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Sperm competition and the evolution of gamete morphology in frogs

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Despite detailed knowledge of the ultrastructure of spermatozoa, there is a paucity of information on the selective pressures that influence sperm form and function. Theoretical models for both internal and external fertilizers predict that sperm competition could favour the evolution of longer sperm. Empirical tests of the external-fertilization model have been restricted to just one group, the fishes, and these tests have proved equivocal. We investigated how sperm competition affects sperm morphology in externally fertilizing myobatrachid frogs. We also examined selection acting on egg size, and covariation between sperm and egg morphology. Species were ranked according to probability of group spawning and hence risk of sperm competition. Body size, testis size and oviposition environment may also influence gamete traits and were included in our analyses. After controlling for phylogenetic relationships between the species examined, we found that an increased risk of sperm competition was associated with increased sperm head and tail lengths. Path analysis showed that sperm competition had its greatest direct effect on sperm tail length, as might be expected under selection resulting from competitive fertilization. Sperm competition did not influence egg size. Oviposition location had a strong influence on egg size and a weak influence on sperm length, with terrestrial spawners having larger gametes than aquatic spawners. Our analysis revealed significant correlated evolution between egg morphology and sperm morphology. These data provide a conclusive demonstration that sperm competition selects for increased sperm length in frogs, and evidence for evolutionary covariance between aspects of male and female gamete morphology.

Keywords: sperm morphology; egg size; gamete coevolution; sperm competition; frogs

1. INTRODUCTION

The ultrastructure of spermatozoa has exhibited rapid and divergent evolution. Indeed, sperm morphology has been widely used for the construction of phylogenies, often when whole-organism morphology is inadequate for resolving ancestral relationships between taxa; for example in insects (Jamieson 1987), fishes (Jamieson 1991) and frogs (Lee & Jamieson 1993). Nevertheless, the selective pressures driving sperm evolution remain largely unexplored (Gage 1998).

Much of sperm form and function is likely to be influenced by the requirements of finding, interacting with and fertilizing eggs and should therefore be subject to natural selection. In his original treatise on sperm competition, Parker (1970) argued that there was every reason to suppose that selection acts on individual sperm, so that sperm morphology may also be subject to sexual selection. Theoretical approaches to understanding sperm evolution have thus considered specific fertilization processes and have been based on assumptions concerning the relationship between sperm morphology and parameters such as sperm swimming speed and longevity (Parker 1993; Ball & Parker 1997, 1998). In general these models predict that, across species, increased risk (the species' average probability of double mating by females) and intensity (the species' average number of males involved in a female's reproductive event) of sperm competition can favour increased sperm length. For internal fertilizers there is mounting evidence to support this prediction from insects (Gage 1994), birds (Briskie & Montgomerie 1992; Briskie *et al.* 1997) and some (Gomendio & Roldan 1991), but not all (Hosken 1997) mammals. For external fertilizers, however, the empirical results have been contradictory. Although Stockley *et al.* (1997) found that, across fishes in general, sperm length decreased with increased sperm competition, Balshine *et al.* (2001) found that, across species of African cichlids, sperm length increase with increased sperm competition.

Previous empirical studies have used simplistic measures of morphological variation in sperm, based on the entire length of the sperm. Recently, Anderson & Dixon (2002) found that across primates sperm length *per se* was not associated with sperm competition. Rather, the volume of the midpiece was greater in multi-male than in single-male mating systems, while sperm head and tail volumes were not related to breeding system. This analysis suggests that selection via sperm competition may target specific aspects of sperm morphology. Indeed, Anderson & Dixon (2002) interpret their findings as indicating that sperm competition favours an increase in energy allocation to sperm motility (for a similar analysis across shorebirds see Johnson & Briskie (1999)). Nevertheless, Gage & Freckleton (2003) found no general influence of sperm competition (estimated from variation in testes mass) on head, midpiece or flagellum lengths, across 83 mammalian species.

There is every reason to expect that sperm morphology should be influenced by female reproductive anatomy, egg morphology and/or the fertilization environment (Gomendio & Roldan 1993; Pitnick *et al.* 1999; Miller &

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Pitnick 2002). For example, across species of fish, Stockley *et al.* (1996) found that the sperm of internally fertilizing species are generally longer than those of externally fertilizing species, and that, among external fertilizers, sperm longevity is positively related to egg size. By contrast, in his comparative analysis of 445 mammals, Gage (1998) was unable to attribute variation in sperm head volume, midpiece volume or tail length to variation in body size or the duration of female oestrus (see also Hosken (1997) for an analysis restricted to bats).

Selection for enhanced fertilization is expected to act on both male and female gametes. In marine invertebrates, for example, sperm limitation can be a significant selection pressure favouring increased egg size (Levitan 1996, 1998; Levitan & Irvine 2001) and/or accessory structures such as jelly coats that enhance rates of egg–sperm collision (Farley & Levitan 2001; Podolsky 2001, 2002). However, we should not expect egg morphology to be influenced by sperm competition.

Here, we present a comparative analysis of gamete morphology across 100 species of Australian myobatrachid frogs. The ultrastructure of frog sperm has been the subject of detailed observation and has been used to infer phylogenetic relationships within the group (Lee & Jamieson 1992, 1993; Kwon & Lee 1995; Scheltinga *et al.* 2002). Nevertheless, there has been no detailed attempt to understand the adaptive significance of this variation. Species differ in their fertilization environments, spawning directly into the environment or into foam nests, in either aquatic or terrestrial habitats, and it has been suggested that some of the variation in sperm ultrastructure may be associated with differences in spawning environment (Garrido *et al.* 1989; Scheltinga *et al.* 2002). Nevertheless, no formal comparative test of this hypothesis has been made. Spawning environment is also expected to influence egg characteristics, which may in turn impose selection on sperm morphology. Elsewhere (Byrne *et al.* 2002) we have shown how sperm competition across the Myobatrachidae strongly influences testis size and might also favour sperm with characteristics that enhance competitive fertilization (Ball & Parker 1997). Specifically, based on the hypothesis that swimming speed is a positive function of the length of the propulsive tail (Katz & Drobnis 1990; Gomendio & Roldan 1991), we predict that sperm competition should impose greater selection on the length of the sperm tail than on the size of the sperm head, and should have no influence on egg size. Thus, we seek relationships between gamete characteristics, sperm competition, fertilization environment and body size while controlling for common ancestry within our target taxa.

2. MATERIAL AND METHODS

(a) *Taxa investigated*

The study was based on 100 species, from 17 genera in the family Myobatrachidae (see electronic Appendix A available on the Royal Society's Publications Web site). Specimens were obtained from collections in the Australian, Queensland, Victorian, South Australian and West Australian museums. Species used in the analysis were those where phylogenetic status had been resolved, information was available on breeding biology and adequate samples could be obtained (see electronic appendices A2 and A5 in Byrne *et al.* 2002).

(b) *Egg size*

Data on egg size (specifically the diameter of the ovum and not of the gelatinous egg capsule), clutch size and spawn location were collated from reports in the literature and can be found in electronic appendix A2 in Byrne *et al.* (2002). Where a range of values was obtained for a species for egg size or clutch size, the median value was used. Where several medians were obtained from different references, an average was made of the median values. For spawn location we placed species into one of four categories: aquatic; aquatic in foam; terrestrial; and terrestrial in foam.

(c) *Technique for measuring sperm morphology*

Sperm were obtained from the testes of preserved frogs. As the testes of anurans may regress outside the breeding season (Lofts 1974), we recovered sperm from specimens only if they had been collected within their respective breeding seasons. Information on breeding season was obtained from the literature (see electronic appendices A2 and A5 in Byrne *et al.* 2002). Frogs were removed from collections, towel dried to remove excess alcohol, weighed and their snout–vent lengths (SVLs) measured. They were dissected and their left and right testes were removed, blotted dry and weighed to ± 0.1 mg. Testes were then re-hydrated by running them through four solutions of decreasing alcohol concentration (75% ethyl alcohol (EtOH), 50% EtOH, 25% EtOH and 0% EtOH (distilled water), respectively). Testes remained in each of these solutions for a period of *ca.* 15 min. This rehydration procedure is a standard technique used to obtain anuran sperm for morphological analysis from preserved material (D. M. Scheltinga, personal communication).

Following rehydration, whole testes were macerated in distilled water and the suspension was spread finely on a slide and left to air dry. Sperm from testis smears were then viewed under a Leica compound microscope (100× magnification) and images were captured using the image-analysis program Optimas v. 6.5. Individual sperm were chosen for measurement by scanning the sample image. To control for any edge effects, sperm were systematically chosen from positions ranging across the entire smear. Any damaged sperm (e.g. head and tail separated) were avoided. Myobatrachid sperm possess an elongated and sharply pointed head (Lee & Jamieson 1992). The tail consists of a major axial fibre and a minor juxtaxonemal fibre attached to the axoneme. The major and minor fibres are joined by an undulating membrane, which affords motility (Lee & Jamieson 1992). For each sperm we measured both head length and tail length to the nearest 0.01 μm. Head length was measured from the apex of the head to the junction of the head with the tail. Tail length was measured from the junction of the head and tail to the apex of the tail. The mitochondrial region (mid-piece) lies at the base of the tail, and is not always readily distinguishable from the rest of the tail so that separate measurements of this region were not possible. Measurements were made of 10 sperm from each of two to six males per species (depending on availability). The data on body and sperm morphology can be found in electronic Appendix A. Data on testis weights can be found in electronic appendix A2 in Byrne *et al.* (2002).

We performed a preliminary investigation to determine whether measurements of sperm recovered from preserved specimens gave reliable estimates of live sperm morphology. We compared the sperm head and tail lengths of preserved (average values obtained from two to six individuals per species) and freshly field-collected males (average values obtained from two

to 11 individuals per species) for 17 species from seven genera (see electronic Appendix A). All live specimens were collected in Western Australia during their respective breeding seasons and were euthanased by double pithing within 24 hours of collection. Sperm were obtained from testes crushes and measured in the same way as those obtained from preserved material. Mean sperm lengths did not differ between preserved and freshly collected material (mean difference between preserved and freshly collected across 17 species: head length, -0.41 ± 0.55 μm, $t_{16} = 0.752$, $p = 0.231$; tail length, 0.05 ± 0.91 (s.e.) μm, $t_{16} = 0.061$, $p = 0.524$) so we are confident that preservation did not affect our assessment of sperm morphology.

Finally, to test whether the morphology of sperm collected from testis crushes was representative of the morphology of sperm released by males under natural conditions, we compared ejaculated sperm with sperm obtained from fresh testes crushes for one species, the quacking frog *Crinia georgiana*. To obtain sperm from ejaculates, 31 mating pairs, recently formed in the field, were placed in circular transparent plastic containers (one pair per container) with a known volume of water. Following oviposition and pair breakup, water containing sperm was transferred into plastic vials and returned to the laboratory, where aliquots of the sperm solution were placed onto slides using a pipette and left to air dry. We measured 10 sperm per ejaculate and compared these with 10 sperm obtained from testes crushes from each of 24 freshly euthanased males. Controlling for SVL, sperm head length did not differ between ejaculated sperm and sperm from testes crushes (SVL $F_{1,52} = 4.72$, $p = 0.034$; ejaculated (25.19 ± 0.19 (s.e.) μm) versus testes crush (25.30 ± 0.22 μm), $F_{1,52} = 0.14$, $p = 0.711$), but ejaculated sperm tended to have shorter tails (SVL $F_{1,52} = 0.23$, $p = 0.637$; ejaculated (43.83 ± 0.32 (s.e.) μm) versus testes crush (45.19 ± 0.32 μm), $F_{1,52} = 9.29$, $p = 0.004$). Thus, measures obtained from testes crushes may overestimate the lengths of sperm tails in fresh ejaculates.

(d) *Index of sperm-competition risk*

We used the index of sperm-competition risk developed in Byrne *et al.* (2002). Briefly, the index is based on the assumption that species where breeding occurs in dense aggregations, within which males are in close proximity to each other and interact intensely during breeding, will have a higher likelihood of group spawning and thus, sperm competition (cf. Halliday 1998). Five ranked categories of increasing sperm-competition risk were created:

(i) 0 = males solitary or widely spaced and/or show site fidelity within breeding choruses, no interaction between males reported;
(ii) 1 = males aggregate to breed but are highly spaced and/or show site fidelity within choruses, no interaction between males reported;
(iii) 2 = aggregations are dense (less than 0.5 m between males) and there have been reports of inter-male acoustic interaction, physical display or minor direct physical interaction;
(iv) 3 = aggregations are dense and there is direct physical interaction between males in the form of fighting or wrestling and/or males show indiscriminate clasping behaviour;
(v) 4 = aggregations are dense, direct physical interaction occurs between males and there are observations of multiple males amplexing single females.

