Sperm competition and the evolution of precopulatory weapons: Increasing male density promotes sperm competition and reduces selection on arm strength in a chorusing frog

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Sperm competition theory assumes a trade-off between precopulatory traits that increase mating success and postcopulatory traits that increase fertilization success. Predictions for how sperm competition might affect male expenditure on these traits depend on the number of competing males, the advantage gained from expenditure on weapons, and the level of sperm competition. However, empirical tests of sperm competition theory rarely examine precopulatory male expenditure. We investigated how variation in male density affects precopulatory sexual selection on male weaponry and the level of sperm competition in the chorusing frog *Crinia georgiana*, where males use their arms as weapons in male–male combat. We measured body size and arm girth of 439 males, and recorded their mating success in the field. We found density-dependent selection acting on arm girth. Arm girth was positively associated with mating success, but only at low population densities. Increased male density was associated with higher risk and intensity of sperm competition arising from multimale amplexus, and a reversal in the direction of selection on arm girth. Opposing patterns of pre- and postcopulatory selection may account for the negative covariation between arm girth and testes across populations of this species.

KEY WORDS: Anura, contest competition, multiple amplexus, polyandry, precopulatory sexual selection.

Sexual selection arises from nonrandom variation in reproductive success, generated by competition for mates (Darwin 1871; Andersson 1994). Despite acting on both sexes, sexual selection is generally stronger on males, because male fitness depends more strongly on mating success than female fitness, which is typically more dependent on fecundity and longevity (Bateman 1948; Arnold and Duvall 1994). Male secondary sexual traits that are the product of sexual selection include weaponry used in direct male–male combat (intrasexual selection; Emlen 2008) and ornaments used in displaying to females (intersexual selection or mate choice; Andersson and Simmons 2006). Weapons and ornaments are primarily involved in the early stages of mate competition, including the establishment of territories and/or monopolization of resources that are important to females, and in attracting females for mating. These stages precede copulation, so that male weapons and ornaments evolve under precopulatory sexual selection (Andersson 1994).

The females of many species will mate with two or more males before producing offspring, so that the sperm from different males must compete to fertilize a limited supply of ova. Parker (1970) thus recognized that sexual selection could continue after copulation, imposing selection on traits that affect a male's

competitive fertilization success. Postcopulatory sexual selection can act through sperm competition (Simmons 2001) and cryptic female choice (Eberhard 1996), favoring the evolution of male behavioral, morphological, and physiological traits that influence female remating, and the storage and utilization of sperm. Because mating does not necessarily equate with fertilization, postcopulatory sexual selection can be an important mediator of precopulatory sexual selection (Kvarnemo and Simmons 2013).

A considerable body of theoretical work has focused on how postcopulatory sexual selection should affect the evolution of male expenditure on the ejaculate (Parker 1998; Williams et al. 2005; Fromhage et al. 2008; Tazzyman et al. 2009; Parker and Pizzari 2010). Sperm competition games (Parker 1998; Parker and Pizzari 2010; Parker et al. 2013) assume a trade-off between male expenditure on postcopulatory traits that promote fertilization success (testes size, sperm production, ejaculate expenditure) and expenditure on the acquisition of mates, an assumption for which there is accumulating evidence (Kvarnemo and Simmons 2013). Mate acquisition is modeled as a linear function of search effort (scramble competition). The models predict that male expenditure on the ejaculate should increase among species, and among populations within species, as selection from sperm competition increases, a prediction supported by a large body of evidence (Simmons and Fitzpatrick 2012). The converse, that male expenditure on mate search should decline, has rarely been studied. Moreover, while an assumption of scramble competition offers heuristic value in generating tractable models, it ignores the fact that many species engage in direct contest competition with associated costs of exaggerated weaponry. In their most recent model, Parker et al. (2013) examined how predictions from sperm competition games were affected by incorporating a range of male–male competition from direct contest competition to scramble.

Sperm competition games that incorporate male contest competition yield generally similar predictions to traditional models, in that increased sperm competition is predicted to favor an increased expenditure on the ejaculate and a decreased expenditure on weaponry (Parker et al. 2013). Nonetheless, Parker et al. (2013) urged caution in accepting the generality of this prediction because it will depend on the relationships between three parameters: the number of males in competition, the competitive advantage gained per unit expenditure on weapons, and the level of sperm competition. For example, as male density increases, the efficiency of male contests in monopolizing females may decrease (Knell 2009), or the number of encounters between males and females, and thus mating opportunities, may increase (Kokko and Rankin 2006). The direction of these relationships are expected to affect how males allocate resources to pre- and postcopulatory traits, making empirical studies of these relationships essential for accurate predictions to be made (Parker et al. 2013). We explored how male density affects the strength of precopulatory

sexual selection acting on male weaponry and the level of sperm competition experienced by males in natural populations of the Australian quacking frog, *Crinia georgiana*. We provide an empirical, within-species test of predictions arising from Parker et al.'s (2013) sperm competition games for precopulatory male–male competition.

Crinia georgiana is a small myobatrachid frog (19–47 mm snout-vent length) from the southwest of Western Australia (Smith and Roberts 2003b), where adults are reproductively active during most of the wet season, from May (late-autumn) to October (mid-spring), a period in which males form choruses in very shallow and temporary bodies of water (Ayre et al. 1984). Matings occur during the night, mostly in peaks of reproductive activity that frequently occur after major rain events (Byrne 2002). Males normally defend small depressions (approximately 40 mm across in larger, shallow water bodies up to 1 m across, and less than 2 cm deep) on granite or moss beds, from where they broadcast their advertisement calls (Byrne and Roberts 2004), but can also remain silent within a chorus (Byrne and Roberts 2004). When a female arrives at a chorus (Smith and Roberts 2003a), she typically moves under the body of a calling male, which then embraces her in inguinal amplexus. Females may also be intercepted and grasped by silent males when they are seemingly on their way to a calling male (Byrne and Roberts 2004). Multi-male amplexus occurs when a mating pair is joined by nearby males that either grasp the female in a different position (ventral or lateral), or sometimes one of the other males already involved in the mating (Roberts et al. 1999; Byrne and Roberts 2004). In such cases, all males may ejaculate and share paternity of the resulting clutch (Roberts et al. 1999; Dziminski et al. 2010a).

Sexual dimorphism in forelimb morphology is common in frogs, where males frequently have longer and/or enlarged forelimbs, reflecting the greater development, and therefore strength, of the flexor muscle in males (Oka et al. 1984; Navas and James 2007). Males use their arms in wrestling matches over territory ownership (Howard 1978) and/or the possession of females prior to or during spawning (Davies and Halliday 1979; Wells 2007). The enlarged arms of males are thus thought to be the product of precopulatory sexual selection (Emerson 1991), serving to increase success in direct competition between males for access to females. Our study of *C. georgiana* provides the first formal analysis of precopulatory sexual selection acting on arm strength in an anuran amphibian. We gain insight into the relationship between the number of males competing for females and the competitive advantage associated with male expenditure on weaponry. We show that the advantage of arm strength is density-dependent, so that an increase in male density relaxes selection favoring male investment in premating contests and elevates the level of postcopulatory sperm competition.
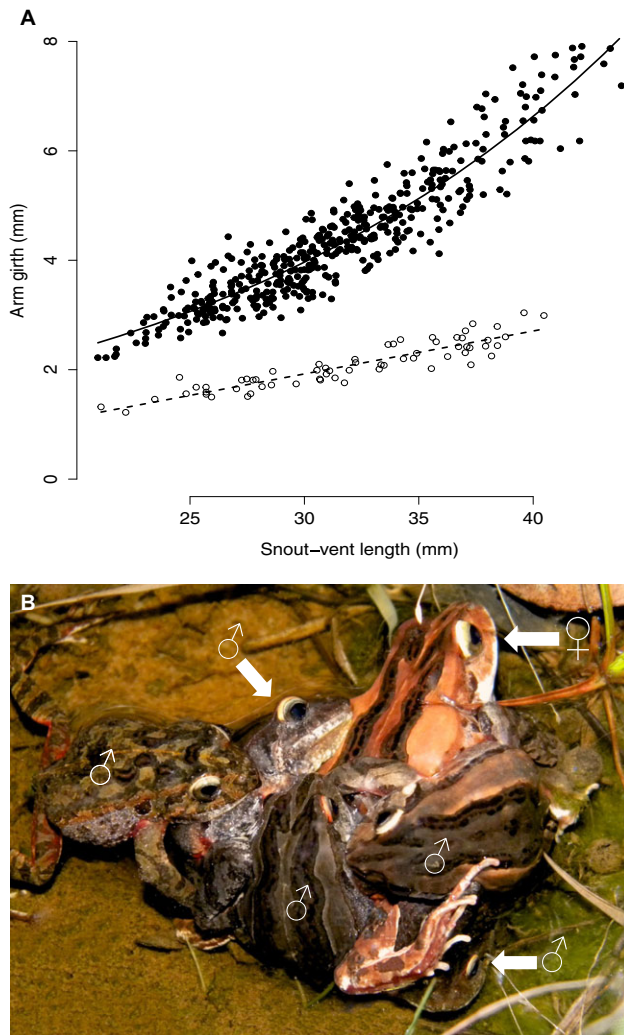
Figure 1. (A) The black curve depicts the model that best fit the allometric relationship between body size (measured as snout-vent length; *SL*) and arm girth (*AG*) for males (full circles) of the Australian quacking frog *Crinia georgiana* ($AG = 0.847 \ e^{\left(\frac{SL}{19.439}\right)}$, where *e* is the Euler's number). The dashed line depicts the model that best fit this relationship for females (open circles; $AG = 0.079 \times SL - 0.431$). (B) A multimale amplexus in *C. georgiana* involving a female and five different males.

Materials and Methods
STUDY SITES AND SAMPLING METHOD

We made behavioral observations in five different natural populations of *C. georgiana* in Western Australia. Three in the urban area of the city of Albany (35° 01′ 22″ S; 117° 52′ 53″ E), one near William Bay (34° 59′ 05″ S; 117° 15′ 56″ E), approximately 10 km west of the town of Denmark, and the fifth at Kangaroo Gully (32° 06′ 35″ S; 116° 08′ 54″ E), approximately 30 km southeast of the city of Perth. All locations consisted of granite outcrops or moss beds with depressions that accumulated rainwa-

ter in small puddles (less than 10 cm deep), where females of *C. georgiana* normally lay their eggs.

We sampled choruses during two consecutive breeding seasons: June to September, 2013 and 2014. In each location, we looked for mating individuals by active searching throughout the chorus and by following females that entered the chorus. We observed each mating event encountered until the female was released from amplexus, after which we weighed all individuals involved (a female and from one up to nine males in the case of multimale matings) to the nearest 0.01 g with a compact digital scale (A&D HT-120). We also measured individuals for snout-vent length and arm girth to the nearest 0.01 mm using a digital caliper (Vernier Craftright). Finally, during the mating we carefully scanned a quadrat of 1 m² area around the mating (with the exact location where the mating started at the center of the quadrat), recording whether peripheral males were calling or silent. On completion of the mating, these peripheral males were also captured, weighed, and measured. In total, 68 mating events were observed.

