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The involvement of prolactin in avian molt: the effects of gender and breeding success on the timing of molt in Mute swans (*Cygnus olor*)

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Abstract
 The aim of the study was to test the hypothesis that decreasing plasma prolactin stimulates or permits the initiation of avian molt. Changes in the concentration of plasma prolactin in Mute swans (*Cygnus olor*) were compared in non-breeding singletons and breeding pairs. In breeding swans, the onset of molt is delayed compared to non-breeders, and is delayed further in breeding males compared to their female partners. The seasonal decrease in prolactin in non-breeding birds of both sexes started at the end of May and was associated with the initiation of molt 4 weeks later. The decrease in plasma prolactin in incubating females was more pronounced, as a consequence of increased prolactin secretion associated with incubation behaviour, but also started at end of May, and was associated the onset of moult 6 weeks later. In breeding males, plasma prolactin increased at the end of May when they started to care for their newly hatched cygnets. Correspondingly, prolactin began to decrease 3-5 weeks later in males than in females. These males started to molt in mid August, at least 4 weeks later than females. It is concluded that molt is related to decreasing plasma prolactin, and is inhibited when plasma prolactin is increasing or high.

 Keywords: molt, prolactin, incubation, Mute swan

1 Introduction

It is important that birds replace their plumage at regular, normally annual, intervals in order to maintain its functional integrity. The period during which feathers are replaced, the molt, tends to be separated from times when birds are breeding (e.g. Svensson and Nilsson, 1997) or migrating (Barta et al., 2008) because all three activities are energetically demanding and also, presumably, because it would be disadvantageous to be caring for young or migrating when flight efficiency is impaired by missing or growing feathers. At the same time, it is advantageous to make maximal use of the time available to replace feathers, so there is often a close relationship between the end of breeding and the start of molt, and the end of molt and the start of migration. Appropriate timing of molt is therefore crucial. Despite the importance of molt and the significance of its timing, little is known about its physiological control. Several hormone systems have been implicated in molt: thyroid hormones, gonadal steroids and prolactin (Payne, 1972; Kuenzel, 2003), but a common factor appears to be prolactin. Studies on wild free-living species (Lincoln et al., 1980; Dawson and Goldsmith, 1982; Deviche et al., 2000) and wild species held in captivity under natural conditions (Dawson and Goldsmith, 1984; Williams et al., 1987; Boswell et al., 1996) have shown that peak prolactin concentrations coincide with the start of molt. Also, active immunization of starlings against vasoactive intestinal polypeptide, the prolactin releasing hormone in birds, inhibited the photoperiodically-induced increase in prolactin and prevented molt (Dawson and Sharp, 1998). However, it may not be the case that high concentrations of prolactin stimulate molt. Indeed the reverse may be true. Prolactin drives incubation behavior (El Halawani et al., 1986; Sharp et al., 1988; review: Sharp, 2008) and probably parental behaviour after hatching. Prolactin concentrations are high in incubating birds and, certainly in altricial species, remain high during the period of parental care (Goldsmith, 1982b; Goldsmith, 1982a; Dawson and Goldsmith, 1985; Hiatt et al., 1987; Wingfield and Goldsmith, 1990). Yet breeding activity can delay the start of molt (Morton, 1992; Hemborg and Merila, 1999; Newton and Rothery, 2005; Meissner, 2007; Allard et al., 2008). High concentrations of prolactin may inhibit molt, so that molt only starts when prolactin begins to decrease (Dawson, 2006). This would still result in a close correlation between peak prolactin and the start of molt and may be a mechanism

that minimizes breeding/molt overlap but at the same time ensures that molt starts as soon as breeding activity has finished.

The pattern of molt in Mute swans (*Cygnus olor*) offers a model to test this. As in many species (see above), molt is delayed in breeding birds. In addition, in successfully breeding swans, males initiate moult 4-6 weeks after their mate (Heinroth, 1911; Scott, 1972; Czapulak, 2002), whereas in non-breeding flocks males and females moult at about the same time (Coleman et al., 2002). In breeding pairs that lose their young, both sexes molt at the same time (McCleery et al., 2007). It is possible that males with dependent young delay their molt until the female has well grown feathers so that at least one of the parents has fully feathered wings to protect the young.

In this study, we collected serial blood samples from breeding pairs, from incubation until molt had started, and samples from randomly caught non-breeding birds during the same period. We could confidently predict that prolactin concentrations would be higher in breeding birds compared to non-breeding birds, but would prolactin concentrations start to decrease later in breeding birds, and in particular, would prolactin concentrations start to decrease later in breeding males than in their female partners?

