

Interestingly, we experienced similar problems recovering DNA from preserved nestling blood of Eastern Kingbirds, but not of Least Flycatchers. We also are aware of similar difficulties with Acadian Flycatchers (*Empidonax vireescens*) and other populations of kingbirds (R. Fleischer, pers. comm.). As yet, we do not know why nestling blood samples of these species should be unusually unstable. We recommend that anyone planning DNA research with tyrant flycatchers conduct their DNA extractions as soon as possible after collection, or test alternate methods of preservation or tissue collection and extraction beforehand.

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WINTER ANDROGEN LEVELS AND WATTLE SIZE IN MALE COMMON PHEASANTS

ANNA PAPESCHI, FEDERICA BRIGANTI AND FRANCESCO DESSI-FULGHERI

*Department of Animal Biology, University of Florence, Via Romana 17, 50125 Florence, Italy,
e-mail: dessi@dbag.unifi.it*

Abstract. We report the results of morphological and hormonal measurements of 101 male Common Pheasants (*Phasianus colchicus*) captured during winter and at the beginning of the breeding season in order to identify correlates of ornament size. Androgen levels in January were bimodally distributed with one group with low hormone levels and a second group with high levels. In February, log transformed androgen levels were normally distributed, with all males showing values similar to the high-level group in January. Wattle size was positively correlated with androgens in January but not in February, suggesting that this male trait can indicate the ability of quality males to start earlier androgen production.

Key words: *androgens, Common Pheasant, ornaments, Phasianus colchicus, sexual selection, wattles.*

Recent experiments have shown that in the Common Pheasant (*Phasianus colchicus*) female choice is influenced by features of some male ornaments, such as tail length and the presence of black points in the wattle (Mateos and Carranza 1995, Mateos 1998), and that male dominance rank depends on wattle size (Mateos and Carranza 1997). Moreover, von Schantz et al. (1989) observed a relationship between male spur length and harem size in natural conditions, even though the role of spur length on female choice in the Common Pheasant has been challenged by the results of recent experiments (Hillgarth 1990, Mateos and Carranza 1996).

It is still not clear what a female gains from ornament-based mate choice in a species like the Common Pheasant nor why male dominance is based on the size of the wattle, a “soft part” that is not used in fighting. Indeed, there is a lack of data on the correlates of male ornaments in this species, especially under natural conditions. Recent studies have failed to clarify the relationships between male ornaments and territory qual-

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ity, and the role of territory quality during female choice (Grahn et al. 1993a, 1993b).

In this paper we report the results of morphological and hormonal measurements of 101 wild Common Pheasants captured during winter and at the beginning of the breeding season. Our aim was to identify physical and hormonal correlates of ornament size. Such correlations could provide some explanation of the possible adaptive aspects of the use of ornaments by females in choosing mates and by males in establishing a hierarchy.

METHODS

STUDY AREA AND PERIOD

Male Common Pheasants were trapped in the Mezzano protected area, near Ferrara in the Po Valley, Northern Italy. This 175 km² area consists of an intensive agricultural environment, mainly cereal fields, and supports a wild population of about 40,000 pheasants.

DATA COLLECTION

Measurements were carried out in two sessions, the first 22–23 January 1990 when we measured 61 males, and the second at the very beginning of the breeding season 20–22 February 1990 when we measured 40 males. None of the males measured in January was recaptured in the second session so that the two data sets were independent. Traps were set in the morning between 10:00 and 11:00 and checked between 17:00 and 18:00. Common Pheasants emerge to feed in the very first and last hours of daylight (Dalke 1937). Although the traps were set for 7–8 hr, we estimated an average time in the trap of 2–3 hr. To minimize stress, traps were small aviaries large enough (2 × 2 × 1.5 m) to allow normal movements, and were provided with water, grass, and branches.

MORPHOLOGICAL MEASUREMENTS

Tail, wing, and tarsus lengths were measured with an accuracy of 1 mm; spur length, tarsus diameter, vertical axis of the wattle, and shaft width of the first proximal primary feather (used only for age determination) were measured with an accuracy of 0.1 mm; body mass was measured to the nearest 10 g. In a preliminary study, we found a significant correlation between the area of the wattle (measured by means of photographs and the image analysis program Optilab) and the length of its vertical axis ($r_{51} = 0.37$, $P < 0.01$). It is almost impossible to measure the engorged wattle of courting males because of its complex surface and the movements of the displaying animals. Thus, we assumed that the length of vertical axis of the resting wattle is a reliable indicator, as a measure of the quantity of tissue, of the size of the engorged wattle during display.