Details of the sperm-competition indices assigned to species of myobatrachid frogs analysed in this study can be found in electronic appendices A2 and A5 in Byrne *et al.* (2002).

(e) *Analyses*

We first performed exploratory analyses using general linear modelling (GLM) techniques with species as independent data points. For those variables showing significant associations, we used comparative analysis by independent contrasts (CAIC; Purvis & Rambaut 1994) to control for common ancestry among the species of myobatrachid represented in the study. A description of the phylogeny used can be found in Byrne *et al.* (2002). Information on branch lengths was not available so we assumed a punctuated model of evolution. To control for interspecific allometric effects, log-transformed variables were used in all analyses. Means are presented with ± 1 s.e.

3. RESULTS

(a) *Species comparisons*

(i) *Egg size*

Across species our predictor variables explained 75% of the interspecific variation in egg size ($F_{6,48} = 24.36$, $p < 0.001$). There was a positive relationship between egg size and body size, but no effect of sperm competition on egg size (table 1). There was a trade-off between egg size and clutch size (least squares mean slope $\beta = -0.205 \pm 0.03$), and terrestrial spawners had larger eggs than aquatic spawners (log least squares means (LSM): aquatic, 0.27 ± 0.02 mm; aquatic in foam, 0.21 ± 0.03 mm; terrestrial, 0.39 ± 0.42 mm; terrestrial in foam, 0.44 ± 0.05 mm; *post hoc* contrast between aquatic and terrestrial, $t = 5.145$, $p < 0.0001$).

(ii) *Sperm morphology*

Preliminary analyses were run on head length and tail length, including all measured predictor variables (sperm-competition index, oviposition location, egg size, SVL, testes mass and clutch size). The analyses explained significant proportions of the variation in these measures of sperm morphology. However, clutch size had no effect on either head length ($p = 0.908$) or tail length ($p = 0.567$) when controlling for the other variables, so clutch size was removed from the model to increase the number of species entered (from 55 species with complete datasets to 71 with complete datasets or lacking only clutch size) and thus the power of the analyses. The final analyses explained 66.8% of the variation in head length ($F_{7,63} = 8.08$, $p < 0.001$) and 67.5% of the variation in tail length ($F_{7,63} = 18.66$, $p < 0.001$). All variables had significant effects on head length (table 2). However, only sperm-competition index, egg size and testis mass significantly influenced tail length, after controlling for the other independent variables (table 2). Thus, after controlling for all other variables, an increase in sperm-competition index was associated with an increase in both the length of the head piece ($\beta = 0.02 \pm 0.01$) and the length of the sperm tail ($\beta = 0.02 \pm 0.006$) across species of myobatrachid frog. Furthermore, species with larger eggs had sperm with longer heads ($\beta = 0.19 \pm 0.09$) and tails ($\beta = 0.17 \pm 0.05$). Species with terrestrial oviposition had longer sperm heads than those with aquatic oviposition (log LSMs after controlling for all other variables: aquatic, 1.16 ± 0.02; aquatic in foam, 1.14 ± 0.03; terrestrial, 1.26 ± 0.02; terrestrial in foam, 1.19 ± 0.05; *post hoc* contrast between aquatic and terrestrial, $t = 2.09$, $p < 0.041$). The log LSMs for tail length exhibited similar but non-significant

Table 1. Results from GLM of factors that contribute to variation in egg size across 55 species of frog from the family Myobatrachidae.
(Abbreviation: SS, sums of squares.)

source	SS	d.f.	F	p
sperm competition	0.0001	1	0.015	0.905
oviposition location	0.2369	3	12.034	< 0.001
log clutch size	0.2724	1	41.507	< 0.001
log SVL	0.2029	1	30.923	< 0.001
error	0.3150	48		

Table 2. Results from GLM of factors that contribute to variation in the lengths of sperm heads and tails across 71 species of frog from the family Myobatrachidae.
(Abbreviation: SS, sums of squares.)

