doi:10.1111/j.1558-5646.2011.01343.x

# POLYANDRY REDUCES SPERM LENGTH VARIATION IN SOCIAL INSECTS

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Received January 29, 2011
Accepted April 20, 2011

**Postcopulatory sexual selection, either in the form of sperm competition or cryptic female choice, is an important selective force that is thought to have generated the enormous variation in sperm morphology observed interspecifically. However, the evolutionary significance of intraspecific variation in sperm morphology, and the role that postcopulatory sexual selection plays in influencing this variation, remains poorly investigated in invertebrates. Here, we tested the hypothesis that postcopulatory sexual selection reduces variation in sperm morphology, both between and within males, in 27 species of eusocial ants and bees. These eusocial species offer an unusual opportunity to assess how selection acts on variance in sperm morphology, as haploid males produce clonal, haploid sperm that does not experience haploid–diploid conflict. We provide solid evidence that males of polyandrous ant and bee species indeed produce less–variable sperm, indicating that sperm competition selected for sperm of superior quality. Our results offer a mechanistic explanation for the evolution of high–quality sperm and provide comprehensive evidence that sperm morphology of social insects is influenced by sexual selection.**

**KEY WORDS:** **Cryptic female choice, postcopulatory sexual selection, sperm competition, sperm length, sperm morphology.**

Sperm and pollen morphology remains a conundrum for evolutionary biologists (Birkhead and Moller 1998; Birkhead et al. 2009). Although sperm are among the most specialized eukaryotic cells known, they exhibit incredible morphological diversity (Jamieson et al. 1999; Pitnick et al. 2009a). This enormous variation in sperm morphology is assumed to evolve in response to variation in postcopulatory sexual selection (Snook 2005; Birkhead et al. 2009), either through sperm competition, the contest between sperm of rival males to fertilize eggs (Parker 1970), or cryptic female choice, where females bias paternity toward preferred males (Eberhard 1996). Indeed, a substantial number of comparative studies have demonstrated effects of postcopulatory sexual selection on the evolution of sperm traits (e.g., Briskie and Montgomerie 1992; Gage 1994; Anderson et al. 2005; Immler and Birkhead 2007; Gomendio and Roldan 2008; Fitzpatrick et al. 2009; Tourmente et al. 2009, 2011). In contrast, the evolutionary significance of the variation in sperm morphology commonly observed, both within species and within males, has received much less attention (Ward 1998; Morrow and Gage 2001; Pitnick et al. 2009a). Postcopulatory sexual selection is hypothesized to reduce variance in sperm traits by favoring an optimal sperm morphology that enhances a male's reproductive success during competitive matings (Parker 1993; Parker and Begon 1993; Birkhead et al. 2005). Recent analyses provided the first evidence supporting this hypothesis, demonstrating reduced variation in sperm morphology in more promiscuous passerine birds species (Calhim et al. 2007; Immler et al. 2008; Kleven et al. 2008). Similarly, reduced variance in sperm traits has also been reported in mammals (Suttle et al. 1988; Breed et al. 2007; Thitipramote et al. 2010), although these studies examined relatively few species and did not take phylogenetic effects into account. However, it remains unclear if a reduction in variance of sperm morphology in the

presence of postcopulatory sexual selection is a general evolutionary phenomenon.

If sperm morphology evolves toward a single phenotype to perform optimally within the arena of postcopulatory sexual selection, variance in sperm morphology should be continuously removed through directional or stabilizing selection (Calhim et al. 2007; Pizzari and Parker 2009). Consequently, explaining the enormous variation typically observed in sperm morphology is a major challenge for evolutionary biologists (Morrow and Gage 2001; Pitnick et al. 2009a). One possible explanation is that variation in sperm morphology is generated continuously by developmental errors during spermatogenesis and postcopulatory sexual selection selects to improve the efficiency of the sperm–producing machinery (Hunter and Birkhead 2002). In this scenario, selection is expected to reduce variance in sperm traits in promiscuous species because paternity success is enhanced when males produce sperm with fewer developmental errors. An alternative, not mutually exclusive, hypothesis is that variation in ejaculate traits result from genomic conflicts when the evolutionary optima for sperm traits differ between the diploid soma and its haploid sperm (Parker 1993; Parker and Begon 1993). Such haploid–diploid conflict may generate variance in sperm traits if haploid gene expression influences sperm morphology (Joseph and Kirkpatrick 2004; Immler 2008; Pizzari and Foster 2008; but see Pitnick et al. 2009b). However, the extent to which ejaculate traits are shaped by haploid gene expression remains unclear (Joseph and Kirkpatrick 2004). Nevertheless, in polyandrous species with intense postcopulatory sexual selection, evolution toward an optimum sperm morphology is expected, irrespective of whether sperm production is under haploid or diploid control (Parker and Begon 1993). In most species studied to date, distinguishing between the evolutionary forces generating variation in sperm morphology has been extremely challenging, as it is difficult to manipulate the potential processes that are predicted to influence the extent of variance in sperm traits.

In this study, we examined the relationship between variance in sperm morphology and postcopulatory sexual selection in 27 species of eusocial bees and ants. In both groups, we collected sperm morphology data from more basal species, characterized by societies with single paternity, as well as from several derived species with exceptionally high paternity frequencies and empirical evidence for postcopulatory sexual selection (den Boer et al. 2010). Consequently, we predicted broad variance in evolutionary pressures arising from postcopulatory sexual selection on sperm morphology between the species investigated. In all the species examined, reproductive females (queens) mate during a short period at the beginning of their lives and store sperm for prolonged periods of time (Baer 2003, 2005). The absence of female remating later in life, together with the enormous need for sperm to be stored and prudently allocated over the queens lifetime (e.g., den Boer et al. 2009), generates intense selective pressure on sperm phenotype to remain in storage while competing with rival sperm during the sperm storage process. This postcopulatory competition is particularly intense in eusocial insects because females can store sperm for up to several decades and more than one sperm is used for each fertilization (Baer 2005; den Boer et al. 2009). All males of the eusocial hymenopterans are haploid, originating from nonfertilized queen eggs. Consequently, males produce clonal sperm resulting in the absence of intraejaculatory sperm competition and haploid–diploid conflicts (Baer 2003). Thus, in male social insects the fitness interests of the adult and its gametes are fully aligned. By examining species with different genetic systems, such as haplodiploids examined here, our aim was to assess how postcopulatory sexual selection influences variation in sperm traits in species where one of the factors thought to generate variation in sperm morphology, namely haploid–diploid conflict, is absent (Pizzari and Foster 2008; Pizzari and Parker 2009). Our phylogenetic analyses reveal a negative relationship between variation in sperm size and paternity frequencies, indicating that sexual selection has shaped sperm length evolution in social hymenopteran insects.

## Methods

### DATA COLLECTION

We combined and reanalyzed datasets from 27 eusocial hymenopterans, comprising 19 species of ants and eight species of bees (see Supporting information for species names and data used in our analyses). Sperm were collected from males of each species (mean number of males per species ± SE: 48.7 ± 7.5, range 5–107) following the methods described in Baer and Schmid–Hempel (2000) and Baer and Boomsma (2004, 2006). Briefly, males were killed and accessory testes were removed and punctured. A subsample of outflowing sperm was collected and smeared onto a microscope slide and air–dried. Mean sperm values were obtained for each individual male by measuring 10 sperm/male for all ant species and bumblebee species. For *Apis* honey bee species 20 sperm/male were measured. However, to ensure that our analyses were not influenced by this extra sampling effort in *Apis* bees we randomly selected 10 sperm/male from each of the *Apis* bee species examined and used these values in our analyses. We obtain qualitatively similar results when we instead used data from all 20 sperm/male collected from *Apis* been in analyses (data not shown). Sperm were measured digitally using the program ImageJ (available at http://rsbweb.nih.gov/ij/). We focused on total sperm length, as the sperm morphology of the species used in our analyses does not allow for clear distinctions between different sperm components (i.e., head, midpiece and flagellum). All sperm samples were collected and measured by the same researcher.

We first quantified variation in sperm length by calculating the coefficient of variation (CV = [standard deviation/mean] × 100) in total sperm length between–males and within–males for each species. Although CV has been used previously as a standard measure of variance in sperm traits (e.g., Malo et al. 2006; Calhim et al. 2007; Immler et al. 2008; Kleven et al. 2008), we were concerned about statistical issues arising when using CV to estimate variance. In particular, variance values are likely to increase with increasing mean values, as is the case when measuring the mean and standard deviation in sperm length between males and within males in this study (between–males: linear regression, $r = 0.48$, $P = 0.01$; within–males: $r = 0.81$, $P < 0.001$). Additionally, the use of ratios, such as CV, in statistical analyses can lead to biased results unless the mean–variance relationship is isometric (intercept of zero and slope of one, Tomkins and Simmons 2002), which we found not to be the case in our sample (slope = 0.55 and intercept = −0.59). Therefore, to evaluate between–male and within–male variance in sperm length, we performed multiple regressions to assess the relationship between the standard deviation of sperm length and queen paternity frequencies while controlling for mean–variance relationships by adding mean sperm length as a variable to our models. We used the standard deviation of mean sperm length for each species in our between–male analyses and the mean of the standard deviation for each male examined per species for our within–male analyses.

