Volume of the Cloacal Protuberance as an Indication of Reproductive State in Male Blue Tits Cyanistes caeruleus

Authors: Schut, Elske, Magrath, Michael J.L., Oers, Kees van, and Komdeur, Jan

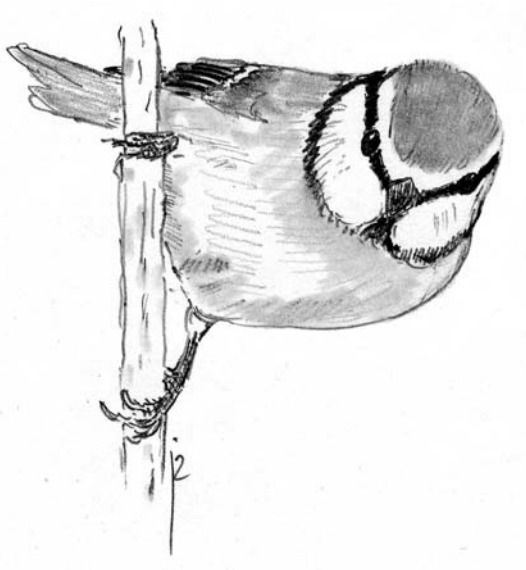
Source: Ardea, 100(2) : 202-205

Published By: Netherlands Ornithologists' Union

URL: https://doi.org/10.5253/078.100.0212

Volume of the cloacal protuberance as an indication of reproductive state in male Blue Tits *Cyanistes caeruleus*

Elske Schut[1,*], Michael J.L. Magrath[2], Kees van Oers[3] & Jan Komdeur[1]



Schut E., Magrath M.J.L., van Oers K. & Komdeur J. 2012. Volume of the cloacal protuberance as an indication of reproductive state in male Blue Tits *Cyanistes caeruleus*. Ardea 100: 202–205.

In male passerines, the accumulation of sperm in the sperm reserves causes the cloaca to become enlarged, forming the cloacal protuberance (CP). In Blue Tits, the timing of breeding differs considerably between pairs. Hence, when catching a male during the breeding season it may be unclear whether he is in a reproductively active state (i.e. producing sperm). Here, we show in captive Blue Tits *Cyanistes caeruleus* that CP volume is increased in males in a reproductively active state when compared to the same males in a reproductively inactive state. However, there was some overlap in CP volume when comparing the values in reproductively active and non-active states. Measurements of CP volume at a single time point, therefore, do not allow the researcher to reliably determine an individual's reproductive status.

Key words: reproductive cycle, sperm production, cloacal swelling, seminal glomerae

[1]Behavioural Ecology and Self-organization, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands; [2]Department of Wildlife Conservation and Science, Zoos Victoria, P.O. Box 74, Victoria 3052, Australia; [3]Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, the Netherlands;
* corresponding author (elske.schut@rug.nl)

Many passerine species are only reproductively active during a short period in spring. At the onset of the breeding season, the male reproductive organs will therefore reach the reproductively active state and will start producing sperm. As soon as the breeding season concludes, sperm production will cease (Wright & Wright 1944, Selander & Hauser 1965, Partecke *et al.* 2004). In most passerines, males store produced sperm in their seminal glomerae (an extension of the ductus deferens) until it is ejaculated. Due to the accumulation of sperm in the seminal glomerae, the cloaca becomes enlarged, forming the cloacal protuberance (CP; King & McLelland 1981). Previous studies have used the CP for sex determination (e.g. Mason 1938) or as an indication that males are in a reproductively active state (e.g. Wolfson 1952, Brouwer *et al.* 2009, Tonra *et al.* 2011). The association between CP volume and stage in the reproductive cycle has been described in the Bearded Reedling *Panurus biarmicus*. In the Bearded Reedling, CP volume followed the reproductive cycle of the social partner, with CP volume reaching its peak on or close to the day of clutch initiation (Sax & Hoi 1998). In a comparison across passerine species, size of the CP was shown to correlate positively with the size of the testes, number of sperm in storage, the length of sperm and copulation rate (Birkhead *et al.* 1993). Furthermore, the association between CP size and numbers of sperm in storage has been demonstrated within-species (Tuttle *et al.* 1996, Peer *et al.* 2000, Tuttle & Pruett-Jones 2004).

