Sperm storage reflects within- and extra-pair mating opportunities in a cooperatively breeding bird

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ABSTRACT

In passerine birds, storage and maturation of sperm takes place in the cloacal protuberance (CP),
25 an external swelling of the reproductive organ. The considerable variation in CP size among
species is presumed to be a consequence of varying levels of sperm-competition, but whether
individual variation in CP size within a species also reflects sperm competition is not well
established. Here, we study temporal variation in male CP size in relation to within-pair and
extra-pair mating opportunities and cuckoldry risk in purple-crowned fairy-wrens *Malurus*
30 *coronatus*. This is a socially monogamous cooperatively breeding passerine that can breed year-
round and has low levels of extra-pair paternity (in 6% of broods). We show that male CP size
sharply increased a few weeks before, and rapidly regressed after his partner laid eggs, consistent
with a cost of its maintenance and/or sperm production. Surprisingly, despite low levels of extra-
pair paternity, CP size of non-breeding and pre-breeding males was positively correlated with the
35 number of breeding females in the population, suggesting that CP size is sensitive to extra-pair
mating opportunities. However, CP sizes do not seem to reflect cuckoldry risk: CP size of
dominant males was unaffected by the presence of a subordinate that was unrelated to the
dominant female, although those subordinates occasionally sire offspring, and had a larger CP
than subordinates living with their mother. Our results suggest that, even in a species with low
40 levels of extra-pair paternity, individual investment in sperm storage reflects both within-pair and,
albeit to lesser extent, extra-pair mating opportunities.

INTRODUCTION

50 Much of the variation in male reproductive anatomy is believed to be the evolutionary result of differences in sperm competition (Birkhead et al. 1991, 1993; Briskie 1993; Tuttle et al. 1996). When more than one male is competing to fertilise the eggs of a female, sperm competition will lead to the evolution of traits that increase a male's chances of achieving fertilization. Since sperm production is limited and costly, males are expected to invest strategically in such traits, according to female promiscuity and additional mating opportunities (Wedell et al. 2002; Pizzari et al. 2003). Female promiscuity in the form of multiple mating is common in birds, even those that are socially monogamous (Griffith et al. 2002), resulting in adaptations to sperm competition (Birkhead and Møller 1998). One adaptation is the cloacal protuberance (CP), an external swelling of the reproductive organs that develops when males breed. The CP forms during seasonal testicular hypertrophy and sperm production, and functions in storage and maturation of sperm (Wolfson 1952). The CP thus enables males to store large numbers of spermatozoa. When sperm competition is high, larger CPs can improve fertilization success by maintaining sufficient stored sperm for frequent copulations or for large sperm transfers (Salt 1954; Wolfson 1954; Lake 1981; Birkhead et al. 1991, 1993). Since maintenance of tissue and sperm production is costly (Dewsbury 1982; Nakatsuru and Kramer 1982; Pitnick and Markow 1994; Olsson et al. 1997), CP size is likely optimised strategically, according to cost-benefit trade-offs. This should not only apply to seasonal enlargement and regression of CPs at the level of the population, but also to individual variation in CP size among males.

Most research has focused on CP size differences among species, showing that species with large CPs often have a polyandrous mating system or mate promiscuously (e.g., Briskie 1993, Tuttle et al. 1996). Because temporal variation in CP size has only been studied in species with rather high levels of sperm competition and/or unusually large CP (e.g., Nakamura 1990; Briskie 1993; Mulder and Cockburn 1993; Sax and Hoi 1998), it is unclear whether and how individual and temporal variation in CP size reflects levels of sperm competition more broadly.

75 Given that extra-pair (EP) copulations can occur throughout the breeding season, it may
be expected that males in socially monogamous species with high levels of EP paternity maintain
large CPs during the entire breeding season to enable successful EP fertilizations (e.g., Low et al.
2005). In superb fairy-wrens *Malurus cyaneus*, a species with intense sperm competition (61-76%
EP offspring; Mulder et al. 1994; Double and Cockburn 2003), CPs remain large throughout the
80 breeding season at the population level, indicating that extra-pair rather than within-pair mating
opportunities explain temporal variation in CP size (Nakamura 1990). In male tree-swallows
Tachycineta bicolor, however, male CPs regress soon after breeding of their social mate
(Lombardo 2001). Although a high proportion of offspring in this species is EP (69%; Barber et
al. 1996), this regression is likely the result of a relatively short and synchronous breeding season,
85 leading to reduced EP mating opportunities when a male's own reproductive attempt is nearly
completed (Lombardo 2001). In bearded tits *Panurus biarmicus*, male CP size also tracks within-
pair mating opportunities, as CPs regress between breeding attempts (although they remain larger
than during non-breeding; Sax and Hoi 1998). As these birds have moderate levels of EP
paternity (14 % EP offspring; Hoi and Hoi-Leitner 1997), the difference between this species and
90 *M. cyaneus* suggests that temporal variation in CP size reflects individual variation in degree of
sperm competition experienced. However, individual variation in CP size has, as far as we are
aware, not been studied in detail in a species with low levels of sperm competition, which will be
useful for making inferences about the relative importance of within-pair breeding and sperm
competition in explaining temporal changes in CP size.

95 In this study, we investigate how individual CP size varies with the stage of the within-
pair breeding cycle, extra-pair mating opportunities and cuckoldry risk in male purple-crowned
fairy-wrens *M. coronatus*. These socially monogamous birds breed cooperatively, with a
dominant breeding pair assisted by subordinate helpers (Rowley and Russell 1993, 1997; Kingma
et al. 2011a). Dominant females can initiate breeding attempts year-round and most offspring are
100 sired by the social father (the dominant male), with only 7% of offspring sired by other males

(Kingma et al. submitted ms). Consequently, we predict that CP size of male *M. coronatus* closely synchronises with the within-pair female's reproductive cycle, rather than with extra-pair mating opportunities. We test this prediction by relating dominant male CP size to breeding stage and days to egg-laying of his social mate, as well as the number of EP mating opportunities in the population. Additionally, since male subordinates that are unrelated to the dominant female can gain within-group paternity (Kingma et al. 2009), we quantify whether CP is affected by such a situation of cuckoldry risk (for dominant males), and within-group mating opportunity (for subordinate males).

METHODS

We studied a colour-banded population of *M. coronatus* resident along Annie Creek and the Adcock River in Mornington Wildlife Sanctuary in Western Australia (S17° 31' E126° 6'), from 2005 to 2010 (Hall and Peters 2008, 2009; Kingma et al. 2010, 2011b). The species is restricted to riparian vegetation and groups maintain all-purpose territories year-round that are linearly arranged along creeks and rivers. *M. coronatus* is a cooperatively breeding species in which the socially monogamous pair may be accompanied by a number of male and female subordinates (Kingma et al. 2011a, b). In our population, birds can breed in any month of the year, with typically a major peak in breeding activity during the wet season (Dec-Mar), and a minor peak in breeding activity in the late dry season in some years (Sep-Oct; Rowley and Russell 1997; unpubl. data). During year-round weekly population censuses, we documented group size, and social status (dominant or subordinate) of each uniquely colour-banded group member based on behavioural cues (the most obvious that only the breeding pair sing duets for cooperative territorial defence; Hall and Peters 2008). Only dominant females build the nest (Rowley and Russell 1993, 1997) and during each census we searched for nests by following the dominant female for at least 20 minutes. Nests were checked regularly to determine laying date and number of eggs (modal clutch size is 3 eggs) and nestlings. We obtained a very accurate estimate of

breeding progress for the population throughout the year: we rarely missed a reproductive attempt, as evidenced by the fact that >88% of nests ($N = 629$) are found during nest building and incubation, and <3% of nests are found by the appearance of fledglings (unpubl. data).

130 Throughout the study period birds were caught using mist nets, and we measured CP size (see below) and tarsus length (± 0.1 mm using a sliding caliper).

