Mellowing with age: older parents are less responsive to a stressor in a long-lived seabird

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Summary

1. Reproductive success often increases with age; however the mechanisms underlying this commonly observed pattern are poorly understood. One mechanism that may be important is a set of physiological responses (the stress response) that allows organisms to evade and cope with stressors, but often inhibits reproduction.

2. If older parents respond less strongly to stressors than younger parents, this age-related difference in the stress response may contribute to the higher reproductive success that often characterizes older parents.

3. Typically the stress response is measured as an increase in plasma glucocorticoid (CORT) concentration, and we have previously reported that stress-induced CORT levels decline with age in the common tern (*Sterna hirundo*). Another hormone, prolactin (PRL), has been reported to decrease in response to stressors in breeding birds and is often positively associated with parental behaviour. We predicted that like the CORT stress response, the PRL stress response would also be suppressed with age.

4. To test this prediction, we captured known-age, incubating common terns ranging in age from 3 to 29 years and measured stress-induced changes in PRL and CORT levels within the same individuals.

5. We found that PRL levels decreased less rapidly in response to capture and restraint stress in older than in younger parents. In these same birds, we also found that stress-induced maximum CORT levels decreased with age, which is consistent with what we have previously reported for this species. Measures of PRL and CORT were not, however, correlated within individuals.

6. Taken together, these results support the hypothesis that modulations of both the PRL and CORT stress response are flexible hormonal mechanisms that help to account for the increase in reproductive success that occurs with age.

Key-words: common tern (*Sterna hirundo*), corticosterone, incubation, life history, parental care, prolactin, reproductive success, stress response

Introduction

Older parents often rear a greater number of offspring to independence than younger parents (Clutton-Brock 1991). This commonly observed pattern has received both theoretical and empirical attention (reviewed in Forslund & Pärt 1995); however, we currently have little information about the underlying physiological mechanisms (Angelier *et al.* 2006, 2007a,b; Heidinger, Nisbet & Ketterson 2006). Knowledge of how age-related changes in reproductive success are physiologically mediated is important for understanding individual reproductive strategies, life-history evolution and age-structured population dynamics (Forslund & Pärt 1995).

One mechanism that may be particularly important in this regard is the set of physiological responses known as the stress response (Ricklefs & Wikelski 2002). In response to stressors such as predation, inclement weather, and reduced food availability, most organisms shift investment away from reproduction and redirect it towards self-maintenance or survival (Wingfield, O'Reilly & Astheimer 1995; Wingfield & Sapolsky 2003). In vertebrates, this stress-induced shift in allocation is

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orchestrated by several hormonal changes, but is typically measured as an increase in glucocorticoids (Sapolsky, Romero & Munck 2000; McEwen & Wingfield 2003; Wingfield & Sapolsky 2003). Elevated glucocorticoid levels are expected to enhance survival by stimulating gluconeogenesis, foraging and escape behaviour (Wingfield *et al*. 1998), but to simultaneously interfere with reproduction by inhibiting gonadotropin production (Wingfield & Sapolsky 2003) and parental behaviour (Silverin 1986; Wingfield, O'Reilly & Astheimer 1995; Kitaysky, Wingfield & Piatt 2001; Wingfield 2003).

In species that care for offspring, changes in prolactin (PRL) may also play a role in redirecting resources away from reproduction in response to stressors (Chastel *et al*. 2005; Angelier *et al*. 2007b; reviewed in Angelier & Chastel 2009). PRL is a pituitary hormone that promotes and maintains diverse parental behaviours in vertebrates (reviewed in Buntin 1996; Freeman *et al*. 2000). Like CORT, PRL is known to be influenced by exposure to both acute and chronic stressors in a variety of taxa (mammals: Armario *et al*. 1986; Freeman *et al*. 2000; Carlson *et al*. 2006, birds: reviewed in Angelier & Chastel 2009 and fish: Pottinger, Prunet, & Pickering 1992). In non-reproductively active mammals and birds, PRL has been reported to increase in response to stressors (Yelvington, Weiss & Ratner 1985; Gala 1990; Manney *et al*. 1999; Freeman *et al*. 2000). In contrast, PRL has been reported to decrease in response to stressors in both pregnant and lactating mammals (Morehead & Gala 1989) as well as in incubating (reviewed in Angelier & Chastel 2009) and chick-rearing birds (Miller, Vleck & Otis 2009). Because PRL is often positively correlated with parental care, the 'PRL stress response hypothesis' posits that a stress-induced decrease in PRL will lead to a reduction in parental behaviour (reviewed in Angelier & Chastel 2009). Consequently, parents that attenuate the PRL stress response (i.e. maintain higher PRL levels or decrease PRL levels more slowly in response to stressors) are expected to provide more care for offspring than parents that respond more robustly to stressors when conditions deteriorate (reviewed in Angelier & Chastel 2009).

When the value of present reproduction is high relative to the value of survival and future reproduction the stress response is often suppressed so that critical resources are not diverted away from reproduction (Wingfield, O'Reilly & Astheimer 1995; Wingfield & Sapolsky 2003). As organisms age, the value of present reproduction is predicted to increase because future reproductive opportunities are expected to decline (Stearns 1992; Roff 2002). Consequently, older parents are predicted to respond less strongly to stressors than younger parents so that reproduction is not inhibited – even if this comes at a potential cost to survival and future reproduction (Wingfield & Sapolsky 2003; Heidinger, Nisbet & Ketterson 2006; Angelier *et al*. 2007b; Angelier & Chastel 2009).

In support of this prediction, we have previously reported that stress-induced maximum glucocorticoid levels decline with age in a long-lived seabird, the common tern (*Sterna hirundo*) (Heