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Yolk testosterone levels and offspring phenotype correlate with parental age in a precocial bird

Floriane Guibert [a,*], Marie-Annick Richard-Yris [a], Sophie Lumineau [a], Kurt Kotschal [b], Erich Möstl [c], Cécilia Houdelier [a]

[a] UMR 6552 Ethologie animale et humaine, CNRS-Université de Rennes 1, 263 avenue du Général Leclerc, 35042 Rennes, France
[b] Konrad-Lorenz-Forschungsstelle, University of Vienna, Fischerau 11, A-4645 Grünau, Austria
[c] Department of Natural Sciences, Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

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ABSTRACT

Parents, and particularly mothers, can influence their offspring's development in non-genetic ways. Maternal effects can occur during the mothering phase as well as during the embryonic phase. Prenatal maternal effects in birds can be mediated by yolk steroid hormones that influence subsequent offspring development. Studies have focused mainly on the influence of laying females' living conditions on yolk hormonal contents, and rarely on the effects of individual characteristics. Here, we investigated prenatal influence of parent age on yolk steroid levels and on offspring phenotype. We compared Japanese quail at two different ages: at the beginning of their reproductive cycle (11 weeks old: *age 1*) and six months later, after egg production peak (37 weeks old: *age 2*). Egg composition, reproductive outcomes, and offspring growth, sexual development and behaviour were studied at both ages. We found that laying rate, fertility and chick survival rates declined between *age 1* and *age 2*. *Age 2* eggs had relatively lighter shells and higher yolk plus albumen contents; they also had lower testosterone contents. *Age 2* offspring weighed more at hatching than did *age 1* offspring; subsequently their growth patterns differed and their sexual development was more precocious. *Age 2* offspring were less emotional than *age 1* offspring when encountering a novel environment, and they appeared more sensitive to social separation. Our study shows, for the first time in a bird species, a strong impact of parental age on offspring phenotype, and especially on behaviour, an impact that is possibly mediated via modulation of yolk testosterone content.

1. Introduction

Parents influence the phenotype of their offspring through genetic inheritance, but also through non-genetic mechanisms. Mothers have been the centre of interest concerning non-genetic inheritance processes and, in many species, they play a fundamental role in influencing their offspring's general development, both during the mothering phase [1–3] and during embryogenesis [4–7]. Prenatal maternal influences in birds have aroused much interest since the discovery that avian egg yolks contain variable levels of steroid hormones of maternal origin that have a strong influence on offspring development [6–8]. Most researchers so far have investigated the effects of yolk androgens on offspring phenotype by injections into eggs and they report strong effects on offspring short and long-term growth, immunity (cellular and humoral immunocompetence) and behaviour including begging, aggressiveness, emotional and social behaviours [6,7,9].

Avian yolk steroid levels appear to be influenced by social factors experienced by laying females, such as breeding density [10–12], frequency of social intrusion [13], social instability [14], social status [15], and male attractiveness [16]. In these social contexts, modulation of yolk androgen levels could have an important adaptive value for the next generation, preparing chicks for the social environments they will encounter either during the rearing phase, after fledging during the juvenile stage or as adults [6].

Effects of the laying females' characteristics on their yolk steroid levels have been investigated to a lesser extent. The genetic origin of a female has been shown to influence the hormonal content of her eggs. Japanese quail (*Coturnix coturnix japonica*) females from a genetic line selected for long tonic immobility duration (corresponding to high inherent fearfulness) laid eggs with lower progesterone and androgen levels than did females from a line selected for short duration of tonic immobility (corresponding to a low level of emotional reactivity) [17]. Lesser black-backed gull (*Larus fuscus*) females in enhanced body condition laid eggs with reduced yolk androgen contents [18]. Finally,

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[*] Corresponding author. Tel.: +33 2 23 23 68 10; fax: +33 2 23 23 69 27.
E-mail address: floriane.guibert@univ-rennes1.fr (F. Guibert).

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females' age appears to modulate yolk androgen levels, although effects seem to differ amongst species. Androgen levels increase with age in European starlings (*Sturnus vulgaris*) [19], whereas they decrease with age in Japanese quail [20].

Parental age has been shown to affect several aspects of bird reproduction, younger and first-time breeders are generally less efficient. In many species, egg weight and clutch size increase with female age [21,22]. Moreover, chicks of younger parents appear to have a lower survival rate than chicks of older parents; this could be linked either to their lower hatching weight (correlated to lower egg weight) that influences their postnatal survival [23] and/or to the reduced offspring-rearing abilities of younger parents compared to older parents [24,25]. Although this last aspect has generally been well analysed in bird species, parental age effects on egg laying and especially egg quality, and their consequences on the general development of young have been neglected [26].

This study investigated the effects of parental age on egg characteristics, especially yolk steroid levels and on the subsequent morphological and behavioural development of chicks. Japanese quail have a relatively short life span. They reach puberty at 6–8 weeks and attain full sexual maturity 2–3 weeks later [27]. We chose to study Japanese quail eggs and chicks at two different reproductive stages: first, at the beginning of the reproductive cycle (11 weeks old) and second, after the egg production peak (37 weeks old). We assayed the three main steroids present in quail yolk: progesterone, androstenedione and testosterone [28]. We hypothesised that yolk steroid levels would vary with parental age and that offspring morphological and behavioural development would differ according to parental age. We expected that yolk testosterone levels would decrease with parental age, as observed in a previous study on quail [20]. Furthermore, as several quail studies have shown that chicks from eggs with lower testosterone levels were less emotive than those from eggs with higher testosterone levels [14,29,30], we expected that chicks of older parents developing in eggs with lower androgen levels would present lower emotional reactivity than chicks of younger parents.

