Sperm competition selects for increased testes mass in Australian frogs

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Keywords:

Anuran amphibians;
ejaculate expenditure;
external fertilization;
sperm competition.

Abstract

Game theory predicts that investment in spermatogenesis will increase with the risk and intensity of sperm competition. Widespread support for this prediction has come from comparative studies of internal fertilizing species reporting positive associations between testes mass and the probability that females mate with more than one male. Data for external fertilizers have generated conflicting results. We investigated how risk of sperm competition affects testes size in two families of Australian frogs: the Myobatrachidae and the Hylidae. We also examined effects of clutch size, egg size and oviposition location as alternative factors that might influence sperm production. Species were ranked according to probability of group spawning, and hence risk of sperm competition. Controlling for body size and phylogenetic relationships, we demonstrated that within the Myobatrachidae, the risk of sperm competition explained a significant amount of variation in testes mass. Oviposition location had a weak influence, with species ovipositing into foam having smaller testes. No significant effects of clutch size or egg size were detected. In hylids, the relationship between testes mass and risk of sperm competition was positive but not significant, again with no predictable effects related to egg size or number. These data provide an important test of sperm competition theory for externally fertilizing taxa.

Introduction

Sperm competition is defined as the competition between the sperm from two or more males for fertilization of a given set of ova (Parker, 1970, 1998). Sperm competition is recognized as a pervasive force in evolution, favouring behavioural tactics such as mate guarding, morphological features such as genital complexity, or physiological properties of ejaculates, which reduce the probability that a male's sperm will have to compete (Parker, 1970; Birkhead & MøIler, 1998; Simmons, 2001).

Since the early 1990s, Parker and colleagues have developed a series of game theoretical models for predicting how sperm competition should influence the amount of resources males expend on their ejaculate (Parker, 1990a,b; for a review see Parker, 1998). The early models were developed specifically for internally fertilizing species and rest on two principal assumptions: (i) that sperm compete in a situation analogous to a raffle, where success in obtaining fertilizations is proportional to a male's representation in the total sperm pool present at the site of fertilization; and (ii) there is a trade-off between expenditure on the ejaculate and other reproductive activities such as mate searching. A male's overall fitness is the product of his fertilization gain per mating obtained and is sensitive to sperm competition, which will devalue fertilization success when females mate more than once. The models predict that, across species, an increased risk (the average probability that females of a species will mate with two or more males) and intensity (the average number of males competing for a given set of ova) of sperm competition should favour an increased expenditure on sperm production. Strong empirical support for the models' predictions comes from comparative studies which show positive

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relationships between sperm competition risk and testes mass (mammals: Harcourt *et al.*, 1995; Rose *et al.*, 1997; birds: Møller, 1991; Birkhead & Møller, 1992; Møller & Briskie, 1995; insects: Gage, 1994).

Sperm competition game models have since been developed specifically for species with external fertilization (Ball & Parker, 1996, 1997, 1998; Parker *et al.*, 1996). These models take into account the fact that gametes are shed simultaneously by males and females. Two forms have been analysed, one in which fertilization is instantaneous and there is no sperm mortality during the fertilization process (Parker, 1993), and one in which fertilization is a continuous-time process and there is sperm mortality during the process. The continuous-fertilization model is perhaps the more biologically realistic, as externally fertilizing species can show appreciable sperm mortality leading to sperm limitation and infertility (Levitan & Peterson, 1995). Both models predict that, across species, expenditure on the ejaculate should increase with increasing risk and intensity of sperm competition.