Several (non-mutually exclusive) hypotheses have been proposed to explain the evolutionary function of the CP (reviewed in Birkhead *et al.* 1993), including the "efficient copulation hypothesis" (proposing that the CP facilitates sperm transfer), the "spermatozoa size hypothesis" (larger CPs are needed to store similar numbers of longer sperm) and the "sperm competition hypothesis" (if sperm competition is strong and copulation is frequent, more sperm must be stored and the CP will be larger). Birkhead *et al.* (1993) found supporting evidence for both the sperm competition and the spermatozoa size hypotheses. In line with the sperm competition hypothesis, Laskemoen *et al.* (2010) demonstrated that the fertilization success of male Tree Swallows *Tachycineta bicolor*, a species with a high sperm competition risk, correlated positively with male fertilization success.

Here we investigate whether CP volume is (as expected) increased in male Blue Tits *Cyanistes caeruleus* in a reproductively active state. Using captive birds allowed us to measure cloacal protuberance during and after the breeding season for the same males, something that is difficult in free-living birds. We are unaware of any previous study describing CP volume in the Blue Tit. Since Blue Tits lay an exceptionally large clutch, are known to copulate frequently during the breeding season (Kempenaers *et al.* 1995) and extra-pair offspring frequently occur (e.g. Magrath *et al.* 2009), we might expect males to store sperm in their seminal glomerae, to ensure the availability of adequate sperm. When comparing between Blue Tit pairs, considerable asynchrony exists in the timing of breeding (range of date of first egg in our population: 4 April – 7 May, SD = 5.46 days in 2008; 10 April – 10 May, SD = 3.67 days in 2009; O. Vedder, unpubl. results). Hence, the timing of the onset of sperm production will differ between males. When catching a Blue Tit male (away from the nestbox) during the breeding season it may therefore be unclear whether he is in a reproductively active state (i.e. producing sperm) or not. The aims of our study are to determine (1) whether a males' CP volume is greater while he is in a reproductively active state when compared to while he is in a reproductively inactive state and (2) whether during the breeding season, individuals in reproductively active and non-active states can be distinguished using a single measurement of CP volume.

Methods

We used 18 male captive Blue Tits to investigate copulation behaviour. All birds were hand reared in 2007 and afterwards housed in aviaries during autumn and winter (3 × 2 × 2 m; for details on the origin of the birds, see Vedder *et al.* 2010). Hence, all males were approx. 1 year old , thereby excluding the possibility that age differences bias the results (previous studies demonstrated that older males have a larger CP volume; Kempenaers *et al.* 2002, Laskemoen *et al.* 2008). Birds were housed in individual cages (0.8 × 0.4 × 0.4 m) within an outdoor aviary during spring and summer. Hence, birds were exposed to a natural light schedule and outside temperatures. Birds were fed *ad libitum* with a mixture of commercial bird food and beef heart, supplemented with mealworms, wax moth larvae and caterpillars from the wild. Birds had *ad libitum* access to drinking water. As part of separate studies, males were offered a live stimulus female with which they were free to interact every second day from 30 March until 6 June 2008. A nestbox and nesting material were provided. The male was allowed access to the female at 08:30 (through removal of a sliding panel) and removed to his own cage at 17:00h. We used the occurrence of copulations as an indication that males were in a reproductively active state. Copulations were observed in six of the captive males. Whether the other 12 males came into a reproductively active state or not is unclear, since the occurrence of copulations may also be influenced by female behaviour.