Birds were classified as yearlings (<20 months old) or adults (≥20 months old) on the basis of the shaft width of the first proximal primary feather of the wing (Greenberg et al. 1972).

HORMONE RADIOIMMUNOASSAY (RIA)

Blood samples were taken from each animal via the alar vein between 19:00 and 23:00, and each bird was caged in a box for approximately 2 hr after trapping

and bled at the beginning of the measurements. Plasma androgen levels (testosterone plus 5 α -dihydrotestosterone (5 α -DHT)) were determined in triplicate from a single 200 μ l plasma sample after extraction with methylene chloride. The range of recovery of the internal standard was about 70–80%. RIA was performed on the dried extracts using tritiated testosterone (specific activity 93 Ci mmole⁻¹). Separation of free and antibody-bound steroids was obtained by a charcoal-dextran mixture. The antiserum for testosterone had a very low cross-reaction with other steroids (<0.2%), except for a 100% cross-reaction with 5 α -DHT. We were thus able to measure testosterone plus 5 α -DHT (Lupo et al. 1990). The sensitivity of the assay system was 10 pg, the intra-assay and inter-assay coefficients of variation were 5–7% and 10–12%, respectively. The upper limit in the assay was about 400 pg/tube (= 20,000 pg ml⁻¹).

STATISTICAL ANALYSES

Probability tests are two-tailed. Data were examined for departures from normality by preliminary graphing and testing of data. If necessary, data were normalized by log transformation. Because parametric assumptions were not met, the Mann-Whitney *U*-test was used to compare androgen levels between months. A two-way analysis of variance (ANOVA) was used to test for differences in parametric variables between months and between age classes. Multivariate general linear models were performed to determine ornament correlates (MGLH-module, stepwise option, of SYSTAT). Age was forced in all models to control for any age-dependent variation in male morphology. When a structure was damaged, its measure was eliminated from the data set.

RESULTS

ANDROGEN LEVELS

Androgen levels in January were not normally distributed, even after log transformation (Shapiro-Wilk *W*-test, $W = 0.87$, $P < 0.001$). There was a bimodal distribution with one group of males with low levels of androgens (mode = 60 pg ml⁻¹) and a second group with high levels (mode = 2,200 pg ml⁻¹). In contrast, log transformation resulted in normally distributed androgen levels in February (Shapiro-Wilk *W*-test, $W = 0.96$, $P = 0.20$), with a mode of 1,820 pg ml⁻¹ (Fig. 1).

In February, there was a general increase of androgen levels, with all males showing values similar to the high-level group in January ($Z_1 = -5.0$, $P < 0.001$) and there was a corresponding decrease in variance ($F_{59,38} = 2.3$, $P < 0.01$) (Fig. 1).

MALE MORPHOLOGY

Male morphology did not change significantly from January to February. However, vertical wattle size tended to increase ($P = 0.12$), and tail length to decrease ($P = 0.09$); moreover adults were heavier, had larger vertical wattle, longer spur, and tended to have longer wings and longer and wider tarsus; tail length did not differ between the two age-classes (Table 1).

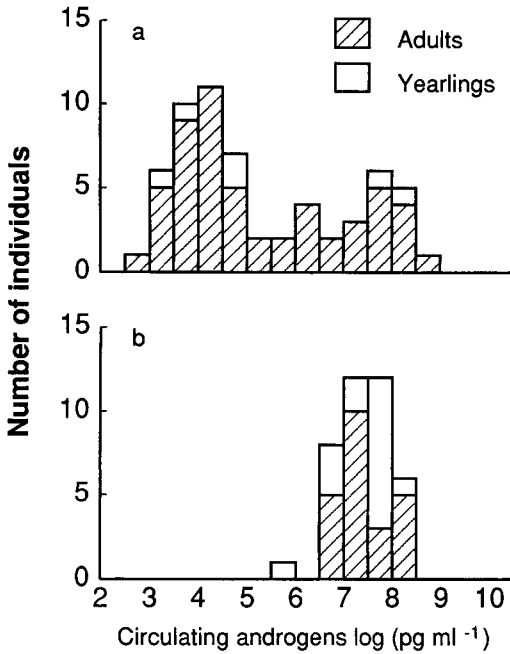


FIGURE 1. Distribution of plasma androgen levels of 101 wild male Common Pheasants measured in (a) January ($n = 61$) and (b) February ($n = 40$) in the Mezzano protected area of Northern Italy, 1990.