source	SS	d.f.	F	p
head length				
sperm competition	0.0259	1	4.348	0.041
oviposition location	0.0890	3	4.974	0.004
log egg size	0.0273	1	4.574	0.036
log testis weight	0.1240	1	20.778	< 0.001
log SVL	0.0330	1	5.528	0.022
error	0.3759	63		
tail length				
sperm competition	0.0244	1	15.656	< 0.001
oviposition location	0.0110	3	2.339	0.082
log egg size	0.0215	1	13.788	< 0.001
log testis weight	0.0146	1	9.325	0.003
log SVL	0.0002	1	0.111	0.740
error	0.0983	63		

Table 3. Results from GLM[a] using independent contrasts to analyse the factors responsible for evolutionary changes in the lengths of sperm heads and tails across the family Myobatrachidae.
(Abbreviation: SS, sums of squares.)

source	SS	d.f.	F	p
head length				
sperm competition	0.0059	1	5.160	0.029
egg size	0.0063	1	5.484	0.024
testis weight	0.0106	1	9.305	0.004
SVL	0.0003	1	0.303	0.585
error	0.0526	46		
tail length				
sperm competition	0.0041	1	14.741	< 0.001
egg size	0.0049	1	17.612	< 0.001
testis weight	0.0006	1	2.223	0.143
SVL	0.0003	1	0.943	0.337
error	0.0129	46		

[a] For testing evolutionary associations, the regression was forced through the origin.

trends across oviposition locations (aquatic, 0.45 ± 0.008; aquatic in foam, 0.43 ± 0.01; terrestrial, 0.48 ± 0.01; terrestrial in foam, 0.44 ± 0.03).

(b) *Comparative analysis by independent contrasts*

(i) *Egg size*

We first analysed the apparent trade-off between egg size and clutch size by generating independent contrasts in log SVL, log clutch size and log egg size using the 'Crunch' algorithm in CAIC. We generated 42 independent contrasts, which revealed a significant evolutionary trade-off between clutch size and egg size when controlling for body size (multiple regression of independent contrasts forced through the origin: whole model, $F_{1,40} = 16.71$, $p < 0.001$; partial effect of SVL, $\beta = 0.561 \pm 0.15$, $F_{1,40} = 13.45$, $p < 0.001$; partial effect of clutch size, $\beta = -0.230 \pm 0.04$, $F_{1,40} = 32.81$, $p < 0.001$).

As oviposition location was neither a continuous variable nor a dichotomous variable, comparative analysis is made more complex (Purvis & Rambaut 1994). We therefore generated a dichotomous variable, categorizing species as being either aquatic or terrestrial since it was this aspect that had the major influence in our cross-species analysis. Independent contrasts in oviposition location, egg size, clutch size and body size were then generated using the 'Brunch' algorithm in CAIC. There were eight independent contrasts, which revealed that a move from aquatic to terrestrial oviposition was associated with an increase in egg size (eight out of eight contrasts were positive and the mean, 0.06 ± 0.02, differed significantly from zero, $t = 3.653$, $p = 0.008$) and a decrease in clutch size (eight out of eight contrasts were negative and the mean, -0.16 ± 0.06, differed significantly from zero, $t = -2.460$, $p = 0.043$). This analysis was not confounded by evolutionary changes in body size since a move from aquatic oviposition to terrestrial oviposition was not associated with a change in body size (the mean contrast in SVL did not differ from zero, -0.01 ± 0.02, $t = 0.374$, $p = 0.719$).

(ii) *Sperm morphology*

The 'Crunch' algorithm of CAIC returned 50 independent contrasts in the dependent variables head length and tail length, and the independent variables testes mass, egg size and SVL. Changes in sperm competition were positively associated with changes in the lengths of sperm heads and tails when other variables were controlled for statistically (table 3 and figure 1). Changes in sperm head length were also associated with changes in testes mass. Changes in body size were not associated with changes in either sperm characteristic (table 3).

We employed path analysis to illustrate the relative influences of evolutionary contrasts in independent variables on contrasts in sperm characteristics (figure 2). The path coefficients show that sperm competition had a greater direct positive influence on the average length of sperm tails than it did on the average length of sperm heads. Nevertheless, sperm competition also had an indirect positive influence on head length because increases in sperm competition were associated with increases in testes mass, which in turn were positively associated with head length. The net association between sperm competition and head length was still less (sum of the products of the chains of path coefficients of 0.37) than that between sperm competition and tail length (0.46). The allometric exponent derived from a regression of log head length on log tail length was greater than 1.0, both across species (exponent 1.534 ± 0.103, $t = 5.181$, d.f. = 98, $p < 0.001$) and across independent contrasts (1.516 ± 0.171, $t = 3.017$, d.f. = 49, $p = 0.004$), indicating that head length increases more rapidly than tail length.