Comparative analyses that test predictions of the external-fertilization models have been restricted to just one group, the fishes. However, results have been inconsistent. Stockley *et al.* (1997) used the method of Burt (1989) of obtaining independent contrasts by pairing species with their closest relative, differing with respect to sperm competition intensity. They obtained 10 independent contrasts between sperm competition intensity and testes mass, and eight contrasts between the mean number of sperm per stripped ejaculate from 89 fish species. Despite the small number of contrasts, the data supported the models in that species with higher sperm competition intensity had larger testes and greater numbers of sperm per stripped ejaculate. Nevertheless, phylogenetic analysis (Pyron, 2000) across 37 species of minnow failed to find a difference in testes mass between group spawners and pair spawners, although sperm competition intensity is predicted to be higher in group spawning situations. Again, Pyron's analysis returned just seven contrasts for paired spawners. Finally, the study of Petersen & Warner (1998) found a positive association between sperm competition intensity and testes mass across four families of tropical reef fishes, although this test is equivocal because it did not control for phylogeny. The inconsistency within fish, coupled with a lack of empirical evaluation for other externally fertilizing groups, makes it questionable whether increased sperm production is a general response to sperm competition under conditions of external fertilization. Clearly, empirical studies are now required for a range of groups to quantify how reproductive effort under conditions of group spawning and sperm competition is allocated towards ejaculate expenditure.

Among anuran amphibians there is considerable potential for sperm competition (Halliday, 1998). Group spawning in frogs has been documented in over 10 species from four different families, with genetic evidence for multiple paternity available for three species (D'Orgeix & Turner, 1995; Laurila & Seppä, 1998; Roberts *et al.*, 1999). A common explanation for group spawning is that intense intrasexual competition drives competitively inferior males to force copulation (Halliday, 1998; Byrne & Roberts, 1999, 2000). The conditions leading to group spawning appear to vary according to temporal breeding pattern. For explosive breeders, where activity lasts hours to days, males compete for direct access to females by actively searching. During this 'scramble competition' multiple-male matings typically arise when indiscriminate males amplex already paired females (Davies & Halliday, 1979; Howard, 1988; Roberts, 1994). In prolonged breeders, where activity lasts months, males usually defend territories and call to attract females. In this situation, group spawns appear to occur when competitively inferior 'satellite males' join mating pairs and steal fertilizations in a fashion analogous to sneaky matings in fish (Pyburn, 1970; D'Orgeix & Turner, 1995; Byrne & Roberts, 2000).

There is some evidence that sperm competition has selected for greater gamete investment in frogs. Kusano *et al.* (1991) compared testes mass among 19 species of Japanese rhacophorids and revealed that species which experienced multiple-male mating had relatively large testes in comparison with species where multiple mating was absent. Similarly, Jennions & Passmore (1993) showed that in African rhacophorids, testes mass was comparatively larger in three species where multiple mating occurred in foam nests than in 31 species where foam nesting (and presumably group spawning) was nonexistent. Phyllomedusine frogs with multiple-male mating also have relatively larger testes than hylid frogs that have not been reported to show such behaviour (Emerson, 1997). None of these studies have controlled for the effects of phylogenetic history. Therefore, their findings provide only superficial tests of theory and an unequivocal association between sperm competition and sperm production remains to be demonstrated in frogs.

Australian anurans provide a model group to investigate how investment in sperm production varies with risk of sperm competition. Across the four endemic families there is a rich diversity of mating patterns arising from extreme interspecific variation in the spatial and temporal distribution of reproductively interacting individuals, the site of oviposition and patterns of egg and tadpole development (Roberts, 1993). Within genera, breeding systems range from those where males form extremely dense choruses and group spawning is frequent (Roberts *et al.*, 1999; Byrne & Roberts, 2000) to those where males are highly solitary and the opportunity for group spawning is likely to be extremely low (Moore, 1961; Cogger, 1992).

Here, we present comparative analyses based on independent contrasts of the influence of sperm competition risk on male gametic investment for two families of

Australian frogs, the Myobatrachidae and the Hylidae. In accordance with sperm competition theory, we predicted a positive association between the risk of sperm competition (group spawning) and investment in spermatogenesis because of direct selection arising from sperm competition.

Materials and methods

Taxa investigated

The study was based on 114 species, from 16 genera in the family Myobatrachidae and 67 species from three genera in the family Hylidae (Appendices S1 and S2). 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 Appendices S1–S5 in web materials).

Technique for measuring testis mass

Frogs were removed from collections, towel dried to remove excess alcohol and weighed. They were dissected and their left and right testis removed, blotted dry and weighed to ±0.1 mg. Measurements were obtained from two to six males per species (depending on availability) and absolute testes mass and body mass calculated as an average across replicates. Because the testes of anurans may regress outside the breeding season (Lofts, 1974), specimens were used only if they had been collected from within their respective breeding seasons. Information on breeding period was obtained from the literature (Appendices S1–S3).