CORRELATES OF ORNAMENT SIZE

The model of vertical wattle size against the predictors was significant ($F_{4,54} = 9.4, P < 0.001$) and explained 41% of the total variance of vertical wattle size. Predictors were body weight ($t = 3.0, P < 0.01$), spur length ($t = 2.2, P < 0.05$), and androgen levels ($t = 2.4, P < 0.02$). Age was forced in the model but was not correlated with vertical wattle size ($P = 0.76$). Wing, tarsus, and tail length, and tarsus diameter did not enter in the model.

The model of spur length against predictors also was significant ($F_{4,55} = 10.9, P < 0.001$) and explained 44% of the total variance of spur length. Predictors were body weight ($t = 2.48, P < 0.01$), tail length ($t = 2.78, P < 0.01$), and vertical wattle size ($t = 2.18, P < 0.05$). Age was forced in the model but was not correlated with spur length ($P = 0.66$). Wing and tarsus length, tarsus diameter, and androgen levels did not enter in the model.

The model of tail length was significant ($F_{3,57} = 10.9, P < 0.001$) and explained 36% of the total variance of tail length. Predictors were spur ($t = 4.43, P < 0.001$) and wing length ($t = 3.15, P < 0.01$). Age was forced in the model but was not correlated with tail length ($P = 0.49$). Neither the other morphological predictors nor androgen levels were correlated with tail length.

The model of vertical wattle size in February against the predictors was significant ($F_{2,37} = 7.7, P < 0.01$) and explained 29% of the total variance of vertical wattle size. Predictors were age ($t = 2.7, P < 0.01$)

TABLE 1. Two-factor ANOVA showing the comparison between January and February male morphology of adult and yearling wild pheasants. Values are means \pm SE.

Variable	January		February		F (month)
	Yearlings (n = 6)	Adults (n = 55)	Yearlings (n = 16)	Adults (n = 24)	
Body weight (g)	1,550.0 \pm 53.9	1,599.4 \pm 17.8	1,433.7 \pm 33.0	1,600.8 \pm 27.0	1.0
Wing length (cm)	24.3 \pm 0.5	25.2 \pm 0.2	24.8 \pm 0.3	25.5 \pm 0.3	1.2
Tarsus length (mm)	91.1 \pm 1.9	93.6 \pm 0.6	91.0 \pm 1.2	93.2 \pm 0.9	0.0
Tarsus diameter (mm)	10.5 \pm 0.3	10.7 \pm 0.1	10.1 \pm 0.2	10.6 \pm 0.1	2.7
Vertical wattle (cm)	3.6 \pm 0.2	3.8 \pm 0.1	3.7 \pm 0.1	4.1 \pm 0.1	2.5
Tail length (cm)	50.7 \pm 2.3	51.2 \pm 0.8	47.5 \pm 1.4	49.2 \pm 1.2	2.9
Spur length (mm)	10.3 \pm 1.0	11.6 \pm 0.3	9.9 \pm 0.6	12.6 \pm 0.5	8.5**
					F (age)
					13.6***
					5.0*
					3.7
					3.6
					4.1*
					0.6
					8.5**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and tail length ($t = 2.6$, $P < 0.02$). Neither the other morphological predictors nor androgen levels entered in the model.

The model of spur length against predictors also was significant ($F_{2,37} = 11.5$, $P < 0.001$) and explained 38% of the total variance of spur length. Predictors were age ($t = 3.43$, $P < 0.001$) and tarsus length ($t = 2.20$, $P < 0.05$). Neither the other morphological predictors nor androgen levels entered in the model.

The model of tail length was significant ($F_{2,37} = 5.6$, $P < 0.01$) and explained 23% of the total variance of tail length. Predictor was body weight ($t = 3.2$, $P < 0.005$); age was forced in the model but was not correlated with tail length ($P = 0.32$). Neither the other morphological predictors nor androgen levels were correlated with tail length.