Paternity analysis showed that extra-pair paternity is rare in *M. coronatus*: of 509 offspring (217 broods) between 2006 and 2010, genotyping showed that 29 (5.7%) offspring from 15 (6.9%) broods were extra-pair. Of these, 25 (4.9%) offspring from 13 (6.0%) broods were
135 sired by an extra-group (EG) dominant male (Kingma et al. submitted ms; see also Kingma et al. 2009 with a smaller dataset). Subordinates are often offspring from the breeding pair and subordinates that are related to the female do not attain within-group paternity. However, due to death or dispersal, subordinates can be unrelated to the dominant female (Kingma et al. 2011a). These helpers do occasionally sire within-group offspring: the same analysis (217 broods, 509
140 offspring) showed that 4 offspring from 2 broods (1%) were sired by a subordinate male within the same group that was not related to the dominant female (Kingma et al. 2011a) and 7% (of 30) such helpers gained within-group paternity. Unrelated helpers thus form a reproductive threat to the dominant male that is of similar magnitude as the risk of cuckoldry by extra-group males.

145 **CP measurements**

The cylinder-shaped CP of males was initially measured as width (w) and length (l) (perpendicular to each other; lateral and antero-posterior axes respectively), using a sliding caliper to the nearest 0.1 mm. The ‘area’ of the CP was calculated using the formula $(w/2 + l/2)^2 * \pi$. Later in the study, we also measured height (h), and calculated CP volume following the
150 formula: $(w/2 + l/2)^2 * \pi * h$ (Briskie 1993). Because area and volume calculations were highly correlated ($r^2 = 0.901$, $N = 158$ birds, volume = $3.897 * \text{area} - 8.133$), we decided to use ‘area’ to allow inclusion of all samples, and we refer to this as ‘CP size’.

Statistical analyses

We related CP size to opportunity for fertilizations and reproductive competition. For dominant males, we examined the relationship with within-pair breeding stage and number of days from the start of egg-laying of their (within-pair) female as well as EP mating opportunities (the number of actively breeding females in the population). We quantified the effect of within-group reproductive competition (a subordinate unrelated to the dominant female) on dominants and subordinates.

For the first set of analyses, those involving within-pair breeding stage, number of days from the start of egg-laying and EP mating opportunities (see below), we used a mixed modelling approach using the nlme-package in R 2.11.1 (Pinheiro et al. 2011, R development core team 2011), including bird-identity as random variable because some males were included in the analyses more than once. We included ‘CP size’ as the response variable, and we excluded non-significant ($P > 0.05$) independent variables (see below) using backwards elimination. Reported values are obtained after re-inclusion in the model with only statistically significant variables. Effect sizes (β) are reported as average $\pm$ SE. Normal distribution and homogeneity of variance of the model residuals were confirmed by visual inspection. CP size was log transformed in all analyses, to normalise model residuals (except for analyses with pre-breeding dominant males only; see below). We included tarsus length in the models to correct for structural size, but this was unrelated to CP size in all analyses (all: $P > 0.14$). Additionally, we included age (days since hatching) in the models using a subset of dominant males with known ages (in 99 of 144 cases we did not know exact age as these males were immigrants or already present in the beginning of the study). In all analyses the effect of age on CP size was non-significant (all: $P > 0.13$).

Within-pair breeding stage

To analyze whether CP size varied with within-pair breeding stage, for each dominant male we
180 classified the breeding stage on his home territory as non-breeding, pre-breeding, incubating, or
with dependent young at time of capture. Because breeding can take place throughout the year,
we based this on the nesting attempt that was closest in time to capture of the male. We classified
birds as *non-breeding* when they were more than 3 weeks before egg-laying (average $\pm$ SE = 106
 ± 7.3 days, range = 26 to 274 days, $N = 59$), after a failed nesting attempt or 6 weeks after
185 fledging (average $\pm$ SE = 135 ± 11.9 days after egg-laying, range = 53 to 250 days, $N = 20$). *Pre-*
breeding was defined as between 3 weeks and 1 day before egg-laying (average $\pm$ SE = 6 ± 0.9
days, range = 1 to 20 days, $N = 33$); this also included nest-building for nests in which no eggs
were laid ($N = 9$). *Incubation* included all males that had a nest with eggs (average $\pm$ SE = 8 ± 1.5
days after start of egg-laying, range = 0 to 14, $N = 9$); we included here two cases where the
190 female was egg-laying since close proximity between partners (physical mate guarding) ceases
when the first egg is laid and within-pair copulations stop occurring 2 days before egg-laying
(Hall and Peters 2009). Finally, we defined *dependent young* as nestlings (average $\pm$ SE = $25 \pm$
 0.6 days after egg-laying, range = 22 to 26 days, $N = 6$), and dependent fledglings (less than 6
weeks after fledging; average $\pm$ SE = 54 ± 3.0 days after egg-laying, range = 45 to 67 days, $N =$
195 7). We excluded one male (with nestlings) from this analysis as this male was identified as an
outlier (using Grubb's test for outliers; Barnett and Lewis 1994) because of an exceptionally large
CP (27.1 mm^2). In a separate model, we analyzed whether the number of days from the start of
egg-laying predicted CP size. Because the range of number of days from the start of egg-laying
was large (see above, and Fig. 2), we log(+1)-transformed number of days from the start of egg-
200 laying. Two non-breeding males did not have a breeding attempt during their tenure as dominant
males, so we could not calculate the number of days from egg-laying for these males.