We took three measurements for CP using sliding callipers (measurements were taken to the nearest 0.1 mm): height, width at base and width at opening. From these measures volume of the CP was calculated, based on a barrel-shape ($\pi \times \text{radius}^2 \times \text{height}$; e.g. Mulder & Cockburn 1993). Radius was calculated as 0.5 × the average of width at base and width at opening. CP was measured during the breeding season (on a day that the male was observed copulating in the morning, between 8 May and 3 June 2008), and again when the breeding season was over and males no longer had access to females (16 June). CP measurements were all taken by the same person (ES). In five out of the six males in a reproductively active state, copulations were recorded on two days during the breeding season and therefore CP volume was measured twice. In those cases, the highest CP volume was used in Figure 1, since we aim to illustrate the difference between the period when the cloaca is maximally enlarged and the period during which birds are not producing sperm. In our statistical analysis both measurements were taken into account. On 16 June, all CP measures (height, width at base and width at opening) were taken twice immediately after each other for 17 males (one male had died during the course of the experiment). To ensure the reliability of the measurements, measurements were taken blind (i.e. the callipers were read by another observer). These consecutive measurements allowed us to calculate the repeatability following Lessells and Boag (1987). Averages of the two measurements were used as the CP volume 'after breeding'. We used a mixed model with individual as a random factor to assess the relationship between male reproductive status and CP volume. This analysis was performed twice: once with all 18 individuals in our dataset and once with only the six individuals observed copulating. Analyses were performed in MLwiN 2.23 (Rashbash *et al.* 2011).

Results

Our consecutive measurements after the breeding season on 16 June demonstrated that our measures of CP volume were repeatable (R = 0.81 ±0.08 SE, Figure 1 in the appendix). Furthermore, we found that the CP was significantly larger when males were in a reproductively active state compared to after the breeding season (all males: $P = 0.008$; copulating males only: $P = 0.011$, Table 1; Figure 1). On average, CP had decreased after the breeding season by 10.30 mm^3 in all males (range –6.3–53.8 mm^3; average decrease 17.8%) and by 25.0 mm^3 when only copulating males were included (range 5.8–53.8 mm^3; an average decrease of 54.4%). Between individuals there was overlap in CP values during and after the breeding season (all males: range while in sexual active state: 11.8–76.0 mm^3; range after breeding season 11.3–43.6 mm^3; copulating males: range while in sexual active state: 20.9–76.0 mm^3, range after season: 15.1–43.6 mm^3).

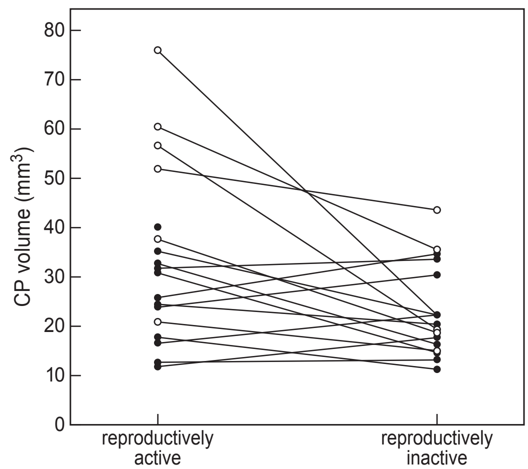


Figure 1. Cloacal protuberance volume for the six males that were known to copulate, comparing measurements taken during the breeding season when males were in a reproductively active state with those taken after the breeding season. Lines connect the two CP values for the same male. Open circles indicate males that were observed to copulate ($n = 6$). Closed circles indicate males for which we are unsure whether they reached a reproductively active state ($n = 12$; one male was measured during sexual activity only).

Discussion

In passerines, the storage of sperm in the seminal glomerae causes the cloaca to become enlarged, forming the cloacal protuberance (CP). The size of the CP may be used for sex determination and to determine reproductive status. Here, we investigated whether CP volume is, as expected, increased in Blue Tit males in a reproductively active state and can be used to determine a male's reproductive status.