For comparative analysis of the influence of oviposition location on sperm traits we again categorized species as aquatic or terrestrial. The 'Brunch' algorithm returned eight contrasts. The mean contrast in head length was 0.032 ± 0.01, which was not significantly different from zero ($t = 2.177$, $p = 0.066$), and the mean contrast in tail length was 0.016 ± 0.006, which was significantly different from zero ($t = 2.537$, $p = 0.039$). Given the low statistical power of these tests, oviposition location seems likely to influence both head length and tail length.

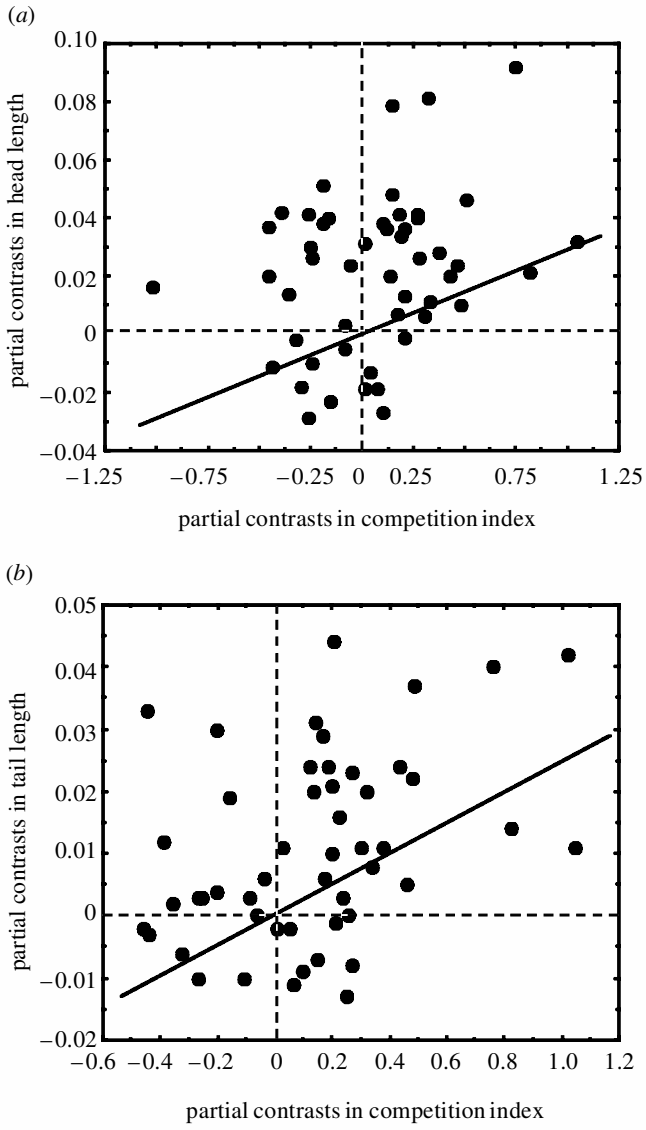

Figure 1. The evolutionary association between sperm-competition index and sperm morphology. Both (*a*) sperm head and (*b*) sperm tail lengths increase with increased sperm-competition index. The data are partial (residual) contrasts in sperm-competition index and partial (residual) contrasts in sperm traits, after controlling for the effects of egg size, testis weight and SVL identified (table 3). The regression line is the least-squares slope forced through the origin.

4. DISCUSSION

Across the species of myobatrachid frogs analysed in this study, sperm head and tail lengths varied by a factor of four; the squelching froglet, *Crinia insignifera* had the shortest sperm, with head and tail measuring 10 μm each, and Lea's frog, *Geocrinia leai* had the longest sperm, with head and tail measuring 32 μm and 45 μm, respectively (see electronic Appendix A). Early studies of mammals suggested that much of the variation in sperm size was associated with variation in body size (Cummins 1983; Cummins & Woodall 1985). More recent analyses have shown that the putative relationship between body size and sperm size is not robust to phylogenetic control (Hosken 1997; Gage 1998). Likewise, after controlling for phylogeny, we found that, across species of myobatrachid

