

crossing a significant marine barrier<sup>1</sup>.

**David W. Krause**

Department of Anatomical Sciences,  
Stony Brook University, Stony Brook,  
New York 11794-8081, USA

e-mail: dkrause@mail.som.sunysb.edu

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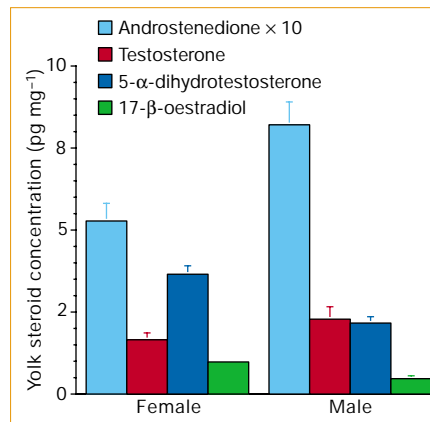
Maternal investment

## Sex differences in avian yolk hormone levels

It has been suggested that female birds put more resources into eggs fathered by attractive males by laying larger eggs<sup>1</sup> or by adding more testosterone<sup>2</sup>, but this inference could be undermined if eggs of different sex are provisioned differently, as these studies did not control for sex differences. Here we compare hormone concentrations in the yolks of male and female eggs and find that these are significantly different. Our results indicate that it is premature to conclude that female birds invest more in eggs sired by a preferred male, and raise the possibility that yolk sex steroids may be part of the sex-determining process in birds.

Cunningham and Russell have shown that female mallards (*Anas platyrhynchos*) produce larger eggs when mated to attractive males<sup>1</sup>, and Gil *et al.* have found that female zebra finches (*Taeniopygia guttata*) put more androgens into the yolk of eggs laid for attractive males<sup>2</sup>. However, neither study controlled for the possible effect of the sex of the eggs in the treatment groups. Females produce more sons when mated to attractive males<sup>3</sup> — for example, it has been shown in zebra finches, using the same colour-band manipulations as Gil *et al.*<sup>2</sup>, that females produce more sons when mated to attractive, red-banded males<sup>4</sup>. If male offspring generally come from bigger eggs or if there are sex differences in the steroid content of eggs, then the results of both studies<sup>1,2</sup> could be attributable to an effect of sex-ratio differences.

We compared the volume (calculated from Hoyt's formula<sup>5</sup>: volume = 0.51 × length × breadth<sup>2</sup>) of a large sample of male and female peafowl (*Pavo cristatus*) eggs and found a non-significant tendency for male eggs to be larger (ANCOVA with laying date as a covariate: main effect sex,  $F_{1,744} = 3.01$ ,  $P = 0.086$ ). We also analysed and compared hormone levels in the yolk of female and male peafowl eggs, in which the embryos had been sexed at an early stage by



**Figure 1** Steroid concentrations (in pg per mg of yolk) in male ( $n = 43$ ) and female ( $n = 45$ ) eggs. Eggs were collected from 46 pens, in which 1 male peacock had been mated to 4 peahens per pen. Eggs were placed in an incubator on day 1 and dissected on day 10 of the 28-day incubation period. A small quantity of embryonic material was collected for sexing and at the same time a sample of yolk was taken for steroid analysis. Yolks were analysed as described<sup>13</sup> without knowing the sex of the egg. Embryos were sexed by using polyacrylamide-gel electrophoresis of DNA amplified by the polymerase chain reaction. Where possible, one egg of each sex from each pen was analysed for hormonal concentration. We used ANCOVA to compare steroid concentrations between sexes with laying date as a covariate (androstenedione,  $F_{1,87} = 10.54$ ,  $P = 0.002$ ; testosterone,  $F_{1,87} = 6.73$ ,  $P = 0.011$ ; 5- $\alpha$ -dihydrotestosterone,  $F_{1,87} = 20.71$ ,  $P < 0.0001$ ; 17- $\beta$ -oestradiol,  $F_{1,87} = 30.86$ ,  $P < 0.0001$ ).

using molecular techniques. We found significant differences in overall levels of yolk steroids, including androgens, for eggs of different sex (Fig. 1).