Preservation of museum specimens may alter tissue mass. To test for such effects we compared relative testes mass (% body weight) between preserved (average of two to six individuals per species) and freshly field collected males (average of 2–11 individuals per species) of 19 species from seven genera. All live specimens were collected in Western Australia during their respective breeding seasons and were killed by double-pithing within 24 h of collection. Testes were immediately removed and weighed. Mean relative testes size did not differ significantly between preserved and freshly collected material (paired by species, $t_{19} = 0.226$, $P = 0.823$), so we are confident that preservation did not affect the size of testes relative to body mass.

Index of sperm competition risk

Information on mating pattern of the species examined was obtained from the published literature (Appendices S1–S3) and through personal communications with Australian herpetologists. Based on these data we constructed an index of risk of sperm competition and allocated a rank to each species (Appendices S1 and S2). The sperm competition index was based on the assumption that species where breeding occurs in dense aggregations, within which males are in close proximity and interact intensely during breeding, will have a higher likelihood of group spawning and thus, sperm competition (cf. Halliday, 1998; Byrne & Roberts, 2000). Within species studies of the myobatrachid *Crinia georgiana* provide empirical support for this assumption; the number of simultaneously amplexed males, and thus intensity of sperm competition, increases with chorus density and male bias in the operational sex ratio (Byrne, 2001). Thus, five categories were created: 0 = males solitary or widely spaced and/or show site fidelity within breeding choruses, no interaction between males reported; 1 = males aggregate to breed but are highly spaced and/or show site fidelity within choruses, no interaction between males reported; 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; 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; 4 = aggregations are dense, direct physical interaction occurs between males and there are observations of multiple males amplexing single females (see Appendices S1 and S2). Sperm competition risk categories were considered, *a priori*, to represent an ordinal variable with risk increasing from 0 to 4.

Alternative factors favouring increased ejaculate expenditure

For external fertilizers several factors relating to oviposition habit may affect ejaculate expenditure and testes size. Larger clutches (ova number) may require larger ejaculates to ensure fertilization, favouring increased gametic investment. In their comparative analysis of testes mass in fishes, Stockley *et al.* (1997) found a positive relationship between the number of sperm per stripped ejaculate and clutch size and Emerson's (1997) analysis of frogs found that, across 11 species of ranids, species with relatively larger clutches also had relatively larger testes. In contrast, larger ova may increase the proportion of successful sperm–egg interactions and select for reduced sperm investment (Levitan, 1993; Podolsky & Strathmann, 1996). It is also conceivable that spawning environment, e.g. aquatic vs. terrestrial egg deposition or eggs in jelly masses vs. floating foam nests, may influence sperm mortality and/or wastage (Levitan, 1998). The relative efficiency of fertilization within environments may influence ejaculate expenditure. Thus, data on yolk size, capsule size, clutch size and spawn location were collated from reports in the literature (Appendices S1 and S2). Where a range of values was obtained for a species for ova size, capsule size and clutch size the median value was used. Where several medians were obtained from different references an average was made of these values. For spawn location we placed species into one of four nominal categories, 1 = aquatic; 2 = aquatic in foam; 3 = terrestrial; 4 = terrestrial in foam (Appendices S1 and S2).

General linear modelling (GLM) techniques were used to test the relative importance of risk of sperm competition, body mass, oviposition location, clutch size, yolk size (mm) and capsule size (mm) on absolute testes mass. In a preliminary analysis the number of species used was restricted to those where information on all six variables was available. Nonsignificant terms were removed sequentially so that the final analysis contained a greater number of species. Data were log-transformed to normalize their distributions.