STATISTICAL ANALYSES

We investigated sexual dimorphism in arm girth (*AG*) by fitting a general linear model with body size (measured as snout-vent length, *SL*), sex, and their interaction as independent variables. Next, because the allometric relationship between body size and arm girth was nonlinear in males, we compared the fit of a linear model [$AG = (0.232 \ SL) - 2.900$], a second degree polynomial model [$AG = (0.006 \ SL^2) - (0.146 \ SL) + 3.021$], and an exponential model [$AG = 0.847 \ e^{\left(\frac{SL}{19.439}\right)}$, where *e* is the Euler's number] on the basis of the bias-corrected version of the Akaike information criterion (*AIC$_c$*), calculated as:

$$AIC_c = -2\ln(L) + 2k\left(\frac{n}{n-k-1}\right),$$

where ln(*L*) is the natural logarithm of the maximum likelihood for the model, *k* is the number of parameters, and *n* is the sample size. We then extracted the residuals of the model with lowest *AIC$_c$* scores and used these as a measure of "relative arm girth" in males. We used the library "*nlrwr*" (Ritz and Streibig 2008) in R version 3.0.2 (R Core Team 2013) to fit the exponential model, and built-in R functions to fit the linear and polynomial models.

To investigate the effect of body size, relative arm girth, and male density on the probability that a male adopts a calling or silent mating tactic, we used generalized linear mixed effects models (GLMMs) with binomial error distributions. Fixed effects included were snout-vent length (*SL*), relative arm girth (*rAG*), male density in the quadrat (*MD*), and every possible interaction between these variables. We built three full models (with all the fixed effects mentioned above) with different sets of random effects: (1) no random effects; (2) quadrat identity (to account for

Table 1. Multimodel inference for the effects of body size (Snout-vent length, *SL*), relative arm girth (*rAG*), and male density (*MD*) on the probability that a male of the quacking frog *Crinia georgiana* adopts a calling or silent mating tactic.

No.	Fixed effects	k	AIC_c	ΔAIC_c	Weight	Log-likelihood	Cumulative weight
1	**SL**	**3**	**388.19**	**—**	**0.28**	**−191.06**	**0.28**
2	SL + MD	4	389.00	0.81	0.18	−190.44	0.46
3	SL + rAG	4	389.40	1.21	0.15	−190.64	0.61
4	SL + MD + rAG	5	390.28	2.09	0.10	−190.04	0.71
5	SL + MD + (SL × MD)	5	391.00	2.80	0.07	−190.40	0.77
6	SL + rAG + (SL × rAG)	5	391.47	3.27	0.05	−190.64	0.83
7	SL + MD + rAG + (MD × rAG)	6	391.71	3.51	0.05	−189.72	0.88
8	SL + MD + rAG + (SL × MD)	6	392.30	4.10	0.04	−190.01	0.91
9	SL + MD + rAG + (SL × rAG)	6	392.36	4.16	0.03	−190.04	0.95
10	SL + MD + rAG + (SL × rAG) + (MD × rAG)	7	393.79	5.59	0.02	−189.71	0.96
11	SL + MD + rAG + (MD × rAG) + (SL × MD)	7	393.80	5.60	0.02	−189.71	0.98
12	SL + MD + rAG + (SL × MD) + (SL × rAG)	7	394.38	6.18	0.01	−190.01	0.99
13	SL + MD + rAG + (SL × MD) + (SL × rAG) + (MD × rAG)	8	395.89	7.70	0.01	−189.71	1.00
14	SL + MD + rAG + (SL × MD) + (SL × rAG) + (MD × rAG) + (SL × MD × rAG)	9	397.94	9.74	0.00	−189.67	1.00
15	MD	3	409.36	21.17	0.00	−201.64	1.00
16	Null	2	410.17	21.97	0.00	−203.06	1.00
17	MD + rAG	4	410.80	22.60	0.00	−201.33	1.00
18	rAG	3	411.56	23.36	0.00	−202.74	1.00
19	MD + rAG + (MD × rAG)	5	412.16	23.97	0.00	−200.98	1.00

All models have the random effect of quadrat identity, because male density was measured in quadrats of 1 m², and hence measures are the same for males found in the same quadrat. We ranked models by increasing AIC_c values. "ΔAIC_c" is the difference between the AIC_c of each model and the AIC_c of model 1, "Weight" is the Akaike weight, k is the number of estimable parameters, "Log-likelihood" is the natural logarithm of the maximum likelihood, and "Cumulative weight" is the cumulative Akaike weight of each model and the models above it. The most parsimonious models are indicated in shaded rows, and the best minimal model is in bold.

the fact that male density was the same for males from the same quadrat); and (3) study site (to account for potential nonindependence of observations in the same study site). In this third model, quadrat identity was nested within study site. We then compared these models on the basis of their AIC_c scores, and determined that no random effect significantly improved the model. We retained quadrat identity as a random effect in all models to avoid pseudoreplicating our observations of male density in each quadrat. Next, we built a set of candidate models that included (1) a null model, (2) seven models with every possible combination of the fixed effects (*SL*, *rAG*, *MD*), (3) 10 models with all combinations of the three fixed effects and every possible combination of the two-way interactions between them, and (4) the fully parameterized model that included all fixed effects, all the two- and three-way interactions. We then compared this set of 19 candidate models on the basis of their AIC_c scores. Finally, we checked the best minimal model for overdispersion by calculating the sum of squared Pearson residuals and comparing it to the residual degrees of freedom of the model. To be consistent in our analytical approach when testing the importance of random and fixed

effects, and because our set of candidate models was relatively large, we chose to use model comparison through AIC_c scores.

