Female choice and the benefits of mate guarding by male mallards

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(Received 12 December 2000; initial acceptance 17 May 2001; final acceptance 18 February 2002; MS. number: A8944R)

Pair formation and breeding in many species of waterfowl are separated both temporally and spatially. Most studies of female choice in this group have focused on male characteristics at the time of pairing, with less attention given to how mate choice affects breeding season outcomes. In this study I compared pairing success, male plasma testosterone level and mate-guarding ability of male mallards, *Anas platyrhynchos*, in two experiments. In the first experiment females and males were group housed with equal sex ratios, thus allowing all of these males to pair. At the same time, an equal number of males was housed in groups without access to females and remained unpaired. In this experiment testosterone levels of paired and unpaired males during autumn (baseline) and spring (breeding) did not differ, indicating that the process of pair formation and breeding does not cause elevated spring testosterone levels in males. However, testosterone did temporarily decrease in paired males during the winter (pair formation) season. In the second experiment groups were male biased, allowing only half of the males to pair. Here paired males had significantly higher testosterone levels than unpaired males during the breeding season, but not during the preceding autumn. Together the results of these experiments indicate that successful pair formation predicts but does not alter male testosterone level during the breeding season. I also found that females paired to males with high levels of testosterone were missing fewer feathers due to forced copulation attempts by nonmates, suggesting that females may choose males based on their mate-guarding abilities.

Numerous studies of mate choice in mallards, *Anas platyrhynchos*, have indicated that the courtship activity level of the male is a consistent predictor of female mate choice (Bossema & Krujit 1982; Bossema & Roemers 1985; Holmberg et al. 1989; but see Brodsky et al. 1988). Other characteristics such as plumage quality (Klint 1980, 1985; Bossema & Krujit 1982; Holmberg et al. 1989; Weidmann 1990), corticosterone (B) level (Sorenson et al. 1997) and body condition (Wishart 1983; Holmberg et al. 1989) may also be important in this and other duck species, but the evidence for body size (Bossema & Roemers 1985; Holmberg et al. 1989) and dominance (Wishart 1983; Brodsky et al. 1988; Sorenson & Derrickson 1994) is more conflicting. However, all of these studies have considered only those characteristics of males displayed at the time of pair formation, with little attention to male phenotype during the breeding attempt. Pairs may remain together for as much as 6 months or longer each year (Hepp & Hair 1983), during which time males and females synchronize activities, and males begin to display mate-guarding behaviour by remaining close to the female and alert for conspecific intrusions and predators. Mate guarding is important to female breeding condition and success (Ashcroft 1976; Ankney & MacInnes 1978; Seymour & Titman 1978; Titman 1981; Gauthier 1987; Lamprecht 1989; Pattenden & Boag 1989) and is energetically costly for males (Ashcroft 1976; Titman 1981; Lamprecht 1989). Furthermore, in many waterfowl species, mate guarding is the only male contribution to the reproductive effort, aside from donating sperm (Goodburn 1984). Therefore, it would seem important to females to choose mates who guard them well. Moreover, as mate guarding is an aggressive behaviour, and aggression is generally under the control of testosterone (T) in birds (reviewed by Harding 1983), mate choice, male T levels and mate-guarding behaviour may all be associated. In this study I explore the possible link between autumn female mate choice and spring mate-guarding ability, as well as possible hormonal
correlates throughout the pair formation and breeding seasons.