Comparative analysis by independent contrasts

To control for phylogeny we used comparative analysis by independent contrasts (CAIC) (Purvis & Rambaut, 1995). We conducted two analyses, one for each of the Hylidae and Myobatrachidae (see Appendices S4 and S5 for phylogenetic codes). Because information on branch lengths was not available the analysis was based on a punctuated model of evolution. The phylogenies used in these analyses combined data from several sources. Generic relationships for myobatrachids were derived from Maxson (1992), Roberts *et al.* (1997), and a recent study on relationships of all genera in the subfamily Myobatrachinae (*Arenophryne*, *Assa*, *Bryobatrachus*, *Crinia*, *Geocrinia*, *Metacrinia*, *Myobatrachus*, *Paracrinia*, *Pseudophryne*, *Spicospina*, *Uperoleia* and *Taudactylus* with *Limnodynastes dumerili* as an outgroup) by Read *et al.* (2001) that was based on DNA sequence data from 12SrRNA and ND2 mitochondrial genes. Relationships within genera were derived from studies by Maxson & Roberts (1984) for *Heleioporus*, Roberts & Maxson *et al.* (1986) and Hutchinson & Maxson (1988) for *Limnodynastes* and *Megistolotis,* Roberts & Maxson (1989) for *Pseudophryne*, Mable & Roberts (1997) for *Neobatrachus* and some unpublished data for *Taudactylus* and *Lechriodus* supplied by Michael Cunningham based on sequence data for mtDNA. No published phylogenies exist for *Philoria* (including *Kyarranus*) or *Mixophyes*. For those genera, geographical proximity or similarity in male-advertisement call was used to predict relationships (Barker *et al.*, 1995). Relationships within the genus *Uperoleia* are also not defined but there is a clear division of species with and without maxillary teeth (Davies *et al.*, 1986) which generates two major polytomies as the only resolution within this genus. Placement of *Rheobatrachus* was based on Hutchinson & Maxson (1987a).

For hylid frogs in the genera *Litoria*, *Cyclorana* and *Nyctimistes*, relationships are based on published phylogenies in Maxson *et al.* (1982, 1985), Hutchinson & Maxson (1986, 1987b) and Roberts & Maxson (1988). These studies do not define relationships of all species. For hylid species where there was no formal phylogenetic analysis but some consensus of species group placement in literature sources (Tyler & Davies, 1978; Barker *et al.*, 1995) species were placed based on either geographical proximity or call similarity. Much of the hylid phylogeny is based on immunological comparisons of albumin. Recent studies on relationships of the myobatrachine genera by Read *et al.* (2001) and Schauble (2000) for the genus *Limnodynastes* both show that relationships determined using DNA sequence data give very much the same relationships as studies using immunological comparisons of albumin (Maxson, 1992; Roberts *et al.*, 1997 for myobatrachine genera; Roberts & Maxson, 1986 for *Limnodynastes*) suggesting that the topologies used here should be robust.

Only factors that had a significant effect on absolute testes mass were selected for inclusion in the CAIC analyses. To control for allometry, analyses were based on the residuals from simple regression analysis of log contrasts in testes mass against log contrasts in body mass, as recommended by Purvis & Rambaut (1995).

Results

Species comparisons

Across the species investigated, testes mass varied greatly (Appendix S1 and S2). GLM showed a highly significant amount of the variation in testes mass was explained by the variables examined (full model, $R^2 = 0.794$, $F_{7,42} = 28.06$, $P < 0.001$; Table 1). However, significant independent contributions to the model were made only by the index of sperm competition risk, body mass and the location of oviposition. Because clutch size, yolk size and capsule size were insignificant, these variables were removed from all further analyses. Testes mass was positively associated with body mass (parameter estimate, 1.19 ± 0.05), and with the index of sperm competition risk (0.17 ± 0.03). Species ovipositing into foam had smaller testes than those ovipositing directly into the environment (least squares means for log testes mass conrolling for other variables in the model: aquatic = –2.41 ± 0.03, aquatic in foam = –2.73 ± 0.104, terrestrial = –2.30 ± 0.07, and terrestrial in foam = –2.78 ± 0.21), suggesting that foam increases the efficiency of fertilization and so reduces the amount of expenditure required on the ejaculate.

Comparative analysis by independent contrasts

The ‘Crunch’ algorithm in CAIC generated 63 independent contrasts of log body weight, log testes weight and sperm competition index across the myobatrachid phylogeny. Contrasts in testes weight varied isometrically with contrasts in body weight (slope, 1.29 ± 0.31,

Table 1 Results from the general linear modelling analysis of factors that might contribute to variation in testes mass across two families (Myobatrachidae and Hylidae) of Australian frogs (A: full model; B: reduced model).