We used the same approach to investigate the effect of body size, relative arm girth, and male density on the probability that a male was involved in a mating, as an analysis of mating success. This time, the random effect of quadrat identity significantly improved the model, and was retained for the rest of the analysis. Next, we focused on the subset of males that were mated, and used binomial GLMMs to investigate the effect of body size, relative arm girth, and male density on the probability that there were other males involved in the mating, resulting in sperm competition. In this analysis, the random effect of study site significantly improved the models, so we retained it in all models. We also retained the random effect of quadrat identity (nested within study site) to avoid pseudo replication in the measures of male density. The comparison between the sets of 19 models with different combinations of fixed effects and the diagnostics of overdispersion for these analyses were performed as described above. We fitted mixed effects models using the library "*lme4*" (Bates et al. 2013); compared candidate models using the library "*AICcmodavg*"
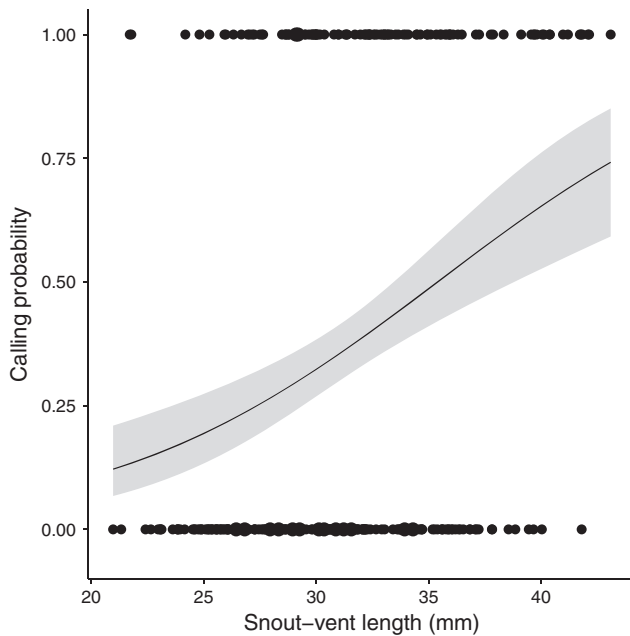
Figure 2. Males of the Australian quacking frog *Crinia georgiana* present two alternative mating tactics: calling to attract females (coded 1) or acting as a silent sneaker/satellite (coded 0). Our results indicate that the probability of adopting the calling tactic increases with a male's body size (measured as snout-vent length, *SL*). Predicted probabilities (curve) and 95% confidence intervals (shaded area) were generated with a generalized linear model with only male *SL* as a fixed effect, equivalent to model no. 1 in Table 1, but without random effects.

(Mazerolle 2014); and performed model validation and plotting using the libraries "*car*" (Fox and Weisberg 2011), "*akima*" (Akima et al. 2013), "*plyr*" (Wickham 2011), and "*ggplot2*" (Wickham 2009), all in R version 3.0.2 (R Core Team 2013).

Finally, we used Poisson GLMMs to analyze the effect of male density on the total number of males involved in each mating. Here no random effects significantly improved the model, but we again retained quadrat identity as a random effect to avoid pseudo replicating male density for matings that occurred in the same quadrat. We compared a model with male density as a fixed effect against a null model through their AIC_c values, and checked the best model for overdispersion using the library "*AER*" (Kleiber and Zeileis 2008) in R version 3.0.2 (R Core Team 2013).

Results

Arm girth was strongly sexually dimorphic (Fig. 1a); there was a significant effect of sex ($F_{1,498} = 1830.1$, $P < 0.0001$) and a significant interaction effect between sex and body size ($F_{1,498} = 169.5$, $P < 0.0001$) on arm girth. Moreover, in males the exponential model ($AIC_c = 482.8$) provided a better fit than a linear ($AIC_c = 541.9$) or a second degree polynomial model ($AIC_c =$

485.8) for the allometric relationship between arm girth and body size. Therefore, hereafter relative arm girth was measured as the residual from the exponential relationship (Fig. 1a).

We recorded the mating tactic of 314 male *C. georgiana* that were found in choruses. The silent mating tactic was the most common behavior (64% of all males observed) employed by males in choruses. The best minimal model describing the probability of calling to attract females only included the effect of a male's snout-vent length (Table 1, Fig. 2), indicating that body size positively influenced calling probability (Fig. 2). There was no evidence for effects of male density, relative arm girth, or any interaction between these variables on the probability that a male was calling (Table 1).

Of the 439 males found, 125 mated successfully, either by amplexing a female on their own, or by joining a multimale amplexus (Fig. 1b). The best minimal model that described mating success included the effects of relative arm girth, male density, and the interaction between relative arm girth and male density (Table 2, Fig. 3). The relative importance of this interaction (sensu Burnham and Anderson 2002) was revealed by the sum of the Akaike weights of models that included this parameter, resulting in a relative importance of 81.5% (Table 2). A visual inspection of predicted values indicates that mating success was higher for males with relatively thicker arms in lower male densities, but this relationship completely reversed in high densities, where males with thicker arms had lower mating success (Fig. 3).

We observed a total of 33 multimale and 35 single-male matings. The number of males involved in a multimale amplexus varied from two to nine (Fig. 1b; mean of three). There was no single, best, minimal model describing the probability that a given mating transitioned to multimale amplexus (Table 3). Instead, two simple models were both ambiguously within a ΔAIC_c of less than 2 from the best model, one of them including only the effect of the males' snout-vent length, and the other including only the effect of male density (Table 3; Fig. 4). However, the relative importance (sensu Burnham and Anderson 2002) of male density (*MD*; 82.4%) was higher than the relative importance of snout-vent length (*SL*, 77.5%) for explaining the probability of multimale amplexus. Finally, male density had a significant effect on the number of males involved in a mating (Fig. 5), because the model with the fixed effect of male density had a lower AIC_c than the null model ($\Delta AIC_c = 6.60$).

Discussion

Recent theoretical models of sperm competition have addressed explicitly how male expenditure on weaponry used in direct contest competition for access to mating opportunities should affect male expenditure on the ejaculate (Parker et al. 2013). The model predictions depend critically on the relationship between

Table 2. Multimodel inference for the effects of body size (snout-vent length, *SL*), relative arm girth (*rAG*), and male density (*MD*) on the probability that a male of the quacking frog *Crinia georgiana* is involved in a mating, as an analysis of mating success.

No.	Fixed effects	k	AIC_c	ΔAIC_c	Weight	Log-likelihood	Cumulative weight
1	**MD + rAG + (MD × rAG)**	**5**	**475.43**	**—**	**0.28**	**−232.65**	**0.28**
2	SL + MD + rAG + (MD × rAG) + (SL × MD)	7	476.08	0.65	0.20	−230.91	0.48
3	SL + MD + rAG + (MD × rAG)	6	477.11	1.68	0.12	−232.46	0.60
4	SL + MD + rAG + (MD × rAG) + (SL × MD) + (SL × rAG)	8	477.51	2.08	0.10	−230.59	0.70
5	SL + MD + rAG + (MD × rAG) + (SL × rAG)	7	478.08	2.65	0.07	−231.91	0.77
6	MD + rAG	4	478.93	3.50	0.05	−235.42	0.82
7	SL + MD + rAG + (SL × MD) + (SL × rAG) + (MD × rAG) + (SL × MD × rAG)	9	479.20	3.77	0.04	−230.39	0.86
8	MD	3	480.11	4.68	0.03	−237.03	0.89
9	SL + MD + rAG	5	480.54	5.11	0.02	−235.20	0.91
10	SL + MD + rAG + (SL × rAG)	6	480.60	5.16	0.02	−234.20	0.93
11	SL + MD + rAG + (SL × MD)	6	480.68	5.25	0.02	−234.24	0.95
12	SL + MD + rAG + (SL × MD) + (SL × rAG)	7	480.80	5.37	0.02	−233.27	0.97
13	SL + MD + (SL × MD)	5	481.43	6.00	0.01	−235.65	0.99
14	SL + MD	4	481.55	6.11	0.01	−236.73	1.00
15	rAG	3	491.66	16.23	0.00	−242.80	1.00
16	SL + rAG	4	492.06	16.63	0.00	−241.98	1.00
17	Null	2	492.35	16.92	0.00	−244.16	1.00
18	SL	3	492.41	16.98	0.00	−243.18	1.00
19	SL + rAG + (SL × rAG)	5	492.50	17.07	0.00	−241.18	1.00

All models have the random effect of quadrat identity, because male density was measured in quadrats of 1 m², and hence measures are the same for males found in the same quadrat. We ranked models by increasing AIC_c values. "ΔAIC_c" is the difference between the AIC_c of each model and the AIC_c of model 1, "Weight" is the Akaike weight, *k* is the number of estimable parameters, "Log-likelihood" is the natural logarithm of the maximum likelihood, and "Cumulative weight" is the cumulative Akaike weight of each model and the models above it. The most parsimonious models are indicated in shaded rows, and the best minimal model is in bold.

the number of males competing for mating opportunities, and the competitive advantage gained from expenditure on weapons. However, empirical studies of this relationship from natural populations of animals are rare (Parker et al. 2013). We have shown that among natural populations of chorusing frogs, *C. georgiana*, male density is negatively associated with the selective advantage of arm strength used in obtaining matings, and positively associated with both the risk (probability of multimale amplexus) and intensity (number of males involved in amplexus) of sperm competition.

Sexual dimorphism in arm morphology is widespread in anuran amphibians, and has been taken as evidence of sexual selection acting on male grasping ability during amplexus and/or direct male–male combat (Lee 2001; Wells 2007). We found significant sexual dimorphism in the girth of the arms of *C. georgiana*, and in their scaling relationship with body size. Male arm girth, which reflects the development and thus strength of the flexor muscles (Oka et al. 1984; Navas and James 2007), was greater than female arm girth and large males had relatively greater arm girths than small males. Moreover, we found density-dependent selec-

tion acting on male arm girth. Our study of *C. georgiana* thus provides the first quantitative estimate of the pattern and strength of sexual selection acting on male arms in an anuran amphibian.

Males with relatively thicker arms were more likely to be involved in matings when the density of surrounding males was low. There are two possible mechanisms that could account for density-dependent selection on male arm girth. First, males with greater arm girth might be better able to repel silent males from their territories through the intense wrestling matches that have been described in this species (Byrne and Roberts 2004). Success in male–male combat might thus increase a male's chance of amplexing a female that moves into his exclusive territory. However, the ability of males to monopolize females is expected to decline with increasing male density (Knell 2009), as males become increasingly unable to displace a growing number of silent males from their territories. Indeed, increased male density was associated with increased probability of multimale amplexus and increased numbers of males amplexing. The positive relationship between relative arm girth and mating success at low male density was completely reversed at high male density, suggesting
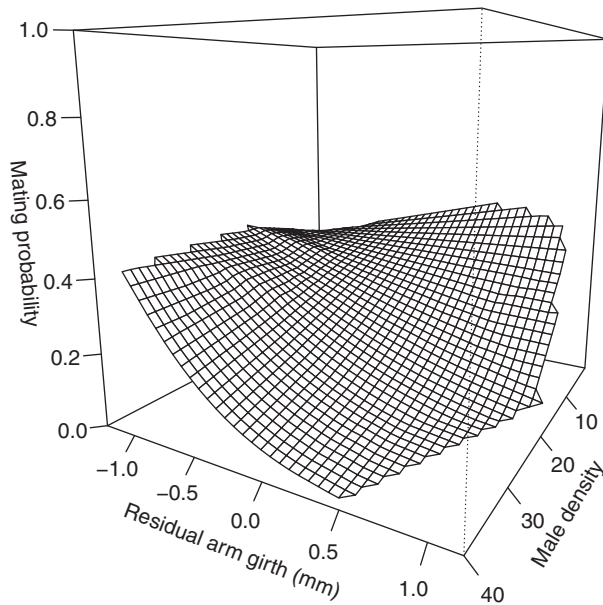
Figure 3. The mating success of males of the Australian quacking frog *Crinia georgiana* depends on an interaction between their relative arm girth (*rAG*, see methods for how this was estimated) and local male density (*MD*). Our results indicate that mating probability increased with *rAG* in low densities of males, but decreased sharply with *rAG* in high densities. At the same time, whereas *MD* had a strong negative effect on the mating probability of males with thick arms, *MD* had little effect on the mating probability of males with thin arms. Predicted probabilities (curved plane) were generated with a generalized linear model with *rAG*, *MD*, and their interaction as fixed effects, equivalent to model no. 1 in Table 2, but without random effects, to allow a 3D visualization of the interaction between *rAG* and *MD*.