2. Methods

The swans used in this study were resident at the Abbotsbury Swannery in the south of England (50.4 °N, 2.4°W). Population dynamics in this flock have been studied for over 25 years (McCleery et al., 2008). Uniquely in the UK, these birds breed colonially (Perrins and Ogilvie, 1981). Each year approximately 150 pairs produce young and another 300 remain as singletons. Almost all birds are banded as cygnets, so their age is known. Most breeding pairs nest near small pools or ditches and their young are exposed to predation. However, approximately 10 selected breeding pairs are isolated in pens, so their young are almost guaranteed to survive. These pairs have a successful breeding record and are sometimes used to foster lost young. Non-breeding birds spend most of their time on or near a lagoon.

At the beginning of May, 2007, six breeding pairs from the pens were caught. The ages of males ranged from 5 to 17 (median 9) and the females 5 to16 (median 10). A blood sample (1-2ml) was taken from a wing vein of both the male and the female within 4 min of capture. All of these birds had eggs at this time. Further blood samples were taken from the same birds at approximately two week intervals until mid-August. The eggs hatched in late May, and at the end of the study, all birds had surviving young. Swans, as many other waterfowl, molt by shedding all of their primary feathers at the same time and replacing them simultaneously. During molt, the length of growing primary feathers was recorded so that the date at which feather growth started could be calculated for each individual by linear regression. On the same dates as breeding pairs were sampled, non-breeding males (age range 2-20, median 5) and females (age range 1-22, median 5) were caught at random and sampled in the same way. The numbers of birds sampled on each date varied from 4-7 for males and 4-9 for females. The length of growing new primary feathers was recorded and the mean date for the start of growth was calculated for each sex by linear regression. Blood samples were allowed to clot at the ambient outdoor temperature for approximately 5 h, centrifuged, and serum stored at -20° until assay. The work was carried out under Home Office Licence 80/1944.

2.1. Prolactin assay

Plasma prolactin was measured in a single assay with an intra- assay coefficient of variation of 7.7% as described by Talbot and Sharp (1994). Swan samples diluted parallel to the standard.

2.2. Statistical analysis

Prolactin data were log transformed before analysis. Differences between male and female non-breeders were assessed by two-way ANOVA and between male and female breeders by two-way ANOVA with repeated measures. Differences with time within male and female breeding birds were assessed by one way ANOVA with repeated measures, and in non-breeding birds by one way ANOVA. Differences between males (breeders and non-breeders) and between females were assessed by two-way ANOVA.

3. Results

There were significant differences in prolactin concentrations between the sexes in breeding birds ($F_{1,70} = 16.9$ $p < 0.001$) but not between the sexes in non-breeding birds ($F_{1,68} = 1.2$ $p = 0.28$). Breeding females were significantly different from non-breeding females ($F_{1,69} = 45.2$ $p < 0.001$) and breeding males were different from non-breeding males ($F_{1,69} = 22.6$ $p < 0.001$).

The first sampling date was in the latter half of incubation. Female swans incubate the eggs; males defend the nest but do not incubate (Cramp, 1977). At this time prolactin was much higher in incubating females than in their partners (, and higher than in non-breeding females ($p < 0.01$; Fig. 1). The eggs hatched at the end of May. At this time, prolactin concentrations decreased in females as they ceased incubation ($p < 0.01$). In males, prolactin increased at the end of incubation ($p < 0.05$) to values similar to females, as they started to share in the care of the cygnets. During the period of caring for cygnets, prolactin was similar in males and females, and gradually decreased during this period. Non-breeding birds had similar prolactin concentrations to breeding males at the start of the study. Values in both sexes remained fairly constant during May and then gradually decreased, significantly in males ($p < 0.01$) but not in females (because of the final values which showed an unexpected non-significant increase). Non-breeding females started to molt on June 27 ± 4 (S.E.) days and non-breeding males on 3 July ± 4 days. Breeding females started molt on 14 July ± 8 days. Two breeding males had shed their primaries on 16 August, but the other four had not started to molt at this time

4. Discussion

The patterns of molt between the four groups were as expected (see Introduction). There was no significant difference in the time of the start of molt between the sexes of non-breeding birds while breeding females began to molt about 2 weeks later than non-breeding birds. It was not possible to assign an exact mean start date for breeding males because only two of the six birds had dropped their primaries by the last sampling date, but this means that the mean start date must be at least 4 weeks later than their partners, and this is in agreement with Czapulak (2002).