Mate Guarding and Testosterone

Mate guarding in waterfowl is a behaviour that is likely to increase both male and female fitness by serving primarily to increase female foraging efficiency (Ashcroft 1976; Seymour & Titman 1978; Titman 1981; Gauthier 1987; Lamprecht 1989), which may occur via increased male vigilance (Lamprecht 1989) or the dominance of pairs over unpaired birds (Raveling 1970; Paulus 1983; Hepp & Hair 1984). Females with increased nutrient reserves, in turn, lay eggs earlier in the breeding season (Pattenden & Boag 1989) and are more likely to survive the breeding effort (Ankney & MacInnes 1978). In some species mate guarding also protects females from forced extrapair copulation (FEPC) attempts by nonmates (Barash 1977; Mineau & Cooke 1979; Goodburn 1984; Seymour 1990; Sorenson 1994a, b), thereby also protecting paternity. In addition, it has been proposed that mate guarding may help to maintain the pair bond and protect the female from predation (McKinney 1988). In this study, I used protection against FEPC attempts as a proxy for overall mate-guarding ability because birds in a provisioned, captive setting spend less time actively foraging, and therefore foraging differences are not easily measured. Forced extrapair copulation, however, is very common in such densely populated groups (Titman & Lowther 1975; Seymour 1990; personal observation), and protection from FEPC attempts rather than increased foraging efficiency is likely to be the major benefit of mate guarding that females receive in a captive population.

Mate guarding is an aggressive activity, and aggression in mallards is generally well correlated with testosterone (Etienne 1964; Balthazart & Stevens 1975; Schmedemann & Haase 1984); it is therefore likely that mate guarding may be influenced by T as well. Conversely, there are several ways in which experience can influence T levels. For example, during the breeding season, the T levels of territorial males rise in response to a male intruder, a phenomenon known as the ‘challenge effect’ (Wingfield & Moore 1987; Wingfield et al. 1990; Wingfield 1994). Testosterone level may also be influenced by stress. The hormone corticosterone (B) increases quickly in response to stresses such as handling, and can suppress T levels when elevated (Siegel 1971, 1980; Harvey et al. 1980), or B may directly affect behaviour. Care must be taken therefore when attempting to evaluate the influence of endogenous T levels on behaviour. It is possible that mate-guarding behaviour may be under the control of T, but it is important to demonstrate that neither stress nor activities such as pair formation, copulation and mate guarding are affecting T levels.

This study examines two main questions that serve to link mate choice to mate-guarding behaviour. First, do males chosen by females have higher T levels than males not chosen? Second, are males with higher T levels better at mate guarding?

METHODS

Study Site and Population

All experiments were conducted at the Hillebrand Rare Bird Ranch in Cross Plains, Wisconsin, U.S.A. In spring 1997, I raised ducklings from eggs collected from nests on the property for experiment 1. Owing to high nest depredation that year, it was also necessary to purchase 40 day-old ducklings from Whistling Wings Mallard Farm in Hanover, Illinois, U.S.A. I used only first-year birds in the study to control for age and experience effects. Offspring of these birds were used beginning in the spring of 1998 for experiment 2.

In the autumn of each year, all males and females were individually and redundantly marked with coloured nasal markers and matching leg bands. All birds were pinioned and housed in covered, wire mesh outdoor pens with unlimited access to food and to water in small wading pools for drinking and bathing. To control for bird density, pens containing both males and females as described below were approximately twice as large (100 m² and 110 m²) as pens containing males only (50 m² and 56 m²). The birds were exposed to ambient light and temperatures throughout the study. Water in the wading pools was heated as necessary to prevent freezing.

Pairing Effects: Experiment 1

Experiment 1 was conducted to determine whether pair formation or breeding activities can affect male T levels during the breeding season. Here I randomly assigned males to be either paired or unpaired. In November 1997, during the autumn courtship season, each of 34 males was assigned to one of four adjacent home pens without regard to relatedness, as it was largely unknown. There were nine males in each of two pens and eight in each of the other two pens. Each pen was visually isolated from all other pens by means of opaque barriers. After giving the males 2 weeks to become accustomed to the pen and group, I took baseline blood samples from each male to ensure that there were no hormone differences between groups at this time. As mentioned above, stress can affect steroid hormone levels. To avoid possible stress effects due to prolonged disturbance within a pen, I sampled blood as described below from only one randomly selected male per pen per day beginning 21 November until they all were sampled (2 December). All males were bled between 1 and 3 h postsunrise throughout the entire study to minimize the effects of diurnal fluctuations in T seen in this species (Balthazart 1976; Balthazart & Hendrick 1979). Mean sampling duration for each sampling period is reported in Table 1.