Table 1. Summary of the mixed model investigating the association between reproductive status (i.e. while in reproductively active- or inactive states) and volume of the cloacal protuberance (CP volume), including a) all 18 individuals in our dataset, b) the six males observed copulating.

Dependent variable: CP volume (all individuals included)				
Predictor	Estimate (SE)	χ^2	*df*	*P*
Reproductive status	12.55(4.75)	6.99	1	0.008
Dependent variable: CP volume (active individuals only)				
Predictor	Estimate (SE)	χ^2	*df*	*P*
Reproductive status	20.98 (8.25)	6.48	1	0.011

We found that CP volume was significantly larger while males were in a reproductively active state than while they were in a non-active state. However, there was overlap in CP volume between individuals in reproductively active and non-active states. This overlap may in part be caused by the fact that during the breeding season, we measured CP on a day that copulation was observed, rather than randomly or at regular intervals during the breeding season. We may have been sampling at times when CP volume was lower than average, because males had only recently lost sperm through ejaculation. When catching a male in the field, the same bias may arise.

Our findings indicate that Blue Tit males show visible signs of the storage of sperm in their seminal glomerae during the breeding season. However, measuring CP volume does not allow the researcher to unequivocally determine whether a wild-caught male is in a reproductively active state.

The authors would like to thank one anonymous referee for helpful comments on an earlier version of the manuscript. The authors would further like to thank Oscar Vedder, Anna Harts, Giuseppe Boncoraglio and Janne Ouwehand for practical assistance. ES received funding from the Schure- Beijerinck-Popping Fonds. The animal experiments committee of the University of Groningen granted permission to raise Blue Tits in captivity and for the experimental procedures (no. D4753A).

References

Birkhead T.R., Briskie J.V. & Møller A.P. 1993. Male sperm reserves and copulation frequency in birds. Behav. Ecol. Sociobiol. 32: 85–93.

Brouwer L., Groothuis T.G.G., Vedder O., Eikenaar C., Richardson D.S. & Komdeur J. 2009. Do primary males physiologically suppress subordinate males? An experiment in a cooperatively breeding passerine. Ethology 115: 576–587.

Kempenaers B., Peer K., Vermeirssen E.L.M. & Robertson R.J. 2002. Testes size and asymmetry in the tree swallow *Tachtcineta bicolor*: a test of the compensation hypothesis. Avian Sci. 2:115–122.

Kempenaers B., Verheyen G.R. & Dhondt A.A. 1995. Mate guarding and copulation behaviour in monogamous and polygynous blue tits: do males follow a best-of-a-bad-job strategy? Behav. Ecol. Sociobiol. 36: 33–42.

King, A.S. & McLelland, J. 1981. Form and function in birds. Academic Press, New York.

Laskemoen T., Fossøy F., Rudolfsen G. & Lifjeld J.T. 2008. Age-related variation in primary sexual characters in a passerine with male age-related fertilization success, the bluethroat *Luscinia svecica*. J. Avian Biol. 39: 322–328.

Laskemoen T., Kleven O., Fossøy F., Robertson R.J., Rudolfsen G. & Lifjeld, J.T. 2010. Sperm quantity and quality effects on fertilization success in a highly promiscuous passerine, the tree swallow *Tachycineta bicolor*. Behav. Ecol. Sociobiol. 64: 1473–1483.

Lessells C.M. & Boag P.T. 1987. Unrepeatable repeatabilities: a common mistake. Auk 104: 116–121

Magrath M.J.L., Vedder O., van der Velde M. & Komdeur J. 2009. Maternal effects contribute to the superior performance of extra-pair offspring. Curr. Biol. 19: 792–797.

Mason E.A. 1938. Determining sex in breeding birds. Bird-banding 9: 46–48.