DISCUSSION

The bimodal distribution of androgen levels in January is new and unexpected and indicates that gonad recrudescence takes place very early in some males. In their study of the annual cycles of sex steroids in the Japanese Common Pheasant (*Phasianus colchicus versicolor*), Sakai and Ishii (1986) showed that the level of testosterone in males remains below 200 pg ml⁻¹ until mid-February and then rises above 1,000 pg ml⁻¹ in March. The difference between our results and those of Sakai and Ishii (1986) could be due to the absence of male-male competition in the controlled aviary conditions of their study, in which each male was housed with six females in an aviary separate from the other males. Later in February, plasma androgen levels were high in all males and this is consistent with the well known seasonal increase of circulating androgen levels in male vertebrates in preparation for the reproductive season.

The high androgen levels in some of the males in January raise two major points: (1) why do some males increase their androgen levels so early? and (2) why does this happen only in some males? The relationship among androgen levels, dominance rank, and territoriality is well documented in other galliformes (Watson 1970, Ramenofsky 1984). In the Common Pheasant, the importance of an early gonad recrudescence leading to an early activation of agonistic and territorial behavior is highlighted by the results of Grahn et al. (1993a). They showed experimentally the importance of prior residency in male pheasants, when early released males could not be displaced from their territories by late released males.

Given these advantages, the question remains as to why all males do not start as early as possible. Perhaps maintaining high circulating androgen levels involves high costs that not all males can tolerate for the same period of time. Indeed, high levels of androgens are associated with a variety of costs, such as increased metabolic rate (Vehrencamp et al. 1989), male-male competition (Dufty 1989, Ketterson et al. 1992), decrease fat storage (Ketterson et al. 1991), and partial immunosuppression (Folstad and Karter 1992, Zuk et al. 1995, Salvador et al. 1996). Recent experiments on the Common Pheasant have shown that testosterone administration at the end of the winter has a differential effect on parasite load as a function of male rank:

subordinant, nonterritorial, "sneaker" males suffered a strong increase in parasite load during the breeding season, whereas territorial males tolerated the treatment well (Papeschi 1998, Papeschi et al. 1998).

These studies indicate that high levels of androgens are costly; thus males producing high androgen levels for a longer time may be good quality males. Our data indicate that the early onset of androgen production in January is signaled by an increase in the size of the wattle. In a recent experiment, we showed the effect of testosterone plasma levels on wattle size in male pheasants: an artificial administration of testosterone was followed by a significant increase in wattle size, the only male morphological trait influenced by the hormone. In particular, wattle size increased significantly when the level of T exceeded a threshold of around 1,200 pg ml⁻¹ (Briganti et al. 1999). In the present study, androgen levels of the January high-level group exceeded this threshold. In natural conditions, the variation in wattle size could be due to many factors, not least of which is ambient temperature given that cavernous tissues underlie the wattle (C. Mateos, pers. comm.), and this is likely the reason for the low percentage of wattle size variance explained by the model (41%).

The role of wattles as a male quality signal also is confirmed by the correlation between wattle size and body mass. Because the wattle is not correlated with body size, we can attribute this correlation to the component of body mass that depends on the male's physical condition in terms of fat reserves and muscular development.

Our results suggest the possible advantages males can gain from a wattle-size based hierarchy (Mateos and Carranza 1997). In the Common Pheasant, male-male competition begins at the end of the winter and lasts until the end of March; male fightings are intense and the loser can be severely wounded (Hill and Robertson 1988, Papeschi 1998). Low quality males avoid fights with large wattle males, thereby reducing the risk of severe injuries and defeat. Vertical wattle size measured at the end of the winter is the only reliable predictor of the rank a male gains later in spring (Papeschi 1998).

Later in February, none of the measured male traits was correlated with androgens, and only tail length, among male ornaments, was correlated with body mass. Females selecting long-tailed males may gain the advantage of mating with males in better physical condition. Whether or not these traits are heritable, females gain a direct advantage from preferring such males because these males are a good defense against subordinate sneaker males; in this species sneaker males are particularly aggressive and attack the female in a violent and prolonged manner in the course of the day, significantly disturbing the female's feeding activity during the pre-laying period (Ridley and Hill 1987; Papeschi, pers. observ.).

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