Females were introduced into two of the pens on 6 December (eight and nine females, respectively) such that the sex ratio within those two pens was 1:1. These pens are referred to as ‘paired-male’ pens. To ensure that birds in the paired-male pens actually paired and to identify mates, males in these pens were observed for evidence of pair formation using 3-min focal sampling
techniques. Potential mallard pairs are identified easily by: (1) the female inciting display, which indicates preference for a particular male (Lorenz 1953; Weidmann 1956; Johnsgard 1961; McKinney 1992; Sorenson & Derrickson 1994); (2) precopulatory displays (Weidmann 1956; McKinney et al. 1983); and (3) copulation. Pairing was also assessed by the (4) proximity and (5) synchronization of activities of a male and female (McKinney 1992). A pair bond was considered to have formed if the same two birds met any of these criteria during three successive focal sampling periods. These observations continued at least twice per week through the end of March, and at least once per week thereafter to confirm the identity of pairs using the same criteria as above. Much of the behaviour recorded involved the two latter criteria, but the first three criteria, which are more overt indications of mate choice and pair formation, helped to confirm the identity of pairs. Fifteen of the 17 females incited to their presumed mates, 15 pairs performed precopulatory displays and nine attempted or successfully performed at least one copulation during focal sampling observations. Several instances of mate switching did occur during the observation period, using the criteria described above, but all individuals in the paired-male pens had permanently paired by the beginning of the spring sampling period.

As there is some evidence that male mallards can assess the breeding condition of females (Sorenson 1994a; McKinney & Evarts 1998), it was necessary to allow the unpaired males to observe female activities. Therefore, I removed all visual barriers after the autumn sampling so that males in the two unpaired-male pens were still isolated physically but not visually from females, and were therefore able to observe female behaviour and breeding condition. Also, to assay hormone levels across the pair-formation and breeding seasons, I drew additional blood samples from the males in January, February and April 1998 (see below). I was unable to successfully obtain a January blood sample from one unpaired male.

Data were also recorded on male–male interactions to monitor levels of aggression in the pens and ensure the well being of individuals. Preliminary observations indicated very low levels of male–male interactions during the autumn and winter in the unpaired-male pens, presumably owing to the absence of females. Because overall interactions were infrequent in these pens and due to time limitations, unpaired males were observed systematically in 30-min sessions beginning February using ad libitum sampling techniques. By the beginning of the breeding season, however, male–male aggression increased in one of the unpaired-male pens, and three males were removed from that pen before the study ended because they were severely harassed by other males; the final sample size was therefore 31.

**Table 1. Summary of regression of hormone levels on sampling duration**

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Sampling duration Mean±SE</th>
<th>Testosterone</th>
<th>Corticosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F$</td>
<td>$r^2$</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov (unpaired)</td>
<td>192.9±11.4</td>
<td>0.06</td>
<td>0.002</td>
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<tr>
<td>Jan (early pairing)</td>
<td>173.2±9.6</td>
<td>0.45</td>
<td>0.014</td>
</tr>
<tr>
<td>Feb (late pairing)</td>
<td>155.6±7.8</td>
<td>0.46</td>
<td>0.014</td>
</tr>
<tr>
<td>April (breeding)</td>
<td>137.0±6.3</td>
<td>0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov (unpaired)</td>
<td>128.4±9.9</td>
<td>2.81</td>
<td>0.076</td>
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<tr>
<td>April (breeding)</td>
<td>162.1±12.7</td>
<td>0.54</td>
<td>0.024</td>
</tr>
</tbody>
</table>

