biology letters

Physiology

Biol. Lett. (2011) 7, 790–792
doi:10.1098/rsbl.2011.0196
Published online 20 April 2011

Why do some adult birds skip breeding? A hormonal investigation in a long-lived bird

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Skipping reproduction is often observed in long-lived organisms, but proximate mechanisms remain poorly understood. Since young and/or very old snow petrels (*Pagodroma nivea*) commonly skip breeding, we tested whether they are physiologically able to breed during the pre-laying stage. To do so, we measured the ability of known-age (11–45 years old) petrels to release luteinizing hormone (LH, a crucial driver for breeding), by injecting exogenous gonadotropin-releasing hormone (GnRH). Although young petrels exhibited low baseline LH levels, they were able to elevate LH levels after a GnRH challenge. Moreover, young and very old petrels showed a stronger decrease in LH levels after the 10 min post-GnRH injection compared with middle-aged petrels. Birds that skipped breeding were as able as breeders to release LH after a GnRH challenge, indicating that they had functional pituitaries. However, the decision to skip reproduction was linked to a strong LH decrease after the 10 min post-GnRH injection. Our result suggests that the youngest and the oldest petrels fail to maintain elevated baseline LH levels, thereby do not initiate reproductive activities. Skipping reproduction in long-lived birds probably results from age-related changes in the dynamics of the hypothalamic–pituitary–gonadal (HPG) axis function.

Keywords: intermittent breeding; age; GnRH challenge; luteinizing hormone; snow petrels

1. INTRODUCTION

Skipping reproduction (i.e. non-breeding by individuals that previously bred) is often observed in long-lived organisms [1]. The 'prudent parent hypothesis' posits that non-breeding decision should be favoured, when the value of the immediate reproduction is low relative to the value of future reproductive opportunities and survival [2], as it is expected to be in young adults. Moreover, senescent individuals may take advantage of sabbatical years to recover from previous reproductive efforts [3]. Alternatively, non-breeding events, instead of being strategically decided, might be imposed by physiological constraints in low-quality individuals [1]. Age appears to be the keystone of skipped breeding, since this behaviour is markedly observed in the youngest and in the oldest adult birds [4,5]. Thus, some of the needed physiological requirements for successful reproduction may be deficient in young and senescent adults [6].

Although ultimate causes of skipped breeding have been widely discussed, proximate factors underlying such patterns remain poorly studied [7,8], especially in an age-specific context [5]. In birds, the onset of breeding is under strong hormonal control through the activation of the hypothalamic–pituitary–gonadal (HPG) axis [9]. In response to increased day length in spring, gonadotropin-releasing hormone (GnRH) is expressed and triggers the secretion of LH (luteinizing hormone) by the pituitary gland. LH, in concert with follicle-stimulating hormone (FSH), promotes gonadal maturation, sex steroid secretion and in turn, sexual behaviours [9]. Considering that non-breeding events are proximately regulated by the endocrine system, it is thus conceivable that birds skipping breeding do not appropriately release LH during the pre-laying period.

As very long-lived birds, snow petrels (*Pagodroma nivea*) provide an excellent model system to study non-breeding decision. Indeed, up to 60 per cent of adults can skip breeding in a given year [10], mainly the youngest and the oldest petrels [4,5]. This study was designed to test, in this monogamous species, whether age affects the ability to release LH during the pre-laying period and whether skipping petrels fail to release LH. To do so, we used a common and powerful protocol, the injection of exogenous GnRH during the photosensitive stage, and tested the ability of known-age petrels to release LH [7]. We predicted that young and very old petrels would release less LH after a GnRH injection than middle-aged ones, and that lower LH release would predict high probability to skip breeding. Moreover, LH levels are known to progressively decrease from 10 to 30 min post-GnRH injection and to return to baseline levels [7]. We therefore investigated whether this LH change (i) would be stronger in young and very old petrels than in middle-aged ones and (ii) would predict a high probability to skip breeding.

2. MATERIAL AND METHODS

The study was conducted on Terre Adélie (66°40′ S, 140°01′ E), Antarctica. Snow petrels are very long-lived birds with low fecundity (one egg per year [10]). Many birds were of known age, because chicks have been banded each year since 1964 [10]. Twenty-four males and 17 females, from 11 to 45 years old, were handled during the pre-laying period (i.e. the courtship and mating period), from 11 to 23 November 2008. Only one bird was captured at each nest and birds were observed as breeders in previous years. They were caught by hand and a first blood sample was collected from the alar vein with a syringe immediately after capture (mean $\pm$ s.e.: 3 min and 9 $\pm$ 5 s) to determine baseline LH levels. Then, birds were immediately (5 min 51 $\pm$ 9 s after capture) injected with exactly 0.1 ml of a GnRH solution in the second alar vein ([Gin^8], Sigma Lot 121H04314). The GnRH was dissolved in a physiological solution to yield a dosage of 0.6 µg $(0.1\ \mathrm{ml})^{-1}$ (about 1.5 µg kg^{-1} body mass in 1 ml of 0.9% saline solution, as validated for seabirds [11]). Blood samples were collected from alar veins between 10 and 30 min after the GnRH injection to measure LH levels. LH changes from 0 to 10 min post-GnRH injection $[(LH_{t=10} - LH_{t=0})/LH_{t=0}]$ and LH changes from 10 to 30 min post-GnRH injection $[(LH_{t=30} - LH_{t=10})/LH_{t=10}]$ were examined as individual abilities to release LH. Blood samples were centrifuged and plasma was stored at -20°C. LH levels were assayed by radioimmunoassay at the CEBC (Centre d'Etudes Biologiques de Chizé) [11]. The lowest detectable concentration of LH was 0.05 ng ml^{-1} and the intra-assay coefficient of variation was 6.2 per cent (three duplicates). Following GnRH injections, LH levels