We used three different estimates for the strength of postcopulatory sexual selection. First, we classified species as monandrous (queens produce offspring from a single male) or polyandrous (queens produce offspring of multiple patrilines) mating systems (see Supporting information for additional information). Second, we used the observed paternity frequency, which is the actual number of patrilines found within a queen's helper offspring. Third, we used the effective paternity frequency, a measure of the relative contribution of each male within offspring. Data on observed and effective paternity frequencies for all species used for our analyses were collected from the literature (see Supporting information).

### PHYLOGENETIC ANALYSES

We performed phylogenetically controlled generalized least–squared (GLS) regressions to account for evolutionary relationships between species (Freckleton et al. 2002). GLS regressions use maximum likelihood–ratio tests to estimate a phylogenetic association parameter, λ, which assesses the degree of phylogenetic dependence in the data ($\lambda = 0$: phylogenetic independence, $\lambda = 1$: phylogenetic dependence, Pagel 1999; Freckleton et al. 2002). To account for multiple tests using the same variables, while avoiding the higher probabilities of committing type II errors associated with Bonferroni corrections (Nakagawa 2004), we established the strength of the relationship between variance in sperm length and predictor variables by calculating effect sizes, $r$, and noncentral 95% confidence intervals (CI) for $r$, from $t$ values generated from GLS regressions. All analyses were performed in the statistical package R version 2.10.1 (R Foundation for Statistical Computing 2009). A composite phylogeny for the species used in our analysis was constructed from published phylogenies (Hughes et al. 2008; Schultz and Brady 2008). Additional phylogenetic information needed for some of the ant species was collected from Bacci et al. (2009) and Baer et al. (2009). All branch lengths were set equal to one and all data were log–transformed prior to phylogenetic analyses.

## Results

Males from polyandrous species of ants and bees where queens produced offspring of multiple paternities had less–variable sperm, both between males ejaculates and within each males ejaculate, compared to males of monandrous species where queens produced offspring from a single male (Table 1A). Across species, we found a negative association between sperm variation and paternity frequencies that was remarkably robust (Fig. 1, see Table 1B and C for a summary of different models): reduced variance in sperm length was found when examining between–male or within–male CV of sperm length, as well as when the between–male or within–male standard deviation of sperm length was entered into our models that controlled for mean sperm length, and independently of whether we used observed or effective paternity frequencies (Table 1B). Variance in sperm length was also negatively associated with paternity frequency when we only considered those species where queens are polyandrous (Fig. 1, Table 1C). Again, these results remained significant when we assessed between–male and within–male variance in sperm length and both measures of postcopulatory sexual selection in all of our subsequent models (Table 1C).

On the interspecific level, we did not detect any significant relationship between mean sperm length and either measure of paternity frequency (Table 1).

## Discussion

Our results provide solid evidence that variation in sperm length, both between–males and within–males, is negatively related with paternity frequencies, a proxy measure of postcopulatory sexual selection, in ants and bees. Thus, haploid, clonal sperm of social insects exhibits substantial between–male and within–male variation in sperm morphology in the absence of postcopulatory selective forces that reduced such variation. These results support Birkhead et al.'s (2005) hypothesis that

**Table 1.** Phylogenetically controlled GLS regressions and multiple regressions between measures of variance in sperm length and postcopulatory sexual selection. Results are divided into analyses performed when (A) comparing of variance in sperm length between mating systems (monandrous vs. polyandrous species), and (B) when assessing the relationship between sperm length and variance in sperm length and paternity frequencies using data from all species and (C) data from polyandrous species only. Variance in sperm length between-males and within-males was assessed using the coefficient of variation (Between CV and Within CV) and the standard deviation (Between Stdev and Within Stdev). We used two measures of paternity frequency: the observed paternity frequency (Obs. pat. freq.) and the effective paternity frequency (Eff. pat. freq.). The superscripts after the phylogenetic scaling parameter, λ, indicate if the λ value was significantly different than 0 (first position) and 1 (second position) in likelihood ratio tests. Nonsignificant values are indicated with "ns" and significant ($P < 0.05$) values are indicated by "∗." The effect size, *r*, degrees of freedom, and the noncentral 95% confidence limits (CL) are presented for each model. CLs are considered significant when the range of values do not overlap zero. Significant relationships are presented in bold text.