The main source of steroids in egg yolk is the maternal ovary cells that surround the developing egg until it is ovulated<sup>6</sup>. Embryos are not known to secrete sex steroids into the yolk at an early stage, although there is some evidence for the presence of corticosterone of embryonic origin in the egg yolks of tree lizards, *Urosaurus ornatus*, after day 25 of the 36-day incubation period<sup>7</sup>. Also, embryos of each sex may use maternal steroids in a different way, although this has not been investigated in birds. Avian oocytes undergo the first meiotic division in the ovary after completion of yolk deposition, a few hours

before ovulation. This division consigns one of the sex chromosomes to the polar body, effectively determining the sex of the egg. If the ovulated egg is fertilized, it undergoes the second meiotic division and develops into a male or female embryo<sup>8</sup>.

The existence of sex differences in yolk hormone levels suggests that female birds provide eggs destined to carry male and female embryos with different amounts of steroids. It is unlikely that females can tell the sex of the egg when providing the yolk with hormones, as sex is determined after the yolk is formed. Instead, we propose that maternal steroids influence sex-chromosome segregation at the first meiotic division and are thus part of the sex-determining mechanism<sup>9–11</sup>. Maternally derived yolk hormones are associated with offspring sex ratio in painted turtles (*Chrysemys picta*)<sup>12</sup>, in which sex is environmentally determined.

If zebra-finch eggs show similar sex-related differences in the amount of hormones they contain, then the increased testosterone levels reported by Gil *et al.*<sup>2</sup>, in groups in which females were mated to attractive males, may be a result of the presence of more male eggs in these clutches<sup>4</sup>. We therefore question whether there is differential investment in hormones by females in the eggs of attractive males, as the results may be explained by females biasing the sex ratio of their offspring<sup>3</sup>.

**Marion Petrie\***, **Hubert Schwabl†**, **Nanna Brande-Lavridsen†**, **Terry Burke‡**

\**Evolution and Behaviour Research Group, Department of Psychology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK*  
e-mail: marion.petrie@ncl.ac.uk

†*Center of Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, Washington 99164, USA*

‡*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*

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**Cunningham and Russell reply** — Female birds may invest more in breeding attempts when paired with preferred males and may consequently produce chicks that are in better condition<sup>1</sup>. But if females favour one sex when mated to preferred males, either

**Table 1 Factors affecting egg size and sex**

Response term	Explanatory term	Statistic	d.f.	P value
Embryo sex	Male rank	1.00	1	0.32
Egg volume	Embryo sex	4.27	1	0.04
	Male rank	6.69	1	0.01
	Male rank × offspring sex	0.4	1	0.53
Chick weight	Male rank	6.29	1	0.01
	Embryo sex	0.07	1	0.78
	Male rank × offspring sex	<0.01	1	0.97
Per cent males (2nd clutch)	Per cent males (1st clutch)	2.78	1,15	0.03
Egg volume (2nd clutch)	Egg volume (1st clutch)	3.23	1,15	0.006
Per cent males	Mean egg volume	0.03	1	0.86

Effects of embryo sex and male rank on egg volume and chick weight were investigated using residual maximum likelihood models. There were no differences in chick size at hatching ( $\chi^2 = 0.03$ , d.f. = 1,  $P = 0.47$ ). Generalized linear mixed models with binomial error structure and logit link function were used to investigate the effects of male rank and a female's average egg volume on the proportion of male eggs laid. Repeated measures within females were controlled for in these analyses ( $n = 2$  clutches, 16 females). General linear models were used to investigate the repeatability in sex ratio and egg volume (Genstat 5.4.1, Lawes Agricultural Trust, IACR Rothamsted; 1998) (E.J.A.C., A.F.R., K. Orr, R. Griffiths and D. J. Ross, unpublished results).

in quantity<sup>2,3</sup> or quality, could this explain these differences in investment? We have shown that female mallards lay larger eggs for preferred males but do not produce more sons<sup>1</sup>. This increased investment is not directed at one particular sex, and here we point out the importance of distinguishing between differential investment in the sexes *per se*, as suggested by Petrie *et al.*, and differential investment in the sexes for different males.

Table 1 (top) shows that, within any breeding attempt, female mallards lay larger eggs for male embryos. However, this does not explain the increased investment for preferred males between different breeding attempts. Also, larger eggs for preferred males then produce heavier chicks, irrespective of their sex. Hence, in mallards, both sexes benefit from their mother's increased investment with preferred males.

Maternal characteristics seem to have a strong effect on the sex of their offspring (Table 1, bottom). Females that produce a high proportion of males in their first clutch also produce a high proportion of males in their next clutch, regardless of their partner's rank. Females are also consistent in their egg size after controlling for differential investment for different males, but females that generally produce larger eggs do not produce more males.