that investment in arm strength at high density may even hamper a male's ability to engage in multimale amplexus. Second, female mate choice could contribute to the effect if females preferentially mate with males bearing relatively thicker arms, and if female preference is also more effective at low densities. Females sometimes initiate amplexus in *C. georgiana* by moving under calling males (Byrne and Roberts 2004), suggesting the potential for female choice. A previous study found that significantly more matings are initiated by the female being intercepted by silent males in high densities (68.4%) than in low densities (31.6%), whereas female initiation of mating by moving under a calling male occurs more often in low densities (72.2%) than in high densities (27.8%; Byrne and Roberts 2004). From the patterns of selection we have observed in natural populations of this frog, we conclude that the competitive advantage gained by males from expenditure on arm strength decreases with the density of competing males.

Consistent with previous studies of this species (Byrne and Roberts 2004), male density was found to be positively associ-

ated with the risk and intensity of sperm competition. Byrne and Roberts (2004) reported a negative effect of male density on the proportion of time male *C. georgiana* spend calling. One interpretation of this pattern is that as male density rises, an increasing number of males adopt an alternative tactic of intercepting females and engaging in sperm competition with males that attract females by calling. Although there was no independent effect of male density on the probability of calling in our dataset, there was an effect of male body size; large males were more likely to call and small males more likely to remain silent. Relative arm girth did not influence whether a male was found calling or adopting a silent tactic. Byrne and Roberts (2004) data likewise suggest that large and small males might adopt alternative tactics of calling and silently searching for mating opportunities. Interestingly, Dziminski et al. (2010b) found that smaller males produced ejaculates of higher quality than large males. Sperm competition theory predicts a pattern of differential expenditure on the ejaculate where one tactic specializes on gaining reproductive success through sperm competition (Parker 1990), a prediction for which there is some evidence (Vladic and Järvim 2001; Simmons et al. 2007; Smith and Ryan 2011).

Our data on the probability of being involved in multimale amplexus can shed light on the potential role that arm strength might play in postcopulatory sexual selection in *C. georgiana*. For example, arm girth could potentially increase the probability of mating singly, if stronger males were better equipped to dislodge rival males that attempt to join an amplexus, and hence avoid sperm competition. Thus, intuitively, we might expect arm girth to be more important for mating success at higher densities. However, only male density or body size affected the probability that a mating would involve multiple males, and there was no evidence for an independent effect of relative arm girth on the probability of a mating transitioning to multimale amplexus (Table 3). Thus, while expenditure on arm girth provides males with a competitive advantage in gaining access to mating opportunities at low male densities, it does not help them to avoid sperm competition when male densities are high.

Our estimate of selection acting on arm girth is based on mating success, and it is clear that mating success may not translate into reproductive success because risk and intensity of sperm competition will determine a male's ultimate share in paternity. Postcopulatory sexual selection has the potential to either amplify or attenuate precopulatory sexual selection (Kvarnemo and Simmons 2013). Considering only males involved in mating, and therefore postcopulatory competition, and assuming that paternity is shared equally among males involved in a given spawning event, there does not appear to be selection acting on arm girth during postcopulatory selection that would alter the strength of precopulatory selection acting via mating success (Fig. 6). However, currently we do not know how a male's amplexus position

Table 3. Multimodel inference for the effects of body size (snout-vent length, *SL*), relative arm girth (*rAG*), and male density (*MD*) on the probability that a mated male of the quacking frog *Crinia georgiana* is involved in a multimale amplexus.

No.	Fixed effects	k	AIC_c	ΔAIC_c	Weight	Log-likelihood	Cumulative weight
1	*SL + MD*	5	118.16	—	0.20	−53.83	0.20
2	***MD***	**4**	**119.41**	**1.25**	**0.10**	**−55.54**	**0.30**
3	*SL + MD + rAG*	6	119.79	1.63	0.09	−53.54	0.39
4	*SL + MD + rAG + (MD × rAG)*	7	119.93	1.77	0.08	−52.49	0.47
5	*SL + MD + (SL × MD)*	6	120.11	1.95	0.07	−53.70	0.54
6	***SL***	**4**	**120.14**	**1.98**	**0.07**	**−55.90**	**0.61**
7	*SL + MD + rAG + (MD × rAG) + (SL × rAG)*	8	120.71	2.55	0.05	−51.73	0.67
8	*SL + MD + rAG + (SL × rAG)*	7	121.07	2.91	0.05	−53.06	0.72
9	*MD + rAG*	5	121.36	3.21	0.04	−55.43	0.75
10	*Null*	3	121.60	3.44	0.04	−57.70	0.79
11	*SL + rAG*	5	121.64	3.48	0.03	−55.57	0.82
12	*SL + MD + rAG + (SL × MD) + (MD × rAG)*	8	121.77	3.61	0.03	−52.26	0.86
13	*MD + rAG + (MD × rAG)*	6	121.79	3.64	0.03	−54.54	0.86
14	*SL + MD + rAG + (SL × MD)*	7	121.85	3.69	0.03	−53.45	0.92
15	*SL + MD + rAG + (MD × rAG) + (SL × MD) + (SL × rAG)*	9	122.59	4.43	0.02	−51.51	0.94
16	*SL + rAG + (SL × rAG)*	6	122.74	4.58	0.02	−55.01	0.96
17	*SL + MD + rAG + (SL × MD) + (SL × rAG)*	8	123.00	4.84	0.02	−52.88	0.98
18	*rAG*	4	123.50	5.34	0.01	−57.58	0.99
19	*SL + MD + rAG + (SL × MD) + (SL × rAG) + (MD × rAG) + (SL × MD × rAG)*	10	124.33	6.17	0.01	−51.20	1.00