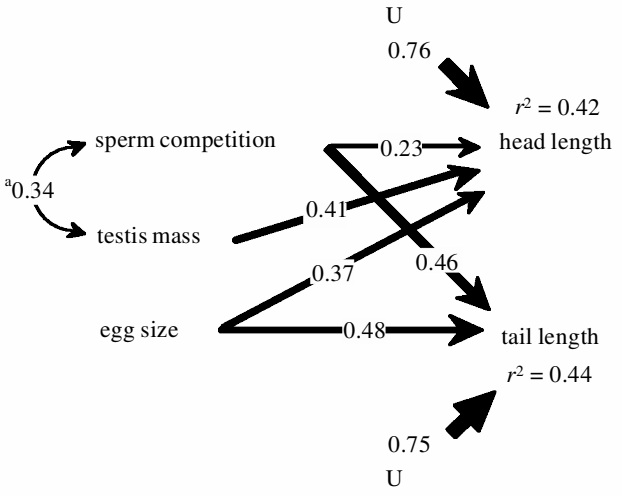

Figure 2. Path analysis of the factors contributing to variation in two dependent variables, head length and tail length of sperm. Path coefficients are the maximum-likelihood estimates of standardized partial regression coefficients from contrasts in the predictor and criterion variables (see table 3). Only significant paths were included in the model, and U represents unexplained or residual variation. In this case some of the unexplained variation is caused by the association between oviposition location and sperm characteristics, which cannot be included in the comparative analysis of continuous traits. The thicknesses of the arrows illustrate the relative strengths of effects on the criterion variables. [a] The correlation coefficient between the two independent variables sperm-competition index and testis mass. The model explained a significant proportion of the variance in sperm head and tail length, $\chi^2_4 = 26.87$, $p < 0.001$.

frogs, sperm head and tail lengths varied independently of body size. As with mammals (Gage 1998), there was a positive allometric relationship between sperm head length and tail length, indicating that sperm heads increase more rapidly in size than do tails. Our study provides evidence for a number of selective pressures that may account for the widespread variation in sperm morphology among myobatrachid frogs.

After controlling for potentially confounding variables, we found strong empirical support for a role of sperm competition in the evolution of sperm morphology. Across species of myobatrachid frog, levels of sperm competition were positively associated with the lengths of both the heads and the tails of sperm. Consistent with the prediction that competitive swimming speed should be the focus of selection under sperm competition, the average length of sperm tails showed the strongest direct relationship with our index of sperm competition. By contrast, the greatest influence on sperm head length was indirect, via the increased testis size that is associated with increased risk and intensity of sperm competition (Byrne *et al.* 2002). Sperm competition had no influence on the size of eggs. Our findings contrast with those of Gage & Freckleton (2003), who concluded that there was no association between sperm-competition risk and sperm morphology across 83 mammalian species. Gage & Freckleton (2003) used testis size as a proxy for sperm-competition risk. However, our path analysis suggests that direct measures of sperm-competition risk may be more reliable for testing evolutionary associations between sperm competition and sperm morphology; although testis size had no influence on sperm tail length, sperm-competition risk did.

The theoretical analysis by Ball & Parker (1997) of sperm competition and sperm morphology was based on a continuous fertilization process during which sperm swimming speed is maximized at the cost of longevity. The model predictions depend critically on the assumed relationship between sperm length and longevity; when negative, increased sperm competition is predicted to favour increased sperm length, but when positive, increased sperm competition is predicted to favour decreased sperm length. Thus, to evaluate this model fully we need data on the relationships between sperm-competition risk and sperm length, and between sperm length and longevity. Currently, we have no knowledge of the relationship between sperm length and longevity for myobatrachid frogs. Across species of fish, Stockley *et al.* (1997) found that evolutionary decreases in sperm longevity were associated with evolutionary increases in sperm length, but increased sperm competition was associated with a decrease in sperm length, rather than the increase predicted by the models. Thus, across fishes the data are inconsistent with the process of selection proposed by Ball & Parker (1997). Our data, and those for fishes, clearly show a role for sperm competition in the evolution of sperm morphology. However, whether the mechanisms proposed by Ball & Parker (1997) are realistic remains to be seen.