2. Materials and methods

2.1. Ethics

All experiments were approved by the departmental direction of veterinary services (Ille et Vilaine, France, Permit number 005283) and were performed in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC).

2.2. Housing of birds and mating

Twenty-two female and 16 male Japanese quail (*Coturnix c. japonica*), obtained from a commercial farm when they were 8 weeks old, were housed in individual cages (22 × 20 × 15 cm). Males and females were housed in different windowless rooms, but under the same conditions: an artificial 14:10 h light:dark cycle and a temperature maintained at 19 ± 1 °C. Water and food were provided *ad libitum.* Three weeks later (when 11 weeks old) when all females had begun to lay, we started the first mating period (*age 1*: February/March 2008). Females met a male three times a week. Pairs stayed together for a few minutes in a small cage until copulation had occurred. Daily copulations are not necessary, as Japanese quail females can store sperm for several days [31]. During the 10 mating sessions realised, female met a different male at each mating session. So a 10-male combination was elaborated for each female. The 10-male combinations implicated that all males ($N = 16$) were mated with at least half of the females (13.75 ± 0.3 females per male) and a similar 10-male combination was used at the most for two females. Eggs were collected daily for 17 days. Each egg was identified according to the female that had laid it and weighed. Six months later (when they were 37 weeks old), after the egg production peak [32], the same individuals (both males and females) were used for a second mating period (*age 2*: August/September 2008). The process was the same as for *age 1* and the same 10-male combinations for each female were used. Eggs were collected daily for 20 days. During the second mating period, 20 of the 22 females still laid regularly (one had stopped laying and one laid only two eggs during the collection period, thus her egg data were not taken into consideration). At the middle of each egg collecting period, one egg per female, collected the same day for all females, was frozen at −20 °C for subsequent hormonal assays. The other eggs collected were stored at 16 ± 1 °C until incubation.

The same experienced individual performed all pairings and egg collection during both periods. During the whole experiment (also between the two periods), we maintained the birds under the same standard conditions (room, photoperiod, temperature, diet) that were controlled with particular attention. Moreover, the same caretaker took care of the birds during the whole experiment.

2.3. Chick rearing

First, 227 *age 1* eggs (*i.e.* 10.32 ± 0.55 eggs/female) and then 236 *age 2* eggs (*i.e.* 11.80 ± 0.72 eggs/female) were collected for incubation. For both periods, the eggs collected were placed in an incubator for 17 days. During the first 14 days, eggs were maintained at 37.7 °C with a relative humidity of 45% and with an automatic rotation of 45° twice a day. During the last three days, temperature was decreased to 37.2 °C, humidity was raised to 60% and egg rotation was stopped. At hatching, leg rings identified chicks according to their mother. At *age 1*, 148 chicks from eggs laid by the 22 females hatched (6.73 ± 0.62 chicks/female). At *age 2*, 117 chicks from only 18 females hatched (6.50 ± 0.72 chicks/female). Unhatched eggs were opened to determine whether they were fertilised or not (*i.e.* presence or absence of an embryo). Fertility (number of fertilised eggs per number of incubated eggs × 100) and hatchability of fertile eggs (number of hatched eggs per number of fertilised eggs × 100) were calculated for each female. Chick mortality during their first week of life was recorded and chick survival rate was then calculated for each female (number of chicks still alive after 1 week per number of hatched chicks × 100).

Seventy-six *age 1* chicks (3.46 ± 0.14 chicks/female) and 75 *age 2* chicks (4.17 ± 0.23 chicks/female) were kept for experimentation. Chicks from different mothers were housed in groups of four in a 100 × 70 × 62 cm cage (for each *age*, one group included only three chicks because of the sudden death of one of the four chicks). Nineteen groups were formed and housed in the same room. A heat lamp (38 ± 1 °C) was placed in each cage to ensure chicks' thermoregulation until they were 10 days old. After this, when chicks were able to regulate their own temperature, the heat lamps were switched off and the temperature in the room maintained at 20 ± 1 °C. Chicks were exposed to a 10:14 h light:dark cycle. Water and food were provided *ad libitum.* To assess weight gain, chicks were weighed on electronic scales once a week, from hatching to 4 weeks old. When they were 3 and 4 weeks old, the length of their cloacal vent was measured with an electronic calliper rule to assess sexual maturation. This measure is an indicator of sexual development in quail (for both males and females) [33–35]. Sex was determined via sexual dimorphic plumage when chicks were 3 weeks old. The chicks' sex ratios did not differ significantly between *age 1* (38 females and 37 males) and *age 2* (30 females and 45 males) (Chi-square test, $\chi^2 = 1.722$, $P = 0.19$).

The same experienced individual was responsible for all chick care and measurements for both *ages*.

2.4. Behavioural tests

Classical ethological tests devised for poultry, based on different social and potentially frightening situations, were used to assess the general emotional reactivity of chicks [36]. *Age 1* chicks ($N = 76$) and