All models have the random effect of quadrat identity, because male density was measured in quadrats of 1 m², and hence measures are the same for males found in the same quadrat. We ranked models by increasing AIC_c values. "ΔAIC_c" is the difference between the AIC_c of each model and the AIC_c of model 1, "Weight" is the Akaike weight, *k* is the number of estimable parameters, "Log-likelihood" is the natural logarithm of the maximum likelihood, and "Cumulative weight" is the cumulative Akaike weight of each model and the models above it. The most parsimonious models are indicated in shaded rows, and the best minimal models are in bold.

might influence his chances of paternity or whether arm girth might, for example, allow males to control access to more favorable fertilization positions in a multimale spawning.

Comparative analyses, from insects to mammals, are revealing patterns of negative covariation between expenditure on weapons for acquiring mates and sperm production for winning fertilizations (Fitzpatrick et al. 2012; Lüpold et al. 2014; Dines et al., 2015). Such macroevolutionary patterns are predicted from opposing patterns of selection acting on weapons and testes. However, within species studies that have documented both pre- and postcopulatory selection acting simultaneously on male weapons and ejaculate expenditure are rare. The only study of which we are aware is Preston et al.'s (2003) study of Soay sheep. They showed that the relative selective advantage of horn length and testes size was dependent on the availability of estrus ewes. Males with longer horns had a greater siring success when the availability of estrus ewes was low and long-horned males could monopolize access to ewes via direct male–male competition. However, as the availability of ewes increased, an effect of testis size on siring success emerged and became stronger because dominant

males became increasingly unable to monopolize access to ewes. Thus, the importance for male reproductive success of male combat relative to sperm competition declined with increasing female availability (Preston et al. 2003). Unlike Preston et al. (2003), we are currently unable to provide quantitative estimates of selection acting concurrently on arm girth and testis size based on siring success. This will require paternity data from clutches produced during group spawnings to confirm how episodes of pre- and postcopulatory selection interact to favor male expenditure on arm girth.

In conclusion, sperm competition games that incorporate male contest competition predict that when the competitive advantage gained from expenditure on weaponry is negatively associated with the number of males competing for mates, as we have found for *C. georgiana*, then increases in the level of sperm competition should favor a decreased investment in armament and an increased expenditure on the ejaculate (Parker et al. 2013). This prediction is supported by negative among-population covariation between arm girth and testes mass in *C. georgiana*; across 10 geographically isolated populations, the density of
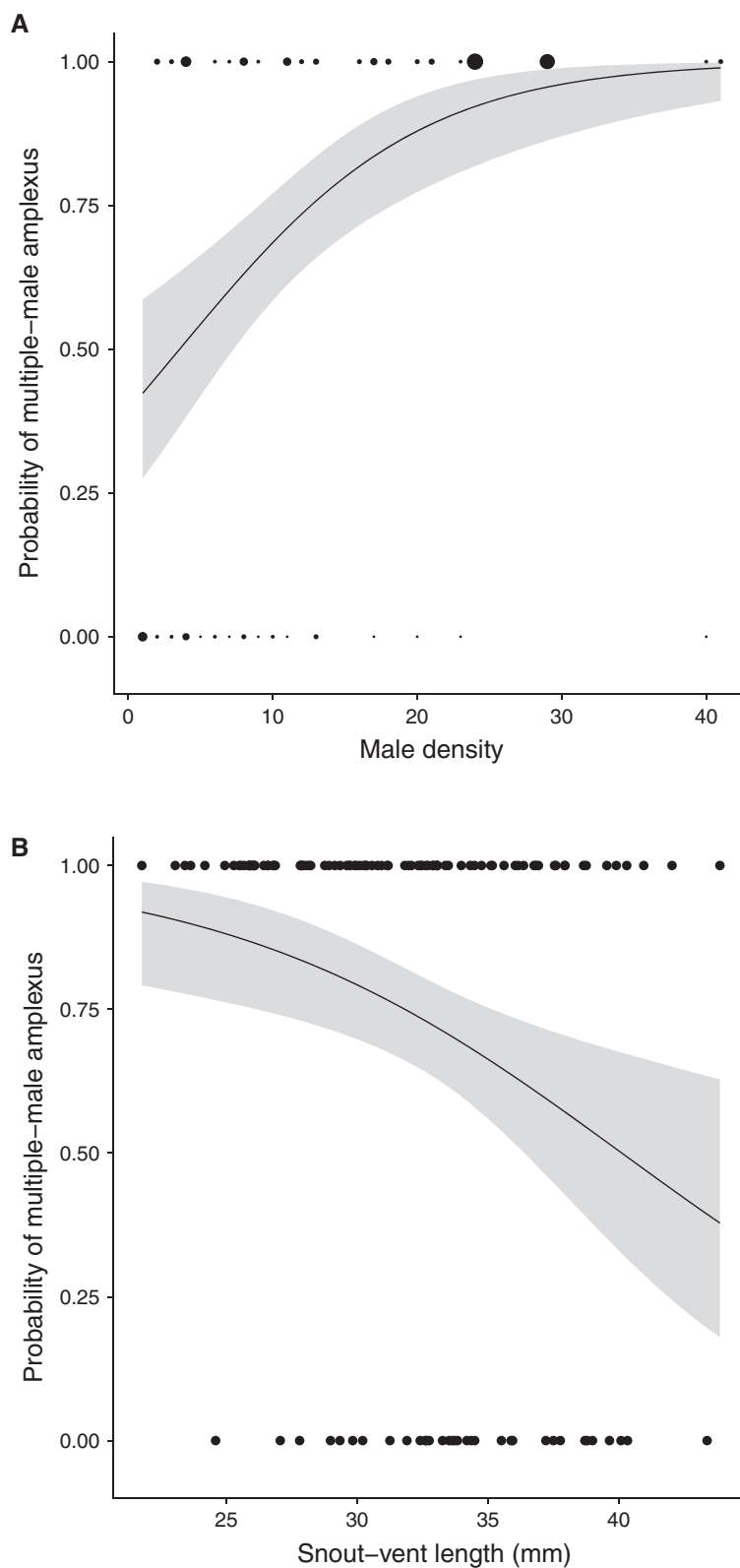
Figure 4. Females of the Australian quacking frog *Crinia georgiana* can mate with only one male at a time (single-male amplexus, coded 0), or with up to nine different males, resulting in polyandry (multimale amplexus, coded 1). Our results indicate that the probability of a multimale amplexus (A) increases with male density (*MD*) and (B) decreases with body size (measured as snout-vent length; *SL*). Predicted probabilities (curve) and 95% confidence intervals (shaded area) were generated with generalized linear models with only *MD* or with only *SL* as a fixed effect, equivalent to models no. 2 and no. 6 in Table 3, but without random effects.
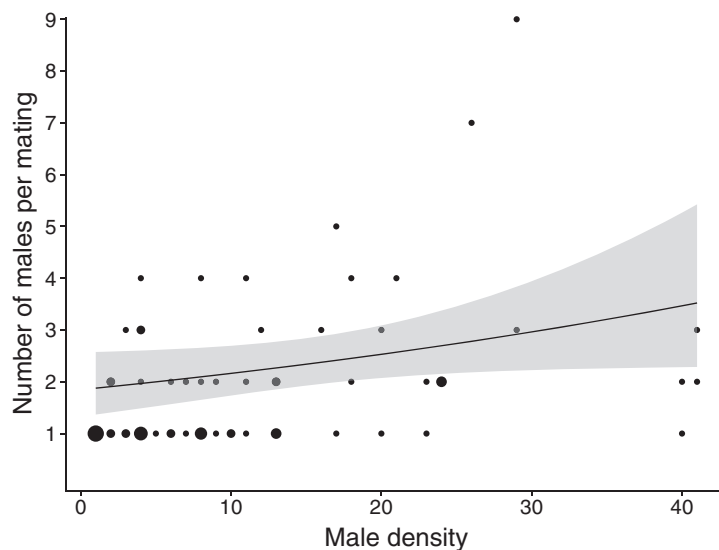
Figure 5. Matings in the Australian quacking frog *Crinia georgiana* can involve from one to up to nine males, and male density *MD* has a positive effect on the number of males involved in a mating. Predicted probability and 95% confidence intervals (shaded area) were generated with a generalized linear model with only *MD* as a fixed effect, without random effects.
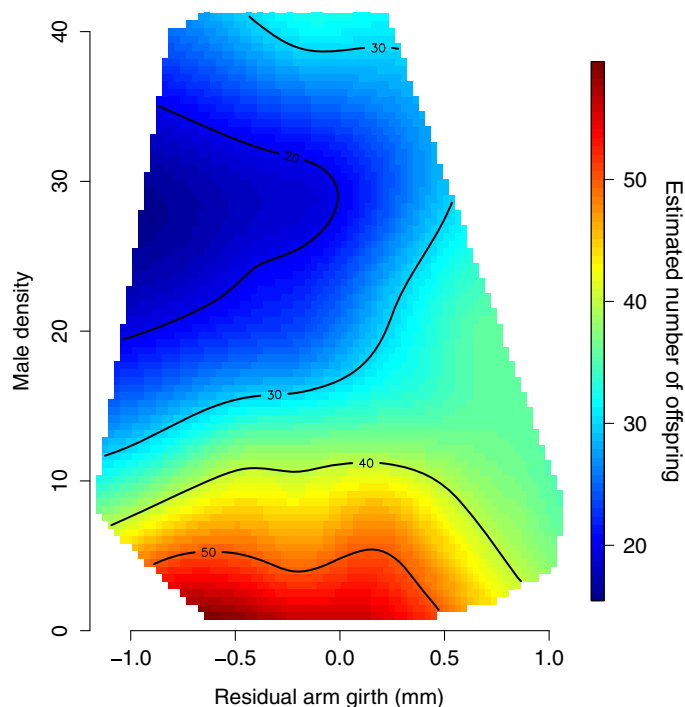

Figure 6. The estimated number of offspring sired by mated males of the Australian quacking frog *Crinia georgiana,* assuming that females lay clutches of 70 eggs on average (Byrne et al. 2002), and that paternity is shared equally among males involved in a multimale amplexus (Dziminski et al. 2010a). The fitness surface is a nonparametric thin plate spline for males of different relative arm girths (*rAG*) and at different male densities varying from 1 to 40 males/m^2.

males in choruses has been found to be negatively associated with arm size, but positively associated with testis mass and sperm production (Dziminski et al. 2010a; Parker et al. 2013). It remains to be seen whether this pattern of covariation represents phenotypic plasticity in male allocation to armaments and ejaculates in response to annually fluctuating chorus densities, or is the result of evolutionary divergence among populations due to consistent differences in chorus densities across generations.

DATA ARCHIVING
The doi for our data is doi: 10.5061/dryad.cr2n4.

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Associate Editor: R. Rodriguez
Handling Editor: M. Servedio