**Pairing Effects: Experiment 2**

Whereas the goal of experiment 1 was to determine whether pair formation and breeding activities could influence T levels, the goal of experiment 2 was to determine whether female choice was associated with either autumn or spring T level. The methodology in the second year was similar to that of the first year overall except that all males were initially housed together and competed for a limited number of females. In November 1998, I assigned 36 males to one large home pen and gave them 2 weeks to become accustomed to the pen and group. I again drew baseline blood samples, as described below, on four different days between 26 November and 6 December 1998. Because the previous results of experiment 1 indicated that neither handling nor sampling duration affected T levels (see Results), I sampled multiple males from the same pen on the same day. After all males were sampled, I placed 18 females into the pen so that the sex ratio was male biased at 2:1, which was similar to the overall sex ratio in experiment 1. To identify pairs as they formed, birds were observed at least two times per week as described above, but this time females were the focal individuals as they were fewer in number. As in experiment 1, most of the data recorded involved more subtle indications of pair formation, but of the 12 pairs that met the pairing criteria, eight of the females had incited to their mates, 11 pairs were observed performing precopulatory displays and four were observed to have attempted or successfully performed at least one copulation. Two females died over the course of the winter from unknown causes and all males survived.

By the beginning of spring, only 12 of the 16 remaining females appeared to be reliably paired. On 23 March, I removed all 24 unpaired males and the four unpaired females from this home pen (now the paired-male pen) to
create a paired-male and unpaired-male group similar to experiment 1. At this time, I also randomly selected 12 of the unpaired males to be placed in an adjacent, unpaired-male home pen. The remaining unpaired birds were transferred to a distant pen that was visually isolated from the two home pens and were not used further in this study. I did not include four of the 24 males in this selection process either because they demonstrated homosexual behaviour such as precopulatory displays towards one another (Schutz 1965), or because they had been paired with one of the females who had died, making their pair status difficult to determine. I took blood samples from the males again in April 1999 to assay breeding season hormone levels.

**Blood Sampling and Hormone Assays**

To draw a blood sample a field assistant and I stood outside of the home pen and first visually located the randomly determined target male. We then entered the pen and the assistant captured the male with a net. The male was rolled up in a towel with only his head and neck exposed, and I then drew a 2.5-cc blood sample from the jugular vein using a heparinized syringe and 22-gauge needle. I timed this process from the moment we entered the pen until the sample was drawn. During the autumn sampling of experiment 2, I sampled subsequent males while remaining in the home pen and timed the process from the point at which the field assistant began to move towards the next target male. Thus, I recorded both the sampling duration (time involved in chasing and sampling a particular male) as well as the time of day that blood was drawn from each male. After all the samples were drawn for the given day, I centrifuged them for 20 min, and transferred the plasma portions to 2-ml storage vials. I placed these vials in a small cooler with ice packs until they could be stored in a −20 °C freezer later that day.

All samples were analysed for T and B in the summer following their collection at the University of Winsconsin Regional Primate Research Center Assay Laboratory. I extracted the samples and separated the progesterone, dihydrotestosterone, T and B fractions by celite chromatography (Abraham et al. 1972) before assaying using radioimmunoassay techniques (Robinson et al. 1975; Moore 1986) with internal recoveries. Testosterone antibody was purchased from Holly Hill Farms in Hillsboro, Oregon, U.S.A. and the B antibody was purchased from Endocrine Sciences Products of Calbasas Hills, California, U.S.A. Laboratory staff conducted the assay validations. Serial dilutions of the plasma pool (N=7) gave parallelism to the standards for T and B with no differences in slopes (P>0.05). Accuracy for T and B was 104.0 and 99.2%, respectively. Sensitivities of the assays were 5 pg/tube and 36 pg/tube for T and B, respectively.