| Trait | λ | Predictor | Slope ± SE | *t* | *P* | *r* | df | CI |
|---|---|---|---|---|---|---|---|---|
| **(A) Comparisons of sperm length variation between monandrous and polyandrous species** | | | | | | | | |
| Between CV | $0.05^{ns,*}$ | Mating system | −0.20 ± 0.07 | −2.82 | **0.01** | −0.49 | 25 | **−0.70 to −0.14** |
| Between Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 0.78 ± 0.20 | 3.91 | **<0.001** | 0.62 | 24 | **0.32 to 0.78** |
| | | Mating system | −0.18 ± 0.07 | −2.37 | **0.03** | −0.44 | 24 | **−0.67 to −0.06** |
| Within CV | $1.0^{*,ns}$ | Mating Behaviour | −0.08 ± 0.03 | −2.40 | **0.02** | −0.43 | 25 | **−0.66 to −0.06** |
| Within Stdev | $1.0^{*,ns}$ | Mean sperm length | 0.91 ± 0.10 | 9.41 | **<0.001** | 0.89 | 24 | **0.78 to 0.93** |
| | | Mating system | −0.07 ± 0.03 | −2.25 | **0.03** | −0.42 | 24 | **−0.66 to −0.03** |
| **(B) Relationship between sperm traits and paternity frequencies: data from all species** | | | | | | | | |
| Sperm Length | $1.0^{*,ns}$ | Obs. Pat. freq. | 0.02 ± 0.07 | 0.31 | 0.76 | 0.62 | 25 | −0.31 to 0.41 |
| Sperm Length | $0.81^{*,ns}$ | Eff. pat. freq. | 0.04 ± 0.09 | 0.47 | 0.64 | 0.10 | 20 | −0.32 to 0.48 |
| Between CV | $< 0.001^{ns,*}$ | Obs. Pat. freq. | −0.27 ± 0.05 | −5.59 | **<0.001** | −0.75 | 25 | **−0.85 to −0.52** |
| Between CV | $< 0.001^{ns,*}$ | Eff. pat. freq. | −0.29 ± 0.06 | −5.04 | **<0.001** | −0.75 | 20 | **−0.86 to −0.49** |
| Between Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 0.92 ± 0.16 | 5.69 | **<0.001** | 0.76 | 24 | **0.54 to 0.86** |
| | | Obs. Pat. freq. | −0.26 ± 0.05 | −4.73 | **<0.001** | −0.69 | 24 | **−0.82 to −0.43** |
| Between Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 0.98 ± 0.19 | 5.17 | **<0.001** | 0.76 | 19 | **0.51 to 0.87** |
| | | Eff. pat. freq. | −0.28 ± 0.06 | −4.44 | **<0.001** | −0.71 | 19 | **−0.84 to −0.42** |
| Within CV | $0.82^{ns,ns}$ | Obs. Pat. freq. | −0.15 ± 0.03 | −5.49 | **<0.001** | −0.74 | 25 | **−0.85 to −0.51** |
| Within CV | $0.21^{ns,ns}$ | Eff. pat. freq. | −0.21 ± 0.02 | −8.65 | **<0.001** | −0.89 | 20 | **−0.94 to −0.76** |
| Within Stdev | $< 0.001^{ns,ns}$ | Mean sperm length | 0.87 ± 0.06 | 15.59 | **<0.001** | 0.95 | 24 | **0.91 to 0.97** |
| | | Obs. pat. freq. | −0.19 ± 0.02 | −10.24 | **<0.001** | −0.90 | 24 | **−0.94 to −0.81** |
| Within Stdev | $< 0.001^{ns,ns}$ | Mean sperm length | 0.87 ± 0.07 | 13.17 | **<0.001** | 0.95 | 19 | **0.89 to 0.97** |
| | | Eff. pat. freq. | −0.21 ± 0.02 | −9.50 | **<0.001** | −0.91 | 19 | **−0.95 to −0.80** |
| **(C) Relationship between sperm traits and paternity frequencies: data from polyandrous species only** | | | | | | | | |
| Sperm length | $0.80^{*,ns}$ | Obs. pat. freq. | −0.02 ± 0.10 | −0.18 | 0.86 | −0.06 | 9 | −0.58 to 0.51 |
| Sperm length | $0.78^{*,ns}$ | Eff. pat. freq. | −0.004 ± 0.10 | −0.04 | 0.97 | −0.01 | 8 | −0.58 to 0.56 |
| Between CV | $< 0.001^{ns,*}$ | Obs. pat. freq. | −0.37 ± 0.09 | −4.35 | **0.002** | −0.82 | 9 | **−0.92 to −0.45** |
| Between CV | $< 0.001^{ns,*}$ | Eff. pat. freq. | −0.34 ± 0.09 | −3.62 | **0.007** | −0.79 | 8 | **−0.91 to −0.31** |
| Between Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 1.03 ± 0.27 | 3.81 | **0.005** | 0.80 | 8 | **0.35 to 0.92** |
| | | Obs. pat. freq. | −0.37 ± 0.10 | −3.89 | **0.005** | −0.81 | 8 | **−0.92 to −0.37** |
| Between Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 1.02 ± 0.31 | 3.27 | **0.01** | 0.78 | 7 | **0.23 to 0.91** |
| | | Eff. pat. freq. | −0.35 ± 0.11 | −3.21 | **0.01** | −0.77 | 7 | **−0.91 to −0.22** |
| Within CV | $0.82^{ns,ns}$ | Obs. pat. freq. | −0.16 ± 0.05 | −3.42 | **0.008** | −0.75 | 9 | **−0.89 to −0.28** |
| Within CV | $< 0.01^{ns,ns}$ | Eff. pat. freq. | −0.21 ± 0.04 | −5.32 | **<0.001** | −0.88 | 8 | **−0.95 to −0.59** |
| Within Stdev | $< 0.001^{ns,ns}$ | Mean sperm length | 0.79 ± 0.11 | 7.39 | **<0.001** | 0.93 | 8 | **0.76 to 0.97** |
| | | Obs. pat. freq. | −0.20 ± 0.04 | −5.27 | **<0.001** | −0.88 | 8 | **−0.95 to −0.58** |
| Within Stdev | $< 0.001^{ns,*}$ | Mean sperm length | 0.80 ± 0.11 | 7.46 | **<0.001** | 0.94 | 7 | **0.76 to 0.98** |
| | | Eff. pat. freq. | −0.19 ± 0.04 | −5.15 | **0.001** | −0.89 | 7 | **−0.95 to −0.56** |

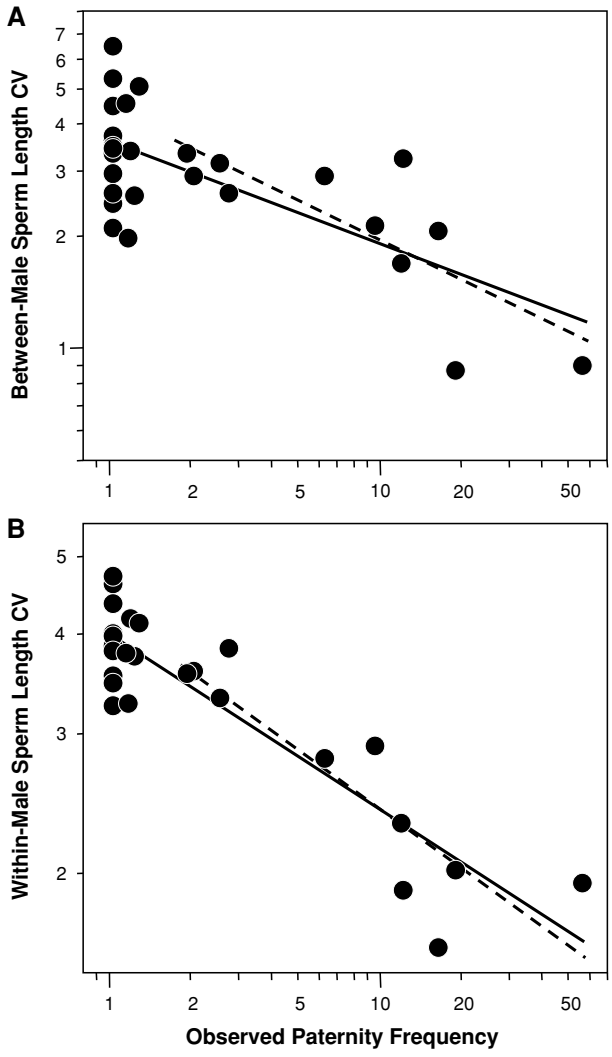


**Figure 1.** **The relationship between variation in sperm length and observed paternity frequencies. Variation in sperm length was measured using (A) between–male CV of total sperm length and (B) within–male CV of total sperm length. The solid line represents the slope of the relationship when all data were included in the analysis whereas the dashed line represents the slope when only polyandrous species were considered. Data in the figures are not controlled for phylogeny.**

postcopulatory sexual selection reduces variance in ejaculate traits and are consistent with previous findings of a negative relationship between intermale variation in sperm length and sperm competition risk in passerine birds (Calhim et al. 2007; Immler et al. 2008; Kleven et al. 2008). However, unlike in passerine birds (e.g., Briskie and Montgomerie 1992; Briskie et al. 1997), and many other taxa (reviewed in Gomendio and Roldan 2008; Montgomerie and Fitzpatrick 2009; Pitnick et al. 2009a), where postcopulatory sexual selection influences sperm length, we found no relationship between sperm length and female promiscuity (similar to Baer et al. 2009 on a smaller dataset). Thus, directional selection does not appear to be acting on sperm length in social insects but stabilizing selection appears to be a salient evolutionary force reducing intraspecific variance in sperm morphology.

Our results also provide insights into the factors that could influence variance in sperm morphology. In diploid organisms, despite evidence of postmeiotic gene expression in spermatozoa (e.g., Wang et al. 2001; Namekawa et al. 2006), sperm phenotypes appear to be determined largely by testicular, and therefore diploid, gene expression (Eddy 2002). Indeed, a recent study demonstrated that haploid gene expression does not influence sperm length variation in *Drosophila melanogaster* and *Scathophaga stercoraria* (Pitnick et al. 2009b). In contrast, in the haplodiploid species studied here, the lack of a diploid phase in the male's life cycle means that selection can act exclusively on haploid genes. Therefore, in male social insects the potential for selection on haploid genes that influence male reproductive success and spermatogenesis is greatly enhanced relative to organisms where the diploid phase predominates and the haploid phase is relatively short lived. Additionally, because sperm production is solely under haploid control in male social insects, the haploid–diploid conflict that can occur in most other sexually reproducing species, and is thought to influence variation in sperm morphology (Parker and Begon 1993), cannot be invoked to explain the reduced variance in sperm morphology that we detected in this study. Thus, by taking advantage of the haplodiploid genetic system that characterizes social insects, in this study we were able to rule out one of the key factors thought to generate variance in sperm traits. Instead, our results suggest that in social insects the increased selective pressures exerted by postcopulatory sexual selection lead to fewer developmental errors during spermatogenesis and/or enhanced maintenance of sperm (sensu Hunter and Birkhead 2002), a hypothesis that should be tested in the future.

The evolutionary reductions in sperm variance we detected indicate that sexual selection has shaped sperm length evolution in eusocial hymenopterans. However, the importance of sexual selection for the mating system evolution of kin–selected species is controversial. On the one hand, kin selection has been predicted to provide few evolutionary opportunities in eusocial insects (Boomsma, 2007), because single paternity is widespread (Hughes, 2008), and females (queens) never remate later in life and/or form monandrous partnerships for life (Boomsma et al. 2005). Furthermore, the evolution of extreme female fecundities is expected to select against any mating costs in eusocial insects, either on females or on competing ejaculates as caused by postcopulatory sexual selection (Boomsma et al. 2005). However, postcopulatory sexual selection, in the form of sperm incapacitation and cryptic female choice, is present in polyandrous ants and bees (den Boer et al. 2009) and we here add a second, independent study, providing evidence that sexual selection influenced

the evolution of sperm morphology in kin–selected species.

If sperm competition and cryptic female choice play a role in shaping ejaculate evolution in social insects, we need further studies to disentangle the relative influence of these selective forces. Given the prevalence of long–term sperm storage by queens and evidence that queens in some eusocial insects may differentially store sperm of a preferred morphology (Baer et al. 2003), the queen's reproductive tract should be an important selective arena for sperm in social insects (as is the case in other insects, e.g., Miller and Pitnick 2002). If queens use reproductive tracts to select sperm of preferred males for storage, competition between sperm from rival males for access to sperm storage sites will likely drive the evolution of less–variable sperm. Although the relationship between sperm variation and fitness remain unclear, recent evidence suggests that males suffer a reproductive cost when they produce more variable sperm. For example, in the flour beetle *Tribolium castaneum*, Michalczyk et al. (2010) recently showed that inbred males have reduced sperm competitive ability and produce more variable sperm. Determining how sperm variance influences fitness in social insects promises to be an exciting avenue for future research. More generally, social insect might offer unique opportunities to disentangle the consequences of variance in sperm morphology on sperm storage and paternity success, especially in species where queens can be artificially inseminated.

## ACKNOWLEDGMENTS

We thank R. Jaffé for his assistance with data collection and helpful discussion, J. Tomkins for helpful discussion and statistical advice and D. Nash for helpful discussion. This research was supported by the Australian Research Council (ARC) Discovery Program (QEII Fellowship to BB and APD Fellowship to JLF), and a Discovery Project to BB and the ARC Centre of Excellence in Plant Energy Biology.

## LITTERATURE CITED

Anderson, M. J., J. Nyholt, and A. F. Dixson. 2005. Sperm competition and the evolution of sperm midpiece volume in mammals. J. Zool. Lond. 267:135–142.

Bacci, M., Jr., S. E. Solomon, U. G. Mueller, V. G. Martins, A. O. R. Carvalho, L. G. E. Vieira, and A. C. O. Silva-Pinhati. 2009. Phylogeny of leafcutter ants in the genus *Atta* Fabricius (Formicidae: Attini) based on mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol. 51:427–437.

Baer, B. 2003. Bumblebees as model organisms to study male sexual selection in social insects. Behav. Ecol. Sociobiol. 54:521–533.

———. 2005. Sexual selection in *Apis* bees. Apidologie. 36:187–200.

Baer, B., and P. Schmid Hempel. 2000. The artificial insemination of bumblebee queens. Insectes Soc. 47:183–187.

Baer, B., and J. J. Boomsma. 2004. Male reproductive investment and queen mating frequency in fungus growing ants. Behav. Ecol. 15:426–432.

———. 2006. Mating biology of the leafcutting ants *Atta colombica* and *A. cephalotes*. J. Morphol. 267:1165–1171.

Baer, B., P. Schmid Hempel, J. T. Hoeg, and J. J. Boomsma. 2003. Sperm length, sperm storage and mating system characteristics in bumblebees. Insectes Soc. 50:101–108.

Baer, B., M. B. Dijkstra, U. G. Mueller, D. R. Nash, and J. J. Boomsma. 2009. Sperm length evolution in the fungus–growing ants. Behav. Ecol. 20:38–45.

Birkhead, T. R., and A. P. Møller, eds. 1998. Sperm competition and sexual selection. Academic Press, London, UK.

Birkhead, T. R., E. J. Pellatt, P. Brekke, R. Yeates, and H. Castillo–Juarez. 2005. Genetic effects on sperm design in the zebra finch. Nature 434:383–387.

Birkhead, T. R., D. J. Hosken, and S. Pitnick, eds. 2009. Sperm biology: an evolutionary perspective. Academic, Burlington, MA.

Boomsma, J. J. 2007. Kin selection versus sexual selection: why the ends do not meet. Curr. Biol. 17:673–683.

Boomsma, J. J., B. Baer, and J. Heinze. 2005. The evolution of male traits in social insects. Annu. Rev. Entomol. 50:395–420.

Breed, W. G., M. Bauer, R. Wade, N. Thitipramote, J. Suwajarat, and L. Yelland. 2007. Intra–individual variation in sperm tail length in murine rodents. J. Zool. 272:299–304.

Briskie, J. V., and R. Montgomerie. 1992. Sperm size and sperm competition in birds. Proc. R. Soc. Lond. B 247:89–95.

Briskie, J. V., R. Montgomerie, and T. R. Birkhead. 1997. The evolution of sperm size in birds. Evolution 51:937–945.

Calhim, S., S. Immler, and T. R. Birkhead. 2007. Postcopulatory sexual selection is associated with reduced variation in sperm morphology. PLoS ONE 2:e413.

den Boer, S. P. A., B. Baer, S. Dreier, S. Aron, D. R. Nash, and J. J. Boomsma. 2009. Prudent sperm use by leaf–cutter ant queens. Proc. R. Soc. Lond. B 276:3945–3953.

den Boer, S. P. A., B. Baer, and J. J. Boomsma. 2010. Seminal fluid mediates ejaculate competition in social insects. Science 327:1506–1509.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton Univ. Press, Princeton, NJ.

Eddy, E. M. 2002. Male germ cell gene expression. Rec. Prog. Horm. Res. 57:103–128.

Fitzpatrick, J., R. Montgomerie, J. K. Desjardins, K. A. Stiver, N. Kolm, and S. Balshine. 2009. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. Proc. Natl Acad. Sci. USA 106:1128–1132.

Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. Am. Nat. 160:712–725.

Gage, M. J. G. 1994. Associations between body size, mating pattern, testis size and sperm length across butterflies. Proc. R. Soc. Lond. B 258:247–254.

Gomendio, M., and E. R. S. Roldan. 2008. Implications of diversity in sperm size and function for sperm competition and fertility. Int. J. Dev. Biol. 52:439–447.

Hunter, F. M., and T. R. Birkhead. 2002. Sperm viability and sperm competition in insects. Curr. Biol. 12:121–123.

Hughes, W. O. H., B. P. Oldroyd, M. Beekman, and F. L. W. Ratnieks. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science. 320:1213–1216.

Immler, S. 2008. Sperm competition and sperm cooperation: the potential role of diploid and haploid expression. Reproduction 135:275–283.

Immler, S., and T. R. Birkhead. 2007. Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. Proc. R. Soc. Lond. B 274:561–568.

Immler S., S. Calhim, and T. R. Birkhead. 2008. Increased postcopulatory sexual selection reduces the intramale variation in sperm design. Evolution 62:1538–1543.

Jamieson, B. G. M., R. Dallai, and B. A. Afzelius, eds. 1999. Insects: their spermatozoa and phylogeny. Scientific Publishers, Enfield, NH.

