Table 1). The box plots show the 10th, 25th, 50th, 75th and 90th percentiles as horizontal lines and all data points outside this range.

Controlling for phylogeny, the relation between sperm length and residual testis mass was significant and positive ($r^2 = .17$, $b = 0.07$, $F_{1,19} = 3.81$, directed $p = .04$, $n = 20$ contrasts; Figure 3). This relation was positive within both buccal ($r^2 = .07$, directed $p = .23$, $n = 12$ contrasts) and substrate fertilizers ($r^2 = .31$, directed $p = .09$, $n = 7$ contrasts), though neither was significant, and statistical power was low. Thus, sperm appear to be longer when sperm competition is more intense, assuming that both mating system and residual testis mass are reliable indices of sperm competition.

Stockley et al. (1996) also found that sperm length in externally fertilizing species was positively correlated with the number of ova available per spawning event but not with ovum size. In our study, sperm length was not significantly related to either the number ($r^2 = .10$, $b = -0.04$, $F_{1,17} = 1.84$, $p = .19$, $n = 18$ contrasts) or diameter ($r^2 = .02$, $b = 0.04$, $F_{1,17} = 0.42$, $p = .53$, $n = 18$ contrasts) of ova at spawning, both analyses controlling for phylogeny. Thus, neither ovum size nor number at spawning would appear to have confounded our analyses of the relations between sperm length and the intensity of sperm competition.

DISCUSSION

We found longer sperm in cichlid fish species with relatively large testes and polygamous (vs. monogamous) mating sys-

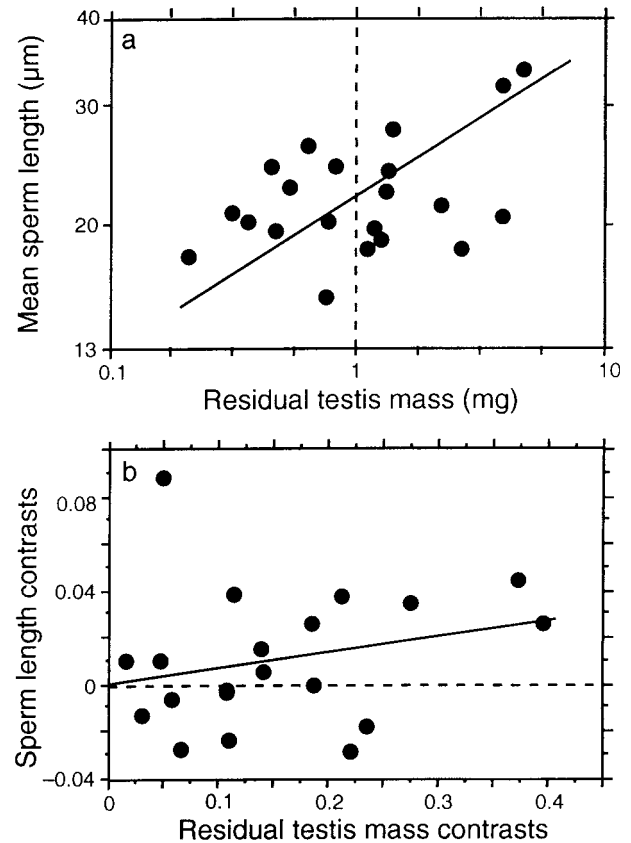


Figure 3
Sperm length in relation to residual testis mass plotted as (a) raw data (note log scales) and (b) independent contrasts with the regression forced through the origin. See text for statistics. Note the regression line drawn in panel a is a model II regression.

tems. This positive relation between sperm length and the intensity of sperm competition (as measured by relative testis mass and mating systems) held true even within fertilization-site categories. This is the first study to find a positive correlation between sperm length and the intensity of sperm competition in fishes. Thus, our results support the predictions of the continuous fertilization model discussed in the introduction. This study also adds cichlid fishes to the growing list of taxa in which variation in sperm length is consistent with the theoretical prediction that selection should favor longer sperm when sperm competition is more intense.

Our results contrast with those of Stockley et al. (1997), who found that fish species experiencing greater sperm competition have shorter sperm. Nor did we find any relation between sperm length and the number of ova as reported by Stockley et al. (1996), though our sample size was small, and this idea certainly deserves further study. Unlike Stockley et al. (1997), we studied only a single family of fishes. By comparing species pairs that have similar ecologies, morphologies, fertilization sites, and phylogenetic histories, but different mating systems, we minimized the risk of finding differences in sperm size that simply reflect differences in life histories. In addition, our measurements were standardized; all samples were taken in exactly the same way, and a single researcher (B.L.) measured all the sperm and testes.