Received 21 February 2011
Accepted 25 March 2011

(baseline: 8.348 ± 0.534 ng ml^{-1}) significantly increased (10 min: 11.544 ± 0.842 ng ml^{-1}), then decreased (30 min: 9.059 ± 0.838 ng ml^{-1}; generalized linear mixed model (GLMM), time as factor: $F_{2,76} = 21.540$, $p < 0.001$, time × sex: $F_{2,76} = 0.244$, $p = 0.784$). The 41 nests were checked every 2 days, until the end of the breeding season, to monitor if birds engaged in breeding ($n = 32$) or if they skipped breeding ($n = 9$).

All analyses were performed using R v. 2.8.0. We used the generalized linear model (GLM) with normal errors and an identity link function to explain absolute LH levels and LH changes as functions of sampling date, sex, age (linear and/or quadratic) and interactions between sex and age (linear and/or quadratic). Similarly, we used GLM with binomial error distribution and a logit link function to test for an effect of sex, LH values and the interaction between sex and LH on the probability to skip breeding. Selected models were checked for assumptions and LH values were log-transformed when necessary.

3. RESULTS

Baseline LH levels were higher in males than in females ($F_{1,38} = 7.338$, $p = 0.010$), and significantly increased with age ($F_{1,37} = 5.517$, $p = 0.024$; figure 1*a*), with no effect of sampling date, age^2 and interactions ($p > 0.16$ for all tests). Absolute LH levels at 10 min were higher in males than in females ($F_{1,34} = 6.412$, $p = 0.016$), with no effect of other variables ($p > 0.07$). LH changes from 0 to 10 min post-GnRH injection were significantly less pronounced with increasing age ($F_{1,36} = 6.640$, $p = 0.014$; figure 1*b*), with no effect of other variables ($p > 0.11$). Absolute LH levels at 30 min increased with sampling date ($F_{1,33} = 11.031$, $p = 0.002$), were higher in males than in females ($F_{1,32} = 15.648$, $p < 0.001$), and tended to be lower in very young and very old petrels than in middle-age ones (age^2: $F_{1,30} = 3.378$, $p = 0.076$), without interaction effects ($p > 0.11$). LH changes from 10 to 30 min post-GnRH injection increased with sampling date ($F_{1,32} = 9.451$, $p = 0.004$) and were significantly more pronounced in young and very old petrels than in middle-aged ones (age^2: $F_{1,30} = 5.439$, $p = 0.027$; figure 1*c*), with no effect of sex and interactions ($p > 0.09$). LH changes from 10 to 30 min post-injection predicted skipped breeding ($n = 37$, $\chi^2 = 4.066$, $p = 0.044$). Skipped breeders showed a stronger LH decrease from 10 to 30 min post-injection ($-35.30 \pm 8.19\%$) compared with breeders ($-16.88 \pm 3.74\%$). Absolute levels and LH changes from 0 to 10 min post-injection did not predict skipped breeding, even when considering an effect of sex ($p > 0.42$).