Mulder R.A. & Cockburn A. 1993. Sperm competition and the reproductive anatomy of male superb Fairy-Wrens. Auk 110: 588–593.

Partecke J., van 't Hof T. & Gwinner E. 2004. Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences? Proc. Roy. Soc. Lond. B 271: 1995–2001.

Peer K., Robertson R.J. & Kempenaers B. 2002. Reproductive anatomy and indices of quality in male tree swallows: the potential reproductive role of floaters. Auk 117: 74–81.

Quay W.B. 1986. Cloacal protuberance and cloacal sperm in passerine birds: comparative-study of quantitative relations. Condor 88: 160–168.

Rasbash J., Browne W., Healy M., Cameron B. & Charlton C. 2011. MLwiN version 2.23. Centre for Multilevel Modelling, University of Bristol.

Sax A. & Hoi H. 1998. Individual and temporal variation in cloacal protuberance size of male Bearded Tits (*Panurus biarmicus*). Auk 115: 964–969.

Selander R.K. & Hauser R.J. 1965. Gonadal and behavioral cycles in the great-tailed grackle. Condor 67: 157–182.

Tonra C.M., Marra P.P. & Holberton R.L. 2011. Early elevation of testosterone advances migratory preparation in a songbird. J. Exp. Biol. 214: 2761–2767.

Tuttle E.M. & Pruett-Jones S. 2004. Estimates of extreme sperm production: morphological and experimental evidence from reproductively promiscuous fairy-wrens (*Malurus*). Anim. Behav. 65: 541–550.

Tuttle E.M., Pruett-Jones S. & Webster M.S. 1996. Cloacal protuberances and extreme sperm production in Australian fairy-wrens. Proc. R. Soc. B 263: 1359–1364.

Vedder O., Schut E., Magrath M.J.L. & Komdeur J. 2010. Ultraviolet crown colouration affects contest outcomes among male blue tits, but only in the absence of prior encounters. Funct. Ecol. 24: 417–425.

Wolfson A. 1952. The cloacal protuberance– a means for determining breeding condition in live male passerines. Bird-banding 23: 159–165.

Wright P.L. & Wright M.H. 1944. Reproductive cycle of the male red-winged blackbird. Condor 46: 46–59.

Samenvatting

Het sperma van mannelijke zangvogels wordt door de vogel opgeslagen tot er een paring plaatsvindt. Het opgeslagen sperma resulteert in een opgezwollen cloaca, de cloacal protuberance (CP) genaamd. Het volume van de CP zou de reproductieve cyclus moeten volgen. Voor het Baardmannetje *Panurus biarmicus* is aangetoond dat dit inderdaad het geval is. Het CP volume kan daarom gebruikt worden voor bepaling van de sekse bij soorten waarbij mannelijke en vrouwelijke uiterlijk sterk op elkaar lijken en om te bepalen of een man in reproductief actieve toestand is (i.e. sperma produceert). In dit artikel tonen wij aan dat de CP van mannelijke Pimpelmezen *Cyanistes caeruleus*, zoals verwacht, vergroot is op het moment dat de vogels in reproductief actieve toestand zijn. Als men echter het CP volume vergelijkt tussen individuen, blijkt er overlap te zijn tussen mannetjes in reproductief actieve en inactieve toestand. Het is daarom niet mogelijk om op basis van een eenmalige meting van het CP volume conclusies te trekken over de reproductieve toestand van een in het wild gevangen Pimpelmeesman.

Corresponding editor: Jouke Prop
Received 6 May 2012; accepted 18 October 2012

Appendix

Figure 1. Repeatability of two consecutive measurements of cloacal protuberance (mm^3); ($F_{1,16}$ = 9.75, $P < 0.001$, R = 0.81 ± 0.08 SE; as calculated following Lessells and Boag 1987). Open circles represent the six males previously observed copulating, closed circles represent the remaining males.