We also found evolutionary covariation between egg and sperm morphologies: evolutionary increases in egg size were associated with evolutionary increases in the lengths of both heads and tails of sperm. Covariation between sperm morphology and female-reproductive-tract morphology has been reported in insects (Pomiankowski 1987) and birds (Briskie *et al.* 1997). However, previous studies of mammals (Gomendio & Roldan 1993) and fishes (Stockley *et al.* 1996) failed to find covariation between sperm and egg traits. Positive covariation between sperm size and egg size might be expected if larger ova have thicker vestments that require larger more powerful sperm for penetration (Gomendio & Roldan 1993). Scheltinga *et al.* (2002) argued that the elongated heads of the sperm of the hylid frog, *Litoria longirostris*, may be a modification for the penetration of the large gelatinous layer surrounding the terrestrially oviposited egg. In general the thickness of the swollen jelly capsule increases isometrically with the diameter of the egg across myobatrachid frogs (data in electronic appendix A2 in Byrne *et al.* 2002). The jelly coat swells after fertilization (Rugh 1977). It seems reasonable to assume that the thickness of the unswollen jelly coat should be proportional to the thickness of the swollen coat, so that longer sperm heads with longer more powerful propulsive tails may have a penetration advantage in species with larger eggs. Furthermore, sperm enter the egg throughout the animal region and must migrate 25° latitude or more towards the animal pole, aided by cortical contractions of the egg, before fusing with the egg nucleus (Elinson 1975; Rugh 1977). Longer sperm heads may well have a selective advantage in travelling the longer distances to the nuclei of larger eggs.

Egg size itself was strongly associated with oviposition location. Species that oviposit in terrestrial habitats have eggs twice as large as species with aquatic oviposition. This trend has been noted previously (reviewed in Bradford 1990) but has not previously been confirmed using appropriate comparative analyses. Despite the small number of contrasts available, this association was robust to control for phylogeny, and evolutionary increases in egg size were associated with evolutionary decreases in clutch size. In general, species with terrestrial oviposition deposit their eggs in areas that subsequently become flooded. Terrestrial eggs hatch at a more advanced stage of development and/or have a period of arrested development while awaiting flooding (Bradford & Seymour 1985; Bradford 1990). Larger eggs are likely to have an adaptive advantage when oviposited terrestrially because the larger yolk content should facilitate the prolonged nourishment of tadpoles as they await unpredictable flooding events. Indeed, Bradford & Seymour (1985) found that, in the terrestrially ovipositing myobatrachid Bibron's toadlet *Pseudophryne bibroni*, yolk reserves of terrestrial embryos lasted 1.5 times as long as those of aquatic hatchlings, illustrating the importance of yolk reserves when hatching is delayed.

Previous studies of sperm ultrastructure in frogs have suggested that sperm morphology also varies consistently between species depending on spawning environment (Garrido *et al.* 1989; Scheltinga *et al.* 2002). Consistent with these studies, using species as independent data points, we found that head length was significantly longer and tail length tended to be longer in terrestrial spawners than in aquatic spawners. Foam nesting did not appear to have any significant influence. After controlling for potentially confounding variables including phylogeny, these patterns were qualitatively, though not quantitatively, similar: evolutionary changes from aquatic to terrestrial oviposition were significantly associated with evolutionary increases in the lengths of sperm tails but the association with changes in head length was not significant. Given the low numbers of contrasts, and thus the low power of these tests, we would suggest that the cross-species patterns may prove robust to phylogenetic control. Nevertheless, the trends are weaker than any of the other effects documented in our analyses and require further study.

This study has generated three important outcomes. First, we have shown that sperm competition has a strong influence on the evolution of sperm morphology across myobatrachid frogs. Moreover, our study is one of the few to have examined selection acting on specific components of sperm morphology (for a study of internal fertilizers see Anderson & Dixon 2002). Second, we have found evidence for coevolution between the male and female gamete morphologies. Although it has been predicted on theoretical grounds, previous studies have failed to find evidence for male–female coevolution in gametic traits (Gomendio & Roldan 1993; Stockley *et al.* 1996). Finally, we have also provided the first robust phylogenetic analyses of the putative associations between oviposition location and egg size and number (Bradford 1990), and between oviposition location and sperm morphology (Garrido *et al.* 1989; Scheltinga *et al.* 2002) that have been suggested from species comparisons of these traits in frogs. While the influence of oviposition location on egg size seems robust to phylogenetic control, its independent influence on sperm morphology remains equivocal.

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