In our comparison of sperm length between mating systems, we controlled for the site of fertilization by comparing taxon pairs that have the same site of fertilization. In addition, we compared the two fertilization sites and found that buccal-fertilizing cichlids had shorter sperm than substrate fertilizers,

making it tempting to suggest that sperm length in these cichlids is also influenced by the site of fertilization. For example, species that spawn in calmer waters have longer-lived sperm compared to species living in turbulent water (Billard, 1987; Leach, 1997). Sperm may not have to travel as far if they are ejected into the buccal cavity or may be less subject to turbulence than those of external fertilizers. However, because our comparison involves only a few taxa, a larger sample needs to be investigated before we can conclude that location of egg fertilization and water currents affect sperm lengths in cichlids. Stockley et al. (1997) categorized buccal-fertilizing cichlids as having little or no sperm competition, whereas our results suggest that sperm competition may occur in this group.

We used both testis size relative to body mass and social mating system as measures of the intensity of sperm competition. Sperm competition, however, may vary within social mating systems, as reproductive sneakers may occur within many socially monogamous species, and in some polygamous species females may mate with only a single male (Taborsky, 1994, 1998). Indeed, socially polygamous males may invest more heavily in gonadal tissue per unit body mass as a tactic to cope with an increased need for either rapid sperm production or larger sperm reserves rather than sperm competition per se. Ideally, to separate this hypothesis from that of sperm competition, we would need to have genetic confirmation of the real relationship between sperm length and mating success.

Although several studies of sperm morphology have been published (for reviews, see Gage, 1998; Jamieson, 1991), much basic information is lacking. For example, we need more information on how sperm lengths affect sperm numbers, mortality, velocity, swimming distances, and swimming direction. To make sense of seemingly contrasting empirical results, we must establish a clearer picture of how sperm size trades off with other components of sperm life history. More work is now needed to see if the pattern we found here holds in other fish taxa and to try to explain the different pattern we see when looking across taxa.

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REFERENCES

- Ball MA, Parker GA, 1996. Sperm competition games: external fertilization and 'adaptive' infertility. *J Theor Biol* 180:141–150.
- Billard R, 1987. Testis growth and spermatogenesis in teleost fish: the problem of the large interspecific variability in testis size. In: Proceedings of the 3rd International Symposium on Reproductive Physiology of Fish, St. John's, Newfoundland; 183–186.
- Birkhead TR, Briskie JV, Møller AP, 1993. Male sperm reserves and copulation frequencies in birds. *Behav Ecol Sociobiol* 32:85–93.
- Birkhead TR, Møller AP (eds), 1998. Sperm competition and sexual selection. London: Academic Press.
- Brichard P, 1989. Cichlids and all the other fishes of Lake Tanganyika. Neptune City, New Jersey: TFH Publications.
- Briskie JV, Montgomerie R, Birkhead TR, 1997. The evolution of sperm size in birds. *Evolution* 51:937–945.
- Burt A, 1989. Comparative methods using phylogenetically independent contrasts. *Oxford Surv Evol Biol* 6:33–53.
- Chao NH, Chao WC, Liu KC, Liao IC, 1987. The properties of tilapia sperm and its cryopreservation. *J Fish Biol* 30:107–118.
- Chao NH, Chen HP, Liao IC, 1975. Study on cryogenic preservation of grey mullet sperm. *Aquaculture* 5:389–406.
- Clutton-Brock TH, Harvey P, 1977. Primate ecology and social organization. *J Zool* 183:1–39.
- Dybas LK, Dybas HS, 1981. Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. *Evolution* 35:168–174.
- Gage MJ, 1994. Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc R Soc Lond B* 258:247–254.
- Gage MJ, 1998. Mammalian sperm morphology. *Proc R Soc Lond B* 265:97–103.
- Gomendio M, Roldan ERS, 1991. Sperm size and sperm competition in mammals. *Proc R Soc Lond B* 243:181–185.
- Goodwin NB, Balshine-Earn S, Reynolds JD, 1998. Evolutionary transitions in cichlid parental care. *Proc R Soc Lond B* 265:2265–2272.
- Harcourt AH, Harvey PH, Larsen SG, Short RV, 1981. Testis size, body weight and breeding system in primates. *Nature* 293:55–57.
- Harvey PH, Pagel MD, 1991. The comparative method in evolutionary biology. Oxford: Oxford University Press.
- Hert E, 1989. The function of egg-spots in an African mouth-brooding cichlid fish. *Anim Behav* 37:726–732.
- Hosken, DJ 1997. Sperm competition in bats. *Proc R Soc Lond B* 264:385–392.
- Hulata G, Rothbard S, Avtalion RR 1981. Evidence for multiple paternity in *Sarotherodon* broods. *Aquaculture* 25: 281–283.
- Jamieson BGM, 1991. Fish evolution and systematics: evidence from spermatozoa. Cambridge: Cambridge University Press.
- Johnson DDP, Briskie JV, 1999. Sperm competition and sperm lengths in waders. *Condor* 101:848–854.
- Keenleyside MHA, 1991. Cichlid fishes: behaviour, ecology and evolution. London: Chapman and Hall.
- Kellogg KA, Markeret JA, Stauffer JA, Kocher TD, 1998. Intraspecific brood mixing and reduced polyandry in a maternal mouth-brooding cichlid. *Behav Ecol* 9:309–312.
- Kenagy CJ, Trombulake SC, 1986. Size and function of mammalian testes in relation to body size. *J Mammal* 67:1–22.
- Kocher TD, Conroy JA, McKaye KR, Stauffer JR, 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. *Mol Phylogenet Evol* 4:420–432.
- Konings A, 1998. Tanganyika cichlids in their natural habitat. El Paso, Texas: Cichlid Press.
- Kuwamura T, 1997. Parental care. In: Fish communities in Lake Tanganyika. (Kawanabe H, Hori M, Nagoshi M, eds). Kyoto, Japan: Kyoto University Press; 59–86.
- Leach B, 1997. Competition and the evolution of sperm characteristics in Pacific salmon (*Oncorhynchus sp*) (MSc dissertation). Kingston, Ontario: Queens University.
- Loiselle PV, 1985. The cichlid aquarium. Melle, Germany: Tetra Press.
- Møller AP, 1989. Ejaculate quality, testis size and sperm production in mammals. *Funct Ecol* 3:91–96.
- Møller AP, Briskie JV, 1995. Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behav Ecol Sociobiol* 36:357–365.
- Mrowka W, 1987. Oral fertilisation in a mouthbrooding cichlid fish. *Ethology* 74:293–296.
- Nishida M, 1997. Phylogenetic relationships and evolution of Tanganyikan cichlids: a molecular perspective. In: Fish communities in Lake Tanganyika (Kawanabe H, Hori M, Nagoshi M, eds). Kyoto, Japan: Kyoto University Press; 3–23.
- Parker A, Kornfield I, 1996. Polygyny and in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environ Biol Fishes* 47:345–352.
- Parker GA, 1970. Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567.
- Parker GA, 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J Theor Biol* 96:281–294.
- Parker GA, 1990a. Sperm competition games: raffles and roles. *Proc R Soc Lond B* 242:120–126.
- Parker GA, 1990b. Sperm competition games: sneaks and extra-pair copulations. *Proc R Soc Lond B* 242:127–133.
- Parker GA, 1993. Sperm competition games: sperm size and sperm number under adult control. *Proc R Soc Lond B* 253:245–254.
- Parker GA, 1998. Sperm games. In: Sperm competition and sexual selection (Birkhead TR, Møller AP, eds). New York: Academic Press; 3–54.