Source	SS	d.f.	*F*	*P*-value
A				
SC index	1.268	1	10.714	0.002
log body weight	7.126	1	60.219	<0.001
log clutch size	0.048	1	0.408	0.526
log egg size	0.011	1	0.093	0.762
log capsule size	0.293	1	2.474	0.123
Oviposition location*	0.845	2	7.137	0.002
Error*	0.118	49		
B				
SC index	4.008	1	31.196	<0.001
log body weight	62.911	1	489.691	<0.001
Oviposition location*	1.746	3	4.531	0.005
Error*	0.129	150		

*degrees of freedom change for the reduced model where more species with complete data sets become available.

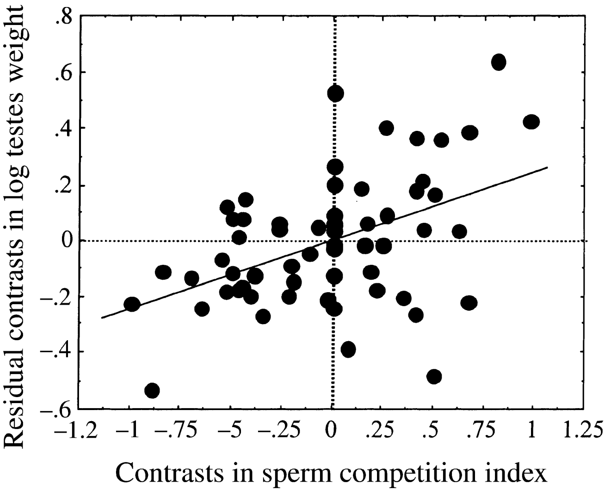

Fig. 1 Relationship between residual contrasts in testes mass and risk of sperm competition across species from the Australian anuran family Myobatrachidae.

$F_{1,61} = 17.26$, $P < 0.001$). Changes in body size were thus controlled for by calculating residual contrasts in testes mass. There was a highly significant positive relationship between residual contrasts in testes mass and risk of sperm competition across the myobatrachids used in this study ($F_{1,63} = 16.43$, $P < 0.001$) (Fig. 1).

To examine the influence of oviposition location in a phylogenetic context, we generated a dichotomous variable by categorizing species as either ovipositing into the external environment or into foam. The 'Brunch' algorithm in CAIC generated just five independent contrasts in oviposition location, four of which were negative and one positive (sign test $P = 0.188$). Thus there is a trend for species ovipositing in foam to have an associated reduction in testes mass. The power of this test is obviously weak because of the low number of contrasts available.

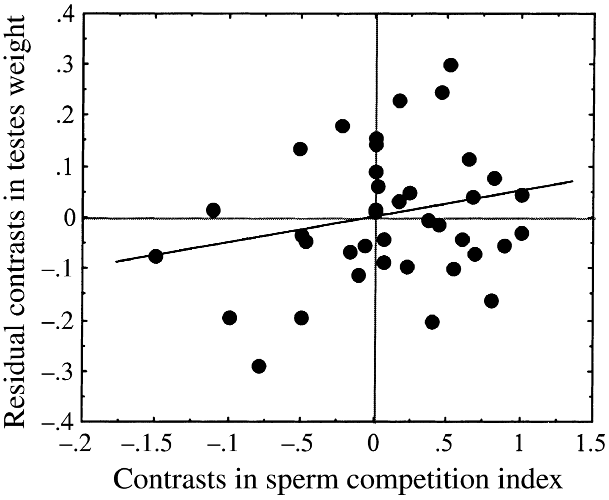

Fig. 2 Relationship between residual contrasts in testes mass and risk of sperm competition across species from the Australian anuran family Hylidae.