Population breeding activity – extra-pair mating opportunities

We assessed whether EP-mating opportunities predict male CP size by relating CP size to the number of females actively breeding in the population. To control for confounding effects of CP enlargement due to their own breeding, we performed two analyses: one including only non-breeding males and one including only pre-breeding males. Additionally, to control for potential co-variation between breeding activity in the population and the likelihood that his own mate was about to initiate a breeding attempt, we included number of days from the start of egg-laying for each focal male in both analyses (absolute or log(+1) transformed; excluding the two males that did not have a clutch (see above)). For each male, we calculated the number of clutches in the population initiated in the month of capture of the focal male (as proxy for EP-mating opportunities; not including within-pair clutches). Because the timing of breeding was slightly different between birds on Annie Creek and the Adcock River (that have different flow regimes; Hall, Kingma and Peters, personal observation), we calculated the number of clutches initiated for both rivers separately. The total number of territories changed slightly over the course of the study and differed between rivers, so we also calculated the proportion of territories that initiated a clutch in each month for each river. Using the latter approach, results were similar (and therefore not reported).

Within-group reproductive competition

To make inferences about CP size and within-group reproductive competition, we included only males in the pre-breeding stage, as direct reproductive competition takes place during this period. We compared groups with related subordinates (living with their mother), and unrelated subordinates (not living with a first or second order relative) that might gain paternity in the group (Kingma et al. 2009, 2011a). CP sizes of subordinate males that were either living with their mother or unrelated to the dominant female were compared using a linear model (we did not use a mixed model as none of the 21 subordinates was included twice). We also included subordinate age in the model (number of days, as independent variable). Additionally, to assess

the effect of cuckoldry risk on dominant males, we compared dominant males living in a group with a subordinate male unrelated to the dominant female to those that did not have subordinates unrelated to the female (in a mixed model, as above, and correcting for the number of clutches in the population initiated in the month of capture of the focal male). For a subset of males for which number of days until egg-laying was known (excluding 9 males that were captured when their female was nest-building but did not lay eggs in that nest), we repeated this model controlling for number of days until egg-laying.

RESULTS

Within-pair breeding stage

CP size of dominant males varied considerably over the course of the breeding cycle on their own territory (Fig. 1). CPs of non-breeding males were significantly smaller than CPs of males with or approaching an active breeding attempt (difference between non-breeding males and pre-breeding males ($\beta = 0.308 \pm 0.0318$, $t = 9.461$, $P < 0.001$), and males with eggs ($\beta = 0.262 \pm 0.0588$, $t = 4.366$, $P < 0.001$)), but CP size of males in nestling and fledgling stage was not significantly different from CP size of non-breeding males ($\beta = 0.0703 \pm 0.0518$, $t = 1.358$, $P = 0.178$).

Number of days from the start of egg-laying was significantly correlated with CP size ($\beta = -0.230 \pm 0.0244$, $N = 133$, $t = -9.450$, $P < 0.001$), because CPs were larger when a male's partner was closer to egg-laying (Fig. 2A).

Population breeding activity – extra-pair mating opportunities

CP size of non-breeding males increased with increasing numbers of females actively breeding in the population (effect of number of clutches initiated in the month of capture of the focal male: $\beta = 0.0277 \pm 0.00925$, $N = 81$, $t = 2.996$, $P = 0.005$; Fig. 3A), but was unrelated to the number of days to egg-laying on their own territory ($\beta = -0.0000054 \pm 0.000372$, $N = 79$, $t = -0.014$, $P = 0.989$; log(+1) transformed, $\beta = -0.0405 \pm 0.0889$, $N = 79$, $t = -0.455$, $P = 0.652$). The effects of

population-wide breeding activity and breeding on their own territory were not confounded: there was no relationship between the number of days to egg-laying (response variable) and the number of clutches initiated in the month of capture (independent variable; $\beta = -2.316 \pm 2.896$, $N = 79$, $t = -0.800$, $P = 0.430$).

260 Males in the pre-breeding stage also had significantly larger CPs when more clutches were initiated in the month of capture of the focal male ($\beta = 0.216 \pm 0.0919$, $N = 42$, $t = 2.354$, $P = 0.036$; Fig. 3B). The significant relationship between focal male CP size and the number of active breeding attempts in the month of capture remained ($\beta = 0.268 \pm 0.101$, $N = 42$, $t = 2.649$, $P = 0.033$) when correcting for the significant effect of number of days to egg-laying on the own
265 territory ($\beta = -0.431 \pm 0.144$, $t = -2.988$, $P = 0.020$; Fig. 2B), using the subset of males for which this was known (see methods, $N = 33$).

Within-group reproductive competition

In the pre-breeding stage, subordinate males that were unrelated to the dominant female had CPs
270 that were on average nearly twice as large (average $\pm$ SE = 11.88 ± 2.06 mm², $N = 6$) as CPs of 15 subordinate males that lived with their mother (average $\pm$ SE = 6.90 ± 0.62 ; $t = -2.829$, $P = 0.011$; Fig 4A). This difference was, however, non-significant ($\beta = -0.0290 \pm 0.109$, $t = -0.267$, $P = 0.793$) when we statistically corrected for the highly significant effect of subordinate age ($\beta = 0.000548 \pm 0.000134$, $t = 4.080$, $P < 0.001$; Fig 4A). Unrelated subordinates were older (average
275 $\pm$ SE = 766 ± 76.9 days old; range = 542-1020) than related subordinates (375 ± 39.2 days old; range = 167-641; $t = 4.523$, $P = 0.002$; Fig 4A).

CP size of (pre-breeding) dominant males that lived in a group with a subordinate male that was unrelated to the dominant female was not statistically different from dominants that did not have any subordinate male unrelated to the dominant female ($t = -0.644$, $P = 0.533$; Fig. 4B).

280 This result was similar ($t = -1.463$, $P = 0.158$) when correcting for the significant effect of

number of days to egg-laying on the own territory (using a subset of males for which number of days to egg-laying was known exactly, see above).

DISCUSSION

285 **Within-pair breeding stage**

CP size in dominant *M. coronatus* males closely tracks the breeding cycle of their social partner. During the 3 weeks before egg-laying, the cloacal swelling increases rapidly as egg-laying approaches (Figs. 1 & 2), and male CP increases in this period to, on average, nearly twice as large as that of non-breeding males (Fig. 1A). This CP enlargement is usually brief: by the
290 nestling/fledgling stage, CPs have regressed to the size of non-breeding males (Fig. 1A). Moreover, CP size of non-breeding males is not correlated with the number of days until egg-laying. This transient and brief increase is in agreement with expectations of strategic allocation to sperm storage: since the maintenance of tissue and sperm production is costly (Pitnick and Markow 1994; Olsson et al. 1997), CPs should be small when the benefits of continued
295 maintenance of storing mature sperm are low. Since female *M. coronatus* can initiate breeding at any month of the year, a strategy of closely tracking his mate's breeding activities ensures male fertility at the time of within-pair copulations without redundant investment at other times. Accordingly, the decline after egg-laying, to non-breeding sized CP, appears sharper than in other species, that maintain a part- (*P. biarmicus*; Sax and Hoi 1998) or even full-sized (*M. cyaneus*;
300 Mulder and Cockburn 1993) CP outside the fertile period of their mate. This is probably related to seasonal breeding with more predictable probability of re-nesting. Additionally, or alternatively, extended maintenance of large CPs could be related to EP mating opportunities, as these species have (much) higher levels of EP paternity (14% and 61-76% of offspring respectively; see Introduction) than *M. coronatus* (5.7 % EP offspring; Kingma et al. submitted
305 ms; see also Kingma et al. 2009).