- Parker GA, Begon ME, 1993. Sperm competition games: sperm size and number under gametic control. *Proc R Soc London B* 253: 255–262.
- Pitnick S, Spicer GS, Markow TA, 1995. How long is a giant sperm? *Nature* 375:109.
- Purvis A, Rambaut A, 1995. Comparative analysis by independent contrasts (CAIC), version 2. Oxford: Oxford University.
- Rice WR, Gaines SD, 1994. 'Heads I win, tails you lose': testing directional alternative hypotheses in ecological and evolutionary research. *Trends Ecol Evol* 9:235–237.
- Rossiter A, Yamagishi S, 1997. Intraspecific plasticity in the social system and mating behaviour of a lek-breeding cichlid fish. In: *Fish communities in Lake Tanganyika* (Kawanabe H, Hori M, Nagoshi M, eds). Kyoto, Japan: Kyoto University Press; 293–318.
- Sanderson MJ, Purvis A, Henze C, 1998. Phylogenetic supertrees: assembling the trees of life. *Trends Ecol Evol* 13:105–109.
- Sokal RR, Rohlf FJ, 1995. *Biometry*, 3rd ed. San Francisco: W.H. Freeman.
- Stockley P, Gage MJG, Parker GA, Møller AP, 1996. Female reproductive biology and the coevolution of ejaculate characteristics in fish. *Proc R Soc Lond B* 263:451–458.
- Stockley PM, Gage JG, Parker GA, Møller AP, 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *Am Nat* 149:933–954.
- Sturmbauer C, Meyer A, 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes from Lake Tanganyika in eastern Africa. *Mol Biol Evol* 10:751–768.
- Sturmbauer C, Verheyen E, Meyer A, 1994. Mitochondrial phylogeny of the Lamprologini, the major substrate spawning lineage of cichlid fishes from Lake Tanganyika in Eastern Africa. *Mol Biol Evol* 11:691–703.
- Taborsky M, 1994. Sneakers, satellites, and helpers: parasitic and cooperative behaviour in fish reproduction. *Adv Study Behav* 23:1–100.
- Taborsky M, 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. *Trends Ecol Evol* 13:222–227.
- Warner RR, Robertson DR, 1978. Sexual patterns in the labroid fishes of the Western Caribbean. I. The wrasses (Labridae). *Smithson Contrib Zool* 254:1–24.