There were 39 contrasts in body mass, testes mass and sperm competition index calculated across the hylid phylogeny. Again, contrasts in testes mass increased isometrically with contrasts in body weight (slope 1.11 ± 0.19, $F_{1,37} = 32.09$, $P < 0\ .001$), so residuals from the regression were used to control for changes in body weight. The relationship between residual contrasts in testes weight and risk of sperm competition was positive, although not significantly so ($F_{1,39} = 2.089$, $P = 0.156$) (Fig. 2). Retrospective power analysis suggests that this analysis lacks statistical power ($1–\beta = 0.29$). The analysis was powerful enough to detect an effect size as large as that found in the myobatrachids (raw effect size 0.103, $1–\beta = 0.99$) but the least significant number of contrasts required to detect an effect size as low as that found for the hylids (0.029) was 76. There was no variation in oviposition location across the hylids studied; all species oviposited directly into water (Appendix S1).

Discussion

Testes size in Australian myobatrachid frogs correlated positively with an index of sperm competition risk based on the probability of group spawning. This supports the general prediction of game-theory models for external fertilizers, that across species increased risk and intensity of sperm competition should favour increased ejaculate expenditure (Parker, 1993; Ball & Parker, 1996, 1997, 1998; Parker *et al.*, 1996). The finding also supports the predictions of Halliday & Verrel (1984) and Halliday (1998), that sperm competition is a potentially important factor affecting the reproductive biology of anuran amphibians.

Multiple paternity has been documented in only one Australian anuran, the myobatrachid *Crinia georgiana* (Roberts *et al.*, 1999). This study shows that group spawning in Australian anurans may be much more common than currently realized. Anecdotal evidence obtained for the analyses showed that multiple-male amplexus occurs in at least one other species of myobatrachid (*N. pelobatoides*) and four species of hylid (*Litoria aurea*, *L. moorei*, *L. phyllochroa* and *L. raniformis*) (Fletcher, 1889; Main, 1965; Ford, 1986; G. Gillespie pers. comm., M. Smith pers. comm.). All these species, with the exception of *L. phyllochroa*, were found to have exceptionally large testes relative to body mass (>1% body weight) (Appendices S1 and S2). Several species where males are known to interact heavily during the breeding season (*Geocrinia laevis*, *G. victoriana*, *N. pictus*, *N. sudelli*, *Pseudophyrne bibroni*, *P. guentheri*, *P. major*, *P. occidentalis*) also showed relatively large testes, suggesting group spawning and sperm competition may also occur frequently in these species (Littlejohn, 1971; Woodruff, 1977; Roberts, 1978; Humphries, 1979; Roberts unpub. data). Two anomalous species in the data set were *C. longipes* and *G. leai*. Although these species were classified as having a moderate risk of group spawning (rank = 2), *C. longipes* had the highest recorded relative testes mass (>2% body weight) of any species examined and *G. leai* also had extremely large testes (>1.5% body weight). Little work has been done on these two species. Therefore, the incidence of sperm competition in these species may be much higher than currently realized.

The strength of the association between testes size and risk of sperm competition differed greatly between the two families examined. Among the Myobatrachidae, sperm competition explained a highly significant amount of variation in testes mass, while among the Hylidae the association was in the expected direction, but was not significant after controlling for phylogeny. Why was such a marked difference detected between these two families? Selection on sperm production via sperm competition may be weak or absent in the Hylidae. This could occur for a variety of reasons. For instance, males may be physiologically incapable of increasing sperm production, multiple-male amplexus may be intrinsically difficult (e.g. because of morphological constraints, all Australian hylids have an axillary amplexus vs. inguinal amplexus in myobatrachids; Roberts, 1993), or multiple-male amplexus may entail large costs to males (e.g. mortality or wasted sperm expenditure). On the other hand, sperm competition may exert a strong force in this phyletic line but has targeted attributes other than increased investment in gametogenesis. For example, behavioural mechanisms may provide a more effective means of excluding rivals and monopolizing fertilizations when the risk of group spawning is high. In several fish species which experience high levels of group spawning, males preferentially channel reproductive effort towards mate acquisition and defence (Bluehead wrasse; Warner *et al.*, 1995: tropical reef fish; Marconato & Shapiro, 1996). The result is that increased expenditure on securing matings is traded off against reduced sperm production. Similar processes could be operating in the Hylidae.