Joseph, S. B., and M. Kirkpatrick. 2004. Haploid selection in animals. Trends Ecol. Evol. 19:592–597.

Kleven, O., T. Laskemoen, F. Fossøy, R. J. Robertson, and J. T. Lifjeld. 2008. Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. Evolution 62:494–499.

Malo, A. F., M. Gomendio, J. Garde, B. Lang–Lenton, A. J. Soler, and E. R. S. Roldan. 2006. Sperm design and sperm function. Biol. Lett. 2:246–249.

Michalczyk, Ł., O. Y. Martin, A. L. Millard, B. C. Emerson, and M. J. G. Gage. 2010. Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. Proc. R. Soc. Lond. B 277:3483–3491.

Miller, G. T., and S. Pitnick. 2002. Sperm–female coevolution in *Drosophila*. Science 298:1230–1233.

Montgomerie R., and J. L. Fitzpatrick. 2009. Testis size, sperm size and sperm competition. pp 1–53 *in* BGM Jamieson, ed. Reproductive biology and phylogeny of fishes, Science Publishers, Enfield, NH, pp 1–53.

Morrow, E. H., and M. J. G. Gage. 2001. Consistent significant variation between individual males in spermatozoal morphometry. J. Zool. Lond. 254:147–153.

Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. 15:1044–1045.

Namekawa, S. H., P. J. Park, L.–F. Zhang, J. E. Shima, J. R. McCarrey, M. D. Griswold, and J. T. Lee. 2006 Post– meiotic sex chromatin in the male germline of mice. Curr. Biol. 16:660–667.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. Camb. Philos. Soc. 45:525–567.

———. 1993. Sperm competition games: sperm size and sperm number under adult control. Proc. R. Soc. Lond. B 253:245–254.

Parker, G. A., and M. E. Begon. 1993. Sperm competition games: sperm size and number under gametic control. Proc. R. Soc. Lond. B 253:255–262.

Pitnick, S., D. J. Hosken, and T. R. Birkhead. 2009a. Sperm morphological diversity. Pp 69–149 *in* T. R. Birkhead, D. J. Hosken, and S. Pitnick, eds. Sperm biology: an evolutionary perspective. Academic, Burlington, MA.

Pitnick, S., R. Dobler, and D. J. Hosken. 2009b. Sperm length is not influenced by haploid gene expression in the flies *Drosophila melanogaster* and *Scathophaga stercoraria*. Proc. R. Soc. Lond. B 276:4029–4034

Pizzari, T. and G. A. Parker. 2009. Sperm competition and the sperm phenotype. Pp 207–245 *in* T. R. Birkhead, D. J. Hosken, and S. Pitnick, eds. Sperm biology: an evolutionary perspective. Academic, Burlington, MA.

Pizzari, T., and K. R. Foster. 2008b. Sperm sociality: cooperation, altruism, and spite. PLoS Biol. 6:e130, 925–931.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schultz, T. R., and S. G. Brady. 2008. Major evolutionary transitions in ant agriculture. Proc. Natl. Acad. Sci. USA. 105:5435–5440.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol. 20:46–53.

Suttle, J. M., A. D. M. Moore, E. J. Peirce, and W. G. Breed. 1988. Quantitative studies on variation in sperm head morphology of the hopping mouse, *Notomys alexis*. J. Exp. Zool. 247:166–171.

Thitipramote, N., J. Suwanjarat, C. Leigh, and W. G. Breed. 2010. Variation in sperm morphology of a murine rodent from South–East Asia: the Greater Bandicoot Rat, *Bandicota indica.* Acta Zoologica, doi: 10.1111/j.1463–6395.2010.00453.x [Epub ahead of print].

Tomkins, J. L., and L. W. Simmons. 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. Anim. Behav. 63:1009–1016.

Tourmente, M., M. Gomendio, E. R. S. Roldan, L. Giojalas, and M. Chiaraviglio. 2009. Sperm competition and reproductive mode influence sperm dimensions and structure among snakes. Evolution 63:2513–2524.

Tourmente, M., M. Gomendio, and E. R. S. Roldan. 2011. Sperm competition and the evolution of sperm design in mammals. BMC Evolutionary Biology 11:12

Wang, P. J., J. R. McCarrey, F. Yang, and D. C. Page. 2001. An abundance of X–linked genes expressed in spermatogonia. Nat. Genet. 27: 422–426.

Ward, P. I. 1998. Intraspecific variation in sperm size characters. Heredity 80:655–659.

Associate Editor: E. Morrow

## *Supporting Information*

The following supporting information is available for this article:

**Table S1.** Sperm traits, observed and effective paternity frequencies, mating system, and sample size (*n*) of the social insect species examined in this study.

Supporting Information may be found in the online version of this article.

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