Whether or not differences in hormonal levels found in clutches sired by different males translate into 'differential investment' is more complex. Overall increases in hormonal level would suggest differential investment. Alternatively, differential allocation of hormones within a clutch could simply be a consequence of biasing the sex ratio in favour of a particular sex. But if the favoured sex is more costly to produce, then this would still represent a form of differential investment.

The key issue is the effect of this bias in investment. Does biasing the sex ratio of offspring increase the overall viability of the clutch because the preferred sex is more

likely to survive? Or does the less preferred sex suffer in the trade-off to the detriment of their survival? Females may bias investment in the sexes for many different reasons, so understanding how they influence the success of their sons and daughters will also be essential in explaining why birds sired by different males differ in their success.

Finally, could maternal provisioning of egg hormone levels be linked to the sex-determining process, as suggested by Petrie *et al.*? First we need to find out whether male phenotype can influence hormonal levels in the maternal body and ovary, and then whether manipulating hormonal levels within the female body and ovary can bias production of the sexes.

**Emma J. A. Cunningham, Andrew F. Russell**  
*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*  
*e-mail: ejac3@hermes.cam.ac.uk*

1. Cunningham, E. J. A. & Russell, A. F. *Nature* **404**, 74–77 (2000).
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Ecology

**Global amphibian population declines**

The decline and disappearance of relatively undisturbed populations of amphibians in several high-altitude regions since the 1970s suggests that they may have suffered a global decline, perhaps with a common cause or causes<sup>1–3</sup>. Houlihan *et al.*<sup>4</sup> examined means of trends for 936 amphibian populations and concluded that global declines began in the late 1950s, peaked in the 1960s, and have continued at a reduced rate since. Here we re-analyse their data using a method that accounts for the sampling of different populations over different time periods, and find evidence of a mean global decline in monitored populations only in the 1990s. However it is calculated, the global mean not only masks

substantial spatial and temporal variation in population trends and sampling effort, but also fails to distinguish between a global decline with global causes and the cumulative effects of local declines with local causes.

The first analytical method used by Houlihan *et al.* evaluated patterns in  $\Delta N$ , the annual change in abundance within populations, where

$$\Delta N_t = \log(N + 1)_{t+1} - \log(N + 1)_t$$

They calculated annual mean changes by averaging  $\Delta N_t$  over populations with recorded abundances in years  $t$  and  $t + 1$ . The temporal pattern of this mean is misleading because each year includes different populations. For example, consider one population studied in years 1, 2 and 3 with  $\Delta N_1 = 0.1$ ,  $\Delta N_2 = 0.3$ , and another studied in years 2, 3 and 4 with  $\Delta N_2 = -0.5$ ,  $\Delta N_3 = -0.3$ . Both populations are doing better with time, but the arithmetical averages for the three years,  $\bar{\Delta N}_1 = 0.1$ ,  $\bar{\Delta N}_2 = -0.1$ ,  $\bar{\Delta N}_3 = -0.3$ , indicate the opposite. The correct approach to estimating  $\bar{\Delta N}_t$  uses least-squares means<sup>5</sup>, which estimate the yearly mean averaged over all populations. The least-squares means of  $\Delta N_t$ ,  $-0.3$ ,  $-0.1$  and  $0.1$ , correctly represent the observed trends.

We used least-squares means to estimate annual mean trends for the combined global data and for several geographical regions<sup>4</sup> (Fig. 1). The global mean trend was significantly positive during 1964–81, indicating that, on average, monitored amphibian populations increased over this period. From 1990 onwards, the trend was significantly and increasingly negative, suggesting a global decline. This decline began more than a decade later than is generally accepted<sup>1,2</sup>, a quarter of a century later than Houlihan *et al.*<sup>4</sup> found, and at around the time that concerns about it were first expressed<sup>3</sup>.

The global mean does not reflect a single worldwide trend. For regions where data allowed separate analysis, trends of the 1990s were significantly negative only in North America and in Central and South America (Fig. 1), where concerns have been raised over amphibian declines<sup>1–3,6,7</sup>. The trend for Asia was significantly negative during 1959–75 and significantly positive after 1983.

The second analysis carried out by Houlihan *et al.* identified significantly more negative than positive correlations of population size with time, and they interpret this as evidence of a global decline. However, this pattern is expected for many amphibians in which recruitment is more variable than survival, and the exact expectation depends on the population biology of each species<sup>1</sup>. It is therefore impossible to establish a correct null hypothesis for the global database.

Extrapolating these results beyond the particular populations studied is tenuous.