Alternatively, the apparent difference in the outcome of our analyses between families may arise because of a lack of data. Few field studies of Australian hylids have conducted detailed observations of breeding behaviour over the entire duration of a reproductive season. Consequently, there is a strong possibility that for many species, risk categories allocated were conservative. Secondly, analysis for the Hylidae was based on a much lower number of independent contrasts than for the Myobatrachidae because phylogenetic relationships are not as well resolved. As a consequence, our analysis lacks the power to accept the null hypothesis of no association between index of sperm competition risk and testis size.

For the Myobatrachidae, the highly significant relationship reported is of considerable interest because it provides the first conclusive demonstration of an evolutionary association between investment in gametogenesis and sperm competition in anuran amphibians. There is evidence to suggest increased gametic investment in response to sperm competition may occur in other anurans. Kusano *et al.* (1991) and Jennions & Passmore (1993) both reported that rhacophorid species with group spawning have exceptionally large testes compared with related species with conventional breeding biologies. Similarly Emerson (1997) showed that phyllomedusine hylid frogs known to group spawn have much larger testes than hylid frogs where this behaviour is absent. However, the exact nature of any association between sperm competition and increased testes size in these groups is uncertain because the comparative analyses employed did not control for phylogenetic inertia. Kusano *et al.* (1991) and Jennions & Passmore (1993) also ignored the possibility that large testes were the outcome of factors other than sperm competition (e.g. oviposition habit). The analysis of myobatrachids suggests that oviposition location can influence testes size, albeit to a lesser extent than sperm competition. Until more rigorous analysis has been conducted it remains unclear whether increased ejaculate expenditure is a common response to sperm competition among anuran amphibians.

Theoretically, increased sperm production in response to sperm competition is predicted for species with both internal and external modes of fertilization (see review of Parker, 1998). There is extensive empirical support for this association in groups with internal fertilization (mammals: Kenagy & Trombulak, 1986; Ginsberg & Rubenstein, 1990; Heske & Ostfeld, 1990; Hosken, 1998; birds: Møller, 1991; Birkhead & Møller, 1992; Møller & Briskie, 1995; insects: Gage, 1994). In contrast, comparative studies for this association among external fertilisers have been restricted to fishes (Stockley *et al.*, 1997; Pyron, 2000). As a result, the generality of the response across reproductive modes is uncertain. Our report of increased ejaculate expenditure under sperm competition across anuran amphibians suggests that mechanisms governing the way sperm compete are probably universal for both internal and external modes of reproduction. Investigation across a diversity of externally fertilizing groups that experience sperm competition is now required to substantiate this trend. Rhacophorid and phylomedusid anurans as well as horseshoe-crabs provide ideal groups for such research (Kusano *et al*., 1991; Jennions & Passmore, 1993; Brockmann *et al*., 1994).

In conclusion, this study has provided two insights. First it provides strong evidence that gamete expenditure in anuran amphibians can evolve under sexual selection generated by sperm competition. Secondly, it supports the argument that increased sperm production in response to sperm competition risk is a universal evolutionary trend across groups with both internal and external modes of reproduction.

Acknowledgments

We sincerely thank the following curators for providing access to museum collections; Patrick Couper (Queensland Museum) Ross Sadlier (Australian Museum) (Victorian Museum) Mark Hutchinson (South Australian museum) and Laurie Smith (Western Australian museum). Thanks to Mike Cunningham for providing data regarding species relationships. Thanks also to Michael and Jody Aird and Glenn Byrne for providing accommodation in the eastern states. This work was supported by a Gene Rodgerson Scholarship to PGB. LWS was supported by the ARC. Research was conducted with permission of the Animal Ethics and Experimentation Committee, University of Western Australia (Approval no. 117/97).

Supplimentary material

The following material is available from http://www.blackwell-science.com/products/journals/suppmat/JEB/JEB409/JEB409sm.htm

Appendix S1 Species and summary of data used in comparative analysis for the family Hylidae

Appendix S2 Species and summary of data used in comparative analysis for the family Myobatrachidae

Appendix S3 Key to references in appendices S1 and S2

Appendix S4 Coding for the phylogeny used in comparative analysis for the family Hylidae

Appendix S5 Coding for the phylogeny used in comparative analysis for the family Myobatrachidae

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Received 28 November 2001; revised 11 January 2002; accepted 26 February 2002