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# Short communications

## Plasma testosterone levels of incubating female Northern Lapwings *Vanellus vanellus*

Gaute Grønstøl<sup>1</sup>, Ole Langeland Myking<sup>2</sup>, Terje Lislevand<sup>1,3</sup> & Ingvar Byrkjedal<sup>3\*</sup>

<sup>1</sup> Department of Biology, University of Bergen, Allégaten 41, N-5007 Bergen, Norway

<sup>2</sup> Institute of Clinical Biochemistry, Haukeland Sykehus, N-5021 Bergen, Norway

<sup>3</sup> Department of Natural History, Bergen Museum, University of Bergen, Muséplass 3, N-5007 Bergen, Norway

\*) Corresponding author; e-mail address: [ingvar.byrkjedal@zmb.uib.no](mailto:ingvar.byrkjedal@zmb.uib.no)

Levels of plasma testosterone (T) have in numerous studies been shown to predict aggression level and dominance of interacting birds (Beletsky *et al.* 1995, and references therein). In birds, T levels vary seasonally and peak in the mating season (Wingfield *et al.* 1987, Beletsky *et al.* 1995). Most studies have focussed on male T levels in monogamous or polygynous species. As in males, female T levels may play an important role in the establishments of female dominance hierarchies within polygynous dyads, and may also be predictive of females' abilities to enforce monogamy through deterrence of prospecting secondary settlers. (Grønstøl 2001). We are only aware of three studies that have addressed T levels in polygynous females; two on the Red-winged Blackbird *Agelaius phoeniceus* (Searcy 1988, Cristol & Johnsen 1994) and one on Dunnocks *Prunella modularis* (Langmore *et al.* 2002). In both species T levels vary over the season in a manner apparently related to intensity of inter-female competition, and experimental elevation of female T levels increased female aggression level in the Red-winged Blackbirds (Searcy 1988).

Northern Lapwings *Vanellus vanellus* share some breeding characteristics with the Red-winged Blackbird, namely resource polygyny (Berg 1993, Byrkjedal *et al.* 1997, Parish *et al.* 1997, Liker & Székely 1999, Hafsmo *et al.* 2001) and inter-female aggression (Liker & Székely 1997, Parish *et al.* 1997, Grønstøl 2001, Grønstøl *et al.* 2003). Northern Lapwing females attempt to evict prospecting secondary settlers. Sometimes they are successful at this, and sometimes they

are not (Grønstøl 2001). On several polygynous territories friction between the females persists throughout the incubation period (Grønstøl 2001). If the T level is a true correlate of abilities to monopolize breeding resources, one should expect that female T levels correlate with condition estimates. If T levels show short-term fluctuations in response to varying intensity of social instability (the Challenge Hypothesis: Wingfield *et al.* 1987) one would expect higher T levels in polygynous females than in monogamous females. We analysed blood samples of 10 females (six from monogamous and four from polygynous pair bonds) for T levels in relation to mating status and size and condition measurements.

We trapped 10 incubating female Lapwings on their nests at two sites in western Norway (six in 1995 at Haukås, 60°19'N, 5°29'E, and four in 1998 at Gimramyra, 58°47'N, 5°37'E). Blood samples were taken within 15 minutes after trapping. Trapping was conducted between 11:00h and 19:00h, and nest age from first egg laid ranged from 11 to 28 days. Samples were centrifuged immediately or stored on ice and centrifuged within four hours. Upon analysis, plasma samples were stored at -20°C.

Testosterone was measured in Lapwing serum by a commercial radioimmunoassay kit (Spectria - Testosterone [<sup>125</sup>I] from Orion Diagnostica, Espoo, Finland). 25 µl serum and 500 µl of <sup>125</sup>I-labelled testosterone were added to tubes coated with polyclonal rabbit anti-testosterone antibody. After incubation and washing away unbound antigen, the bound radioactive testoster-

one in the antibody-coated tubes was counted in a  $\gamma$ -counter. The actual concentration was obtained by means of a standard curve based on known concentrations of an unlabelled antigen run in parallel with the unknowns. The sensitivity of the method is 0.2 nmol/L. Apart from 4.5% cross-reactivity with 5 $\alpha$ -dihydrotestosterone there is no relevant cross-reactivity with other steroid hormones. The intra and inter-assay coefficient of variation were 6.8 and 7.0 % at the level of 5 nmol/L, respectively.

Female T levels averaged 0.65 nmol/l (SD = 0.34), equivalent to a concentration of 0.19 ng/ml, which is two to three fold higher than levels reported for incubating female Red-winged Blackbirds (0.07ng/ml: Cristol & Johnsen 1994). In comparison, the T level of one monogamous Lapwing male was 8.4 nmol/l (2.42 ng/ml). The coefficient of variation of the measurements was high (52%) which indicated a high degree of individual variation.

A positive correlation between body mass and T level is expected if the amount of T-mediated breeding costs is negatively correlated with body mass, i.e. that a heavy female with a high T level would experience lower breeding costs than a small female with the same T level. There was no significant correlation between T level and body mass (Fig. 1, Spearman's Rank test:  $n = 10$ ,  $\rho = 0.53$ ,  $P = 0.11$ ). The heaviest bird showed a low testosterone level, making the corresponding data point appear to deviate from the others. This female was trapped and sampled at a later stage of incubation than the rest (three days before hatching and six days later than the latest of the other nine females). Hence, her T value might have been affected more than the other samples by a general reduction in T levels over the incubation period, which was found to be the case in Red-winged Blackbirds (Cristol & Johnsen 1994). If disregarding this data point, the correlation was significant ( $P = 0.01$ ). There was, however, no reason to suspect that the data point represented an erroneous observation, and it should therefore not be omitted. T levels were not related to body condition, measured as the residual of body mass regressed on wing length (Spearman's Rank test:  $n = 10$ ,  $\rho = 0.24$ ,  $P = 0.50$ ).