I analysed two levels of pooled samples for each hormone for the samples from experiment 1. The intra- and interassay coefficients of variation (CVs) for B were 5.97 and 14.84%, respectively, for the low pool, and 3.19% and 10.77% for the high pool. The intra- and interassay CVs for T were 3.50 and 7.91%, respectively, for the low pool, and 2.02 and 7.42% for the high pool. I analysed one pooled sample for each hormone for experiment 2 samples. The intra- and interassay CVs for B were 4.01% and 18.48%, respectively. The intra- and interassay CVs for T were 4.94 and 9.87%, respectively.

**Mate-guarding Success**

Protection from FEPC attempts may be assessed by measuring the plumage quality of the female. A male typically grabs the head or neck feathers of a female as he mounts to copulate. If the attempt is forced, the female often loses some of these feathers as she struggles to escape. Females that frequently are subjected to FEPC attempts often have bald and sometimes bleeding areas on the head, neck or back (Titman & Lowther 1975; McKinney & Evarts 1998; personal observation). Males often attempt to fend off FEPC attacks on their mates with varying success (Mineau & Cooke 1979; McKinney et al. 1983; personal observation). Therefore, the amount of feather loss shown by a female can be used as an indicator of the ability of her mate to successfully guard her from FEPC attempts. Approximately 1 month after females began laying eggs, I captured each female with the help of a field assistant and measured the length and width of the areas on the head, neck and back of the female from which feathers had been pulled. Feathers pulled from these areas are not replaced until the post-nuptial moult, which follows the breeding season. I then calculated the area of feather loss for each area and summed them to yield one measure of total feather loss for each female. All the females were captured and measured on the same day. Mate-guarding success was measured only during experiment 2.

**RESULTS**

**Handling Effects**

**Sampling duration**

Correlations between sampling duration and hormone levels for both years are reported in Table 1. Of the six sampling periods, B levels were correlated with sampling duration only for the first three sampling periods, presumably because sampling duration decreased with experience, such that sampling was completed in the later sampling periods before B increased significantly. There was no correlation between sampling duration and T for any sampling period. For the sake of further analysis, I used B residuals for all experiment 1 sampling periods presented above.

**Time-of-day Effects**

Mallard T level follows a diurnal cycle, with an early morning peak in winter (Balthazart 1976), and both an early morning peak and higher peak in early afternoon in spring (Balthazart & Hendrick 1979). In the autumn sampling period of experiment 2, I sampled as many as 10 males per day, a process that took nearly an hour. I
therefore examined the data for time-of-day effects. Regression analysis of autumn T level versus time of day as measured by minutes postsunrise revealed a highly significant negative effect ($F_{1,34}=19.9, r^2=0.37, P=0.0001$). However, there was no such effect on B during the autumn ($F_{1,34}=0.862, r^2=0.025, P=0.36$). There was also no effect in the spring on either T ($F_{1,22}=0.0819, r^2=0.004, P=0.78$) or B ($F_{1,22}=0.0247, r^2=0.001, P=0.88$). I used the residuals obtained from this analysis for all subsequent analyses involving autumn T levels reported above.

Overall T levels were significantly higher in the spring of experiment 2 than experiment 1 ($F_{1,5,3}=4.05, P=0.049$). However, birds were sampled on average slightly later in the day in experiment 1 than experiment 2 (mean time postsunrise $\pm$ SD: $138.1 \pm 12.42$ min and $100.1 \pm 23.8$ min, respectively). When sampling time postsunrise was included in the ANOVA model as a covariate, the difference in T level between experiments disappeared ($F_{1,5,2}=1.64, P=0.21$).

**Pairing Effects: Experiment 1**

**Testosterone**

Figure 1 shows the mean plasma T levels for paired and unpaired males across all sampling periods. A nested ANOVA indicated there was no effect due to the individual pens, allowing the four pens to be combined into two treatments: paired and unpaired. Repeated measures ANOVA revealed an overall difference between T levels of paired and unpaired males (ANOVA: $F_{1,28}=6.9581, P=0.014$). Pairwise two-tailed randomization tests between paired and unpaired males showed that there was a highly significant difference in January T levels during early pairing ($P=0.0003$), but no difference for the other three sampling periods ($P=0.50, 0.12$ and 0.63 for November, February and April, respectively).