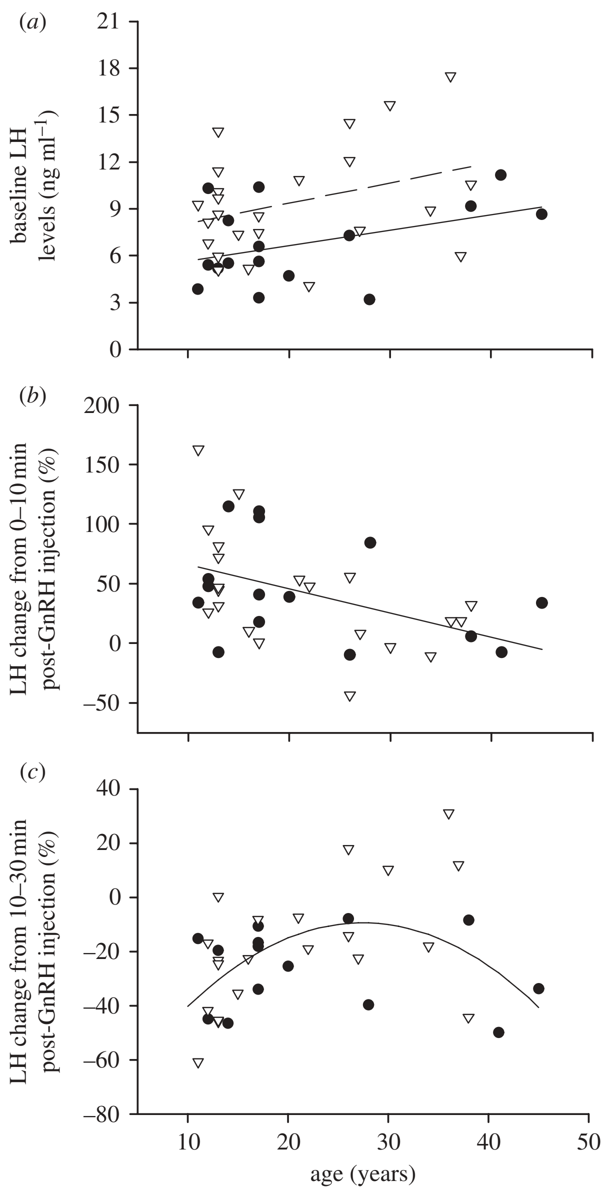

Figure 1. LH levels in relation to age in pre-laying snow petrels. (*a*) Baseline LH levels increased with age in males (open triangles and dashed line) and females (filled circles and solid line). (*b*) LH changes from 0–10 min post-GnRH injection (%) were negatively linked to age. (*c*) LH changes from 10–30 min post-GnRH injection (%) were quadratically linked to age.

4. DISCUSSION

Although young petrels exhibited low baseline LH levels, they showed a substantial increase in LH levels from 0 to 10 min post-injection and reached similar absolute LH levels compared with older petrels at 10 min. LH changes from 0 to 10 min reflect the individual ability to release LH after a GnRH injection, relative to the baseline levels. This indicates that young petrels with previous reproductive experience had functional pituitaries. How, then, is it possible to explain their lower baseline LH levels? Although photoperiod is the primary signal controlling seasonal reproductive activities, social interactions can influence central physiology by modifying GnRH neurosecretory cells, which in turn stimulates the adjustment of reproductive physiology and behaviour [9]. In the monogamous snow petrel, repeated breeding attempts with the same partner could therefore provide important benefits by improving coordination of breeding activities [12]. In this context, low mate stimulations in young and newly paired petrels could explain their lower baseline LH levels. In our study, although sex steroids were not measured owing to small blood samples, we suggest that young females may reduce reproductive costs by delaying oestradiol secretion and ovarian development until establishing long-term pair bond [8]. Contrary to young petrels, old birds showed high baseline LH levels and did not strongly respond to the GnRH challenge. This may suggest that older petrels already secreted LH at their maximum levels, because of strong mate–mate interactions stimulated by long-term partnerships. Alternatively, as the pool of available ova decreases with age, higher LH levels found in old females may have originated from a lower negative feedback at the hypothalamus level, compared with young females [13].

There was considerable inter-individual variation in relative LH changes from 10 to 30 min post-GnRH injection: LH levels rapidly decreased, did not change or continued increasing from 10 to 30 min. Compared with middle-aged petrels, the youngest and the oldest petrels failed to maintain LH release after the 10 min post-injection phase. In a previous study, we found that young and very old petrels were more sensitive to stress than middle-aged ones [5]. We suggest that this high stress response could interfere with the capacity to maintain LH production in an age-specific manner. Thus, the youngest and the oldest petrels would probably downregulate LH production during stressful conditions, such as those simulated by our manipulations. The marked decline in LH levels from 10 to 30 min post-injection in the oldest petrels may originate from a possible physiological senescence of the HPG system, as observed in ageing poultry [14]. These results are the first to show age-related differences in HPG activity of free-living birds, although more observations are needed to confirm that these patterns can be observed over several seasons and in different environmental conditions.

As found in some cooperative breeding bird species [7], skipped breeding in the monogamous snow petrel was not associated with a physiological suppression of LH secretion. However, we found that failure to maintain LH levels in the 10 min post-injection was the main predictor of the decision to skip reproduction. Specifically, the youngest and the oldest individuals, which appear to be more susceptible to stressors than middle-aged ones [5], fail to maintain elevated LH levels during the photosensitive stage and thus would be highly susceptible to skipping breeding. As previously highlighted [1], we suggest that non-breeding in long-lived free-living birds is probably the consequence of some age-related constraints acting on the dynamics of the HPG axis.

The present research project no. 109 was supported by the French Polar Institute (IPEV). A.G. was supported by a grant from CNRS/Région Poitou-Charentes. We thank E. Antoine, V. Lecomte, A. Lacroix and D. Besson for assistance in the field, LH assays and the management of the database, A. Lendvai, F. Angelier, B. Planade and three anonymous referees for useful comments on the article.

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