Extra-pair mating opportunities

Testes and CP in *M. coronatus* are small in comparison to their congeners that have some of the highest rates of EP mating among birds (Kingma et al. 2009). In three fairy-wrens with EP paternity >50% of broods (*M. splendens*, *M. cyaneus* and *M. melanocephalus*) average CP volume in breeding males is 107, 105 and 123 mm³ respectively (Rowe et al. 2008; see also Kingma et al. 2009). During the pre-breeding stage, CP volume of *M. coronatus* males is around half as large (58.2 mm³, n = 42; all calculated as $(w/2 + l/2)^2 * \pi * h$ (Briskie 1993); see methods for details). We argued that this relatively small CP is the result of low competition with other males over fertilizations in *M. coronatus* (Kingma et al. 2009).

Surprisingly however, using detailed information on breeding activity of all females in the population, we show here that when more females in the population were breeding, male *M. coronatus* grew relatively larger CPs: this occurred independent of (Fig. 3A) and in addition to (Fig. 3B) the increase as a result of within-pair breeding. This effect was larger in non-breeding males compared to males with an actively breeding female (compare Fig. 3A and 3B and effect sizes), but not as large as that of within-pair breeding (compare Fig. 3A with Fig. 1: the maximum CP size in non-breeding males is well below the average CP size of breeding males). This increase in CP size in response to general breeding activity is likely related to the increasing number of EP mating opportunities, rather than an effect of males ‘expecting’ a within-pair brood: first, we statistically controlled for the number of days to egg-laying; second, the number of days to egg-laying for a focal male and general breeding activity in the population were not related; and third, in the non-breeding stage, CP size was unrelated to the time until egg-laying. In conclusion, it seems that besides the strong correlation with within-pair breeding stage, a part of the individual variation in CP size consistently reflects opportunities for EP copulations in *M. coronatus*, despite low EP paternity in this species.

Although we have never observed EP copulations in *M. coronatus* (Kingma et al. 2009), in *M. cyaneus*, fertile females initiate clandestine pre-dawn forays for extra-pair copulations

(Double and Cockburn 2000) and males' reproductive state (individual testosterone level) tracks the number of fertile females in the population (Peters et al. 2001). If males similarly track extra-pair opportunities in *M. coronatus*, our results suggest that males maintain an elevated level of 'reproductive-readiness' when the likelihood that EP females visit them for copulations increases. In agreement with this suggestion, in *M. melanocephalus* (attractive) males that have more EP mating opportunities have larger CPs than unattractive males (Karubian 2002; Rowe et al. 2010).

Within-group reproductive competition: subordinate vs. dominant?

Subordinate male *M. coronatus* had smaller CPs than dominant males (Fig. 4), which has also been found in other cooperative breeders where subordinates have limited mating opportunities (e.g. Brouwer et al. 2009). Interestingly, in the closely related *M. cyaneus* and *M. splendens* subordinate males have similarly sized CPs as dominant males (Mulder and Cockburn 1993, Rowe and Pruett-Jones 2006; but see also Rowe et al. 2010). In these species, levels of EP paternity are extremely high, and although subordinates are less successful than dominants, they can still gain fairly substantial reproductive success (Cockburn et al. 2003, 2009, Webster et al. 2004). Similarly, we show here that subordinate male *M. coronatus* that live in a group with an unrelated dominant female had a larger CP than those living with their mother, approaching in size the CP of breeding dominant males (Fig. 4A). This may simply be due to unrelated subordinates being older. However, it also reflects increased mating opportunity since subordinates with an unrelated female occasionally sire offspring (in 7% of 30 cases), whereas subordinates that live with their mother never sire offspring (0% of 92 cases; Kingma 2011a). Greater reproductive readiness in unrelated subordinate males has been demonstrated in other cooperative breeders. For example, in the classic example of the pied kingfisher *Ceryle rudis*, 'secondary helpers' (immigrants) are unrelated to the dominant female and have testosterone levels similar to those of dominant males, whereas 'primary helpers' (offspring of the breeding

pair) express low levels of testosterone, have small gonads and do not produce sperm (Reyer et al. 1986), and they presumably also had smaller CPs.