The T levels of the males in the unpaired-male pens decreased significantly between November and each of the other sampling periods (one-tailed paired t test): $t_{13}=2.499, P=0.012$; $t_{16}=2.36, P=0.001$; and $t_{13}=2.33, P=0.002$ for comparisons with January, February and April, respectively). Likewise, unpaired males had significantly different T levels between January and April ($t_{13}=-2.211, P=0.023$) and between February and April ($t_{13}=-3.07, P=0.029$), but not between January and February ($t_{13}=-0.27, P=0.396$).

Only 14 of the 17 males that were paired by January, 10 showed a decrease in T level from November, whereas two unpaired males showed a decrease in T between November and January.

**Corticosterone**

Figure 2 shows the mean B residuals for paired and unpaired males across all sampling periods. Repeated measures ANOVA yielded no significant difference between paired and unpaired males in their overall B residuals (ANOVA: $F_{1,28}=0.372, P=0.55$). Corticosterone residuals were somewhat larger in April, a fact that is consistent with the lack of significant correlation with sampling duration for that sampling. Similarly, T was not correlated with B for any sampling period (regression: $F_{1,32}=0.866, r^2=0.026, P=0.36; F_{1,31}=0.004, r^2<0.001, P=0.95; F_{1,32}=1.01, r^2=0.031, P=0.32; F_{1,25}=0.323, r^2=0.011, P=0.57$ for November, January, February and April, respectively).

**Pairing Effects: Experiment 2**

Figure 3 shows no association between T and pair status in the autumn (ANOVA: $F_{1,20}=0.917, P=0.35$) for the 24
males used in both autumn and spring of experiment 2. Nor was there an association between autumn T and pair status when the 12 unpaired males used only in the autumn were included in the analysis ($F_{1,34}=0.0229$, $P=0.88$). However, by spring, paired males had significantly higher T levels than unpaired males ($F_{1,20}=6.1$, $P=0.022$), in contrast to experiment 1.

**Mate-guarding Success**

Regression of feather loss on male spring T level did not yield a significant linear relationship ($F_{1,10}=2.61$, $r^2=0.207$, $P=0.14$). However, I also divided the females into two groups based on the median amount of feather loss (Fig. 4), and then calculated the mean T level of their respective mates. Females that were relatively well guarded (feather loss below the median) had mates with significantly higher T levels than females that were less well guarded (feather loss above the median) (two-tailed $t$ test: $t_{10}=2.477$, $N_1=N_2=6$, $P=0.034$). No relationship was observed, however, between B and feather loss (two-tailed $t$ test: $t_{10}=−1.309$, $N_1=N_2=6$, $P=0.24$). Thus, although the relationship was not linear, males with higher T levels appeared to be more successful at mate guarding than males with lower T levels.

**DISCUSSION**

The results of this study indicate that females prefer males during the autumn pair formation season that will have high breeding season T levels in the spring, and that these males are more successful at mate guarding than males with low T. This result is important because it not only identifies one possible function of female choice in waterfowl, but it also describes a choice that is predictive.
That is, females are making decisions in the autumn that bear on their own future reproductive success in the spring.

**Mate Choice and Mate-guarding Success**

Males that paired successfully when competing for a limited number of females during the autumn and winter courtship season (experiment 2) had higher spring T levels than unsuccessful males. This result contrasts with the results of experiment 1: when pair status was randomly predetermined, there was no difference in spring T levels between paired and unpaired males, indicating that pair activities during the breeding season, such as copulation and mate guarding, did not affect T levels, as occurs in numerous other species (for a review see Wingfield 1994). In contrast, Hirschenhauer et al. (2000) found that pair status did appear to modulate T levels in male greylag geese. However, paired and unpaired males in that study were self-selected (as in experiment 2 here), and it is instead possible that the female geese preferred high T males.

It is also possible that the difference in results between experiments 1 and 2 was caused by differences in male–male aggression owing to differences in sex ratios between experiments (1:1 versus 2:1), but this seems unlikely for several reasons. First, when the time of day that sampling occurred was included in the analyses, no difference in spring T levels existed between years, indicating that possible differences in competition between years did not result in overall differences in T levels. Rather, the apparent difference in overall T levels between experiments (cf. Figs 1 and 3) is likely due to the fact that T levels fall throughout the morning during the spring in mallards (Balthazart & Hendrick 1979), and males in experiment 1 were sampled slightly later in the morning on average than males in experiment 2. Second, the winter hormonal profile of paired males in experiment 1, in which the sex ratio was 1:1, closely mirrored the results of Sorenson et al. (1997). In that study the T levels of chosen male northern pintails, *A. acuta*, decreased following mate choice trials in which the sex ratio was 3:1 (discussed more fully below). Thus, the effects of competition on hormone levels found in northern pintails were similar to those observed in experiment 1 of the current study despite the extreme difference in sex ratios between studies. Finally, all unpaired males in experiment 2 were removed and housed separately from the paired birds prior to the spring breeding season, making the sex ratios and presumably the aggression levels during the spring similar in both experiments when the spring blood samples were taken.

Males with higher spring T levels were more successful at mate guarding than males with lower T levels. Several studies of passerine birds have found that male T level is correlated with the amount of time males spend mate guarding (white-crowned sparrows, *Zonotrichia leucophrys*: Moore 1984; barn swallows, *Hirundo rustica*: Saino & Möller 1995). However, the present study is the first in which T levels were compared to a measure of mate-guarding success. That T was associated with mate-guarding success is not a surprising result, as mate-guarding is an aggressive activity, and, as mentioned above, aggression in reproductive birds is typically under the influence of T (Etienne 1964; Balthazart & Stevens 1975; Schmedemann & Haase 1984; Wingfield & Moore 1987; Wingfield et al. 1990).

The data in the current study together suggest that sexual selection may operate on male mate-guarding behaviour in waterfowl. Indeed, it has been proposed that the fitness benefits of mate guarding in migratory waterfowl may drive both the monogamous mating system and early timing of pair formation in this group (Rohwer & Anderson 1988; Oring & Sayler 1992). In addition, Sorenson (1992) found that polygynous white-cheeked pintails, *A. bahamensis bahamensis*, are more effective at mate guarding than males that have only one mate. Assuming that males that attract and secure multiple mates indicate preferred males, her results suggest that selection for mate-guarding behaviour may also operate on sedentary waterfowl species.

It is also possible that some females in the present study were better than others at avoiding FEPC attempts, and that such females paired assortatively with males that had high spring T levels. This interpretation still begs the question of why females would choose high T males if not for their aggressive defence. One possibility is that females are choosing the indirect benefit of good genes. However, this hypothesis appears unlikely in this species, as Cunningham & Russell (2000) showed that differences in offspring condition in this species could not be attributed to paternal effects, and Cunningham & Cheng (1999) demonstrated no postinsemination female preference based on sperm genotype. Moreover, the females of waterfowl species do not solicit extrapair copulations, as might be expected if good genes were important (Birkhead & Möller 1996).

**Autumn T Levels and Pair Formation**

No difference in autumn T levels was found between paired and unpaired males in either year, although autumn T levels were negatively correlated with the time of day that blood was sampled in experiment 2. This correlation was unlikely to be due to a stress response, however, because there was no such association between B and either time of day or sampling duration. Rather, the observed correlation was more likely the result of the natural diurnal fluctuation in T level in mallards (Balthazart 1976; Balthazart & Hendrick 1979), in which winter androgen levels peak in the morning and fall steadily throughout the day.