Prolactin secretion is influenced by both photoperiod and parental behavior (Sharp et al., 1998). Increasing photoperiod stimulates secretion, but a longer photoperiod is required than for the stimulation of gonadotrophin secretion. Consequently, in general, peak prolactin concentrations occur later than peak gonadotrophins, and coincide with decreasing gonadotropin concentrations and gonadal regression (Lincoln et al., 1980; Dawson and Goldsmith, 1982). Breeding activity can stimulate prolactin secretion above that caused by photoperiod alone (Dawson and Goldsmith, 1985). In particular the presence of a nest or eggs can stimulate prolactin secretion (Hall, 1987) so that highest prolactin concentrations are found in incubating birds. However, prolactin concentrations are also high in the non-incubating sex, if they take part in parental care (e.g. Dawson and Goldsmith, 1982).

In the Mute swans, highest prolactin concentrations were found in breeding females while

they were incubating, and concentrations decreased when the young hatched. In their male partners, prolactin was no different from that in non-breeding birds during incubation, but increased when they began to care for the young immediately after they hatched. To the best of our knowledge, prolactin has not been measured in Mute swans previously, but it has been measured in breeding Australian black swans (*Cygnus atratus*). In this species, males share incubation (Brugger and Taborsky, 1994). Nevertheless, the broad pattern of changes in prolactin was similar to that in Mute swans (Goldsmith, 1982a). Concentrations were highest in females during incubation and these decreased when young hatched. Peak values in males occurred at the end of incubation.

In all four groups of Mute swans, i.e. breeding and non-breeding males and females, molt started during a period when prolactin concentrations were decreasing. In non-breeding males and females, prolactin concentrations decreased from the end of May. There was no difference in the timing or magnitude of changes in prolactin between male and female non-breeders, and there was no significant difference in the timing of the start of molt. Both sexes started to molt about 4 weeks after prolactin started to decrease.

In breeding females, peak prolactin occurred in the latter half of May, whereas in breeding males, peak prolactin occurred in mid June, 3-5 weeks later than in females. So, although prolactin concentrations were no different between males and females in the period between mid June and mid August, values in females had been decreasing for 3-5 weeks longer, and molt started in females 4 weeks before any male started to molt. Prolactin concentrations were higher in breeding birds of both sexes at the start of their molt, than in non-breeding birds when they started to molt.

Although the aim of this study was to test the relationship between prolactin and the initiation of molt, it has also yielded interesting information on the relationship between prolactin and reproductive behavior. Prolactin concentrations in incubating females were much higher than in non-breeding females, and higher than in their male partners at the same time. This is hardly surprising; prolactin secretion is known to be stimulated by the presence of a nest or eggs, and prolactin drives incubation behavior. During the period that their female partners were incubating, prolactin concentrations males were no higher than in non-breeding males, but when the eggs hatched, prolactin increased. The presence of chicks appears to have stimulated prolactin secretion, and presumably this coincided with a change in behavior from sexual/territorial to parental/defensive.

During the breeding life-history stage of the annual cycle, resources are devoted to the production of offspring, whereas during the post-breeding stage, the emphasis switches to improving the chances of survival of the individual. There is a trade-off between the two (Stearns, 1992) and this is sometimes apparent in the timing and progress of molt. Birds that prolong the breeding stage start to molt later (Morton and Morton, 1990; Hinsley et al., 2003). Birds that start to molt later may molt more rapidly and this may lead to poorer quality plumage, thus decreasing chances of survival (Nilsson and Svensson, 1996; Dawson et al., 2000). In most birds, molt progresses through the sequential loss of feathers and molt rate is increased by molting more feathers at any one time (Dawson, 2004). In waterfowl such as swans, all of the flight feathers are molted at the same time. Breeding swans started to molt later than non-breeding birds. Whether this results in a more rapid molt, e.g. by growing individual feathers more rapidly is, to the best of our knowledge, not known. Perhaps simply molting later in the year imposes a penalty on individual survival. Whatever the mechanism, prolactin may play a key role in this trade-off (Sockman et al., 2006). Prolactin encourages behaviors related to production of offspring, and a

decrease in prolactin permits processes related to self-preservation.

In conclusion, the data from Mute swans do not disprove the hypothesis that the start of molt is related to the timing of peak prolactin, i.e. the time values start to decrease, rather than to absolute values. Decreasing prolactin concentrations in some way either stimulate or permit molt to start. This is a mechanism that could minimize breeding/molt overlap but at the same time ensure that molt starts as soon as breeding activity has finished. The exact nature of this mechanism remains to be determined.

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Figure legend

Figure 1
Changes in circulating prolactin concentrations in breeding and non-breeding Mute swans from the beginning of May, mid way through incubation, until mid August. For breeding birds, the same pairs were sampled repeatedly (n = 6). For non-breeding birds, individuals were caught at random (n = 4-7 for males and n = 4-9 for females). Each point represents the means ± S.E.M. The solid squares represent the date at which molt started in females and the open squares molt start for males. Breeding males started to molt at least 4 weeks later than their partners but there was no significant difference in molt start date between male and female non-breeders.