360 Dominant males did not show any signs of defensively investing more in sperm competition when the risk of cuckoldry increased: when there was a subordinate in the group that was unrelated to the dominant female (that occasionally gain within-group paternity) the dominant male did not have a larger CP. Possibly males are relying more on behavioural paternity assurance, since males maintain very close proximity to their partners before laying (Hall and
365 Peters 2009). Behavioural strategies to minimise sperm competition are common, and can be effective (Komdeur et al. 1999; Chuang-Dobbs et al. 2001; Brylawski and Whittingham 2004). For example, in *C. rudis*, dominant males do not tolerate secondary helpers during the copulatory phase, although no paternity analysis was performed to quantify cuckoldry (Reyer et al. 1986). Although we occasionally observed displacement of unrelated subordinates by dominant males in
370 *M. coronatus* (Hall and Peters 2009), dominant male behavioural strategies are not 100% effective, as 7% of subordinates that are unrelated to the dominant female do gain within-group paternity (Kingma et al. 2009, 2011a). Possibly, since the unrelated (to the female) subordinate is not only a reproductive competitor, but often also a son of the dominant male (Kingma et al. 2009), there might be reduced fitness incentive for investment in sperm competition.
375 Alternatively, our results could suggest that defensive sperm competition strategies require different investment compared to offensive sperm competition strategies, as has been shown for sperm morphology in *M. cyaneus* (Calhim et al. 2011).

Altogether, our data suggest that, in a species with limited extra-pair paternity, individual
380 variation in sperm storage of dominant and subordinate males reflects primarily the within-pair breeding stage, and to a lesser extent extra-pair mating opportunities, as predicted by strategic investment in sperm competition. Further studies incorporating EP mating opportunities in

explaining individual variation in CP size will need to confirm whether this is a general pattern among species, including those with low levels of EP paternity.

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ETHICAL STANDARDS

All fieldwork was performed with permission from the Max Planck Institute for Ornithology
395 Animal Ethics Committee, the Australian Wildlife Conservancy, the Western Australia Department of Conservation and Land Management (licenses BB002178 and BB002311), and the Australian Bird and Bat Banding Scheme (Authority 2230 and 2073).

The authors declare that they have no conflict of interest.

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510 **FIGURE LEGENDS**

Figure 1. CP size of dominant male purple-crowned fairy-wrens in relation to breeding stage. Breeding stages are categorised as non-breeding, pre-breeding (21 - 1 day before egg-laying), incubation and dependent young. CPs of males captured during the non-breeding stage were significantly smaller than CPs of males in pre-breeding and incubation stage, but not
515 significantly different from CPs of males with nestlings and fledglings. Numbers denote sample sizes (number of observations) and error bars denote standard errors.

Figure 2. CP size of dominant male purple-crowned fairy-wrens in relation to the number of days from (before and after) the start of egg-laying by the within-pair female. (A) CPs of
520 dominant males are larger when the number of days from within-pair females initiating egg-laying is shorter. (B) This effect is driven by an increase in CP size during the pre-breeding stage (21 - 1 day before egg-laying).

Figure 3. CP size of dominant male purple-crowned fairy-wrens in relation to extra-pair mating opportunities. CPs of dominant males are larger, both in the (A) non-breeding stage and
525 (B) pre-breeding stage, when more clutches were initiated along the same river ('population') in the month of capture of the focal male (extra-pair mating opportunities).

Figure 4. CP size of male purple-crowned fairy-wrens in relation to (A) mating opportunities and (B) cuckoldry risk. (A) Subordinates with an unrelated dominant female
530 (filled symbols) had on average a larger CP compared to subordinate males that lived with their mother (open symbols), but unrelated males were also older so that it is unclear whether this is an effect of within-group mating opportunities or the effect of age. (B) The reproductive competition unrelated helpers represent to the dominant male is not reflected in the CP size of the latter:
535 dominant males that did and did not have a subordinate that was unrelated to the dominant female

had similar CP sizes. Numbers denote sample sizes (number of observations; all males captured during pre-breeding) and error bars denote standard errors.

FIGURES

540

Figure 1.

Figure 2.

Figure 4.

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