The lack of association in experiment 2 between autumn T level and pair status was unexpected, given that several studies directly associated autumn and winter T levels with female preference (Klint 1985; Klint et al. 1989), or indicated that the displays themselves may be under the control of testosterone or its metabolite, dihydrotestosterone (Phillips & McKinney 1962; Etienne 1964; Balthazart & Hendrick 1979; Schmedemann & Haase 1985; Klint et al. 1989). There is also evidence that at least one of the major courtship displays in mallards is
used agonistically among males (Davis 1997), indicating that aspects of mallard courtship are aggressive. It may be that female preference is a separate issue from actual pair formation, but despite the lack of evidence in the current study, T may still be a physiological link between courtship, pair formation and mate-guarding behaviour. Regardless, male guarding is an energetically costly activity (Ashcroft 1976; Titman 1981; Lamprecht 1989), and courtship activity is affected by male body condition (Klint et al. 1989; Holmberg et al. 1989). Therefore, courtship activity may be the best predictor females have of both male condition and mate-guarding ability in the spring.

Although the data in experiment 1 indicate that behaviour such as copulation and mate guarding do not cause elevated T levels in paired males during the breeding season, the process of pair formation evidently caused a decrease in T during the autumn and winter courtship season. This result supports the findings of Sorenson et al. (1997), in which the T level of the closely related northern pintail, (A. acuta), showed a significant decrease within 2 h after being introduced to and chosen by a female, whereas the T levels of unchosen males did not show this decrease. Thus, upon being chosen as mates, the T levels of males decreases very quickly and remains lower than unpaired males for at least several weeks.

Together, the results of this study and that of Sorenson et al. (1997) suggest a previously unknown feedback mechanism involving courtship and pair formation in ducks. Although much work on this system is still necessary, the simplest scenario that best reconciles the literature is that: (1) androgens (T or DHT, or both) are positively associated with male courtship display activity in ducks; (2) females prefer males that court actively, and thus, prefer males that are in good condition and have relatively high androgen levels; (3) once a pair bond begins to form, a feedback mechanism triggers a decrease in androgen level in the male; and (4) the male decreases his courtship activity in favour of pair formation activities. Sorenson et al. (1997) posited that the decrease in T was owing to an increase in B levels caused by male–male competition for the female. If so, it is not likely that suppression is maintained by B as paired males in the current study did not have significantly higher B levels than unpaired males in January, although T levels were lower in paired males. Such a feedback mechanism is a classic example of behaviour affecting hormone physiology (see Lehrman 1965 for an early synthesis), but its involvement in waterfowl courtship and pair formation is new.

In summary, males that paired successfully during the autumn had higher spring T levels than unpaired males. Although breeding season activities probably did not elevate T levels in paired males, initial pair formation during the autumn and winter did lead to a temporary decrease in T levels. Also, high T males were better at mate guarding than low T males. These results together suggest that females choose males that will have high T in the spring because they will mate-guard more effectively than low T males.

Acknowledgments

I thank my graduate committee for their patience, advice, guidance and feedback over the years: Jeffrey Baylis, Jack Hailman, Robert Jeanne, Robert Bleiweiss, Charles Snowdon and Thomas Kurtz. I am also thoroughly indebted to Catherine Marler and Lisa Sorenson their help with methodology, and to David Abbott, Toni Ziegler and Daniel Wittwer of the Wisconsin Regional Primate Research Center Assay Services for recognizing that mallards might be distantly related to primates. Helpful comments on earlier versions of this manuscript were made by J. Baylis, C. Marler, Janet Bester-Meredith, Brian Trainor, Laurene Ratcliffe and two anonymous referees. I was also fortunate to have a wonderful group of undergraduate volunteers, who suffered through early mornings in the cold Wisconsin winter. This work was supported by grants from Sigma Xi, the Animal Behavior Society and monies from the UW-Madison Zoology Department.

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