Sample sizes were too low to enable estimation of between-site and between-season effects, but a visual inspection of the data indicated that measurements from the two sites were reasonably well interspersed (Fig. 1).

A comparison of four females mated with polygynous males and six females mated with monogamous males did not reveal signifi-

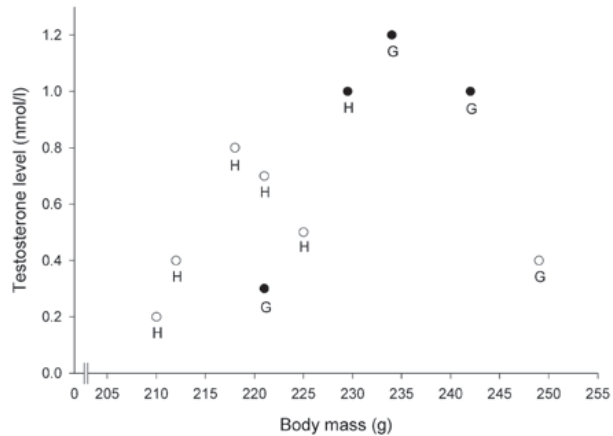


Figure 1. The relationship between body mass and plasma testosterone level in female Northern Lapwings. Open circles: females mated with monogamous males; filled circles: females mated with polygynous males. Letters H and G denote sampling location (Haukås near Bergen and Gimramyra at Jæren).

Forholdet mellom kroppsvekt og nivå av plasmatestosteron hos vipehunner. Åpne sirkler viser hunner i par med monogame hanner og fylte sirkler hunner i par med polygyne hanner. Bokstavene H og G angir lokalitet hvor prøvene ble samlet inn (Haukås og Gimramyra).

cant differences, although a tendency was found for the former to have higher T levels than the latter (Fig. 1, t-test (two tailed):  $df = 8$ ,  $t = 1.95$ ,  $P = 0.087$ ). This tendency, although admittedly very tentative, could be explained by aggressive interactions during incubation being more frequent in females from polygynous matings than in monogamous females. This would be consistent with the Challenge Hypothesis (Wingfield *et al.* 1987), where T levels are thought to increase in periods of high social instability when friction between individuals is frequent. This reasoning assumes that increased T levels confer a cost. If not, females would probably benefit from maintaining constant high T levels. Certain trade-offs between costs and benefits of high T levels seem to exist. Experimental elevation of T levels in females inhibited nest-building and egg-laying in Red-winged Blackbirds (Searcy 1988), and inhibited incubation behaviour in males of the sex-reversed Spotted Sandpiper *Actitis macularia* (Oring *et al.* 1989). If high T levels are incompatible with egg production and incubation behaviour, it should be prudent for females to keep the T level at a minimum during this period. Polygynous females might balance these costs against those of reduced

access to breeding resources (e.g. feeding areas, male attention during incubation and chick rearing) they may have to endure if remaining timid and avoiding confrontations with other females on the territory.

We realize that sample sizes in this study are limited, which yields low testing power. Nevertheless, we think it is important to report T-levels of female members of polygynous species. This has traditionally been given little consideration, even though it is as likely to influence mating dynamics as male T levels. It would probably be rewarding to closer examine female T levels both in Northern Lapwings and other polygynous species, and relate them to individual behaviour and manifestations of conflicts of interests within and between sexes.

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## SAMMENDRAG

### Plasmatestosteron-nivå hos vipehunner i rugetiden

Mange fuglestudier har rapportert plasmatestosteron-nivåer (T) hos polygyne hanner, men bare ganske få har rapportert tilsvarende T-nivåer hos hunner i polygyne parforhold. Det er sannsynlig at T-nivåer spiller en viktig rolle under fastsettelsen av ranghierarkier hos hunner som risikerer å måtte dele hekkeressurser fordi maken deres er sosialt polygam. Hos vipe, hvor polygyni er vanlig, forekommer slik deling ofte. I dette studiet presenterer vi plasmatestosteron-nivåer (T) for 10 hunnviper under rugeperioden på Jæren og ved Bergen, og undersøker i hvilken grad disse T-nivåene varierer systematisk med størrelse, kondisjonsestimater, og monogamt eller polygynt parforhold. Vi sammenligner også hunnenes testosteronnivå med nivået hos en hannvipe.

Testosteronnivået ble målt i blodserum fra viper fanget på reir 11-28 dager etter legging av første egg. Blodprøvene ble sentrifugert og frosset ned. Nivået i prøvene ble fastslått ved hjelp av et kommersielt tilgjengelig sett for radioimmunoassay (Spectria - Testosterone [<sup>125</sup>I]).

Vi fant et gjennomsnittlig testosteronnivå hos vipehunner på 0.65 nmol/l, som er mer enn dobbelt så høyt som det som er rapportert for hunner av den sterkt polygyne arten rødvingetrupial. Hos vipehannen som ble undersøkt lå nivået på 8.4 nmol/l, nesten 13 ganger høyere enn hunnenes gjennomsnitt. Vi fant ingen sammenheng mellom vipehunnens testosteronnivå og deres

kroppsvekt eller kondisjonsmål (basert på residualen fra regresjon av kroppsvekt på vingelengde), men det var en svak tendens til høyere testosteronnivå hos hunner i polygyne parforhold enn hos monogame hunner, noe som kan henge sammen med at hunnene til polygyne hanner ofte er aggressive seg imellom. Materialet er imidlertid for lite til å gi sikre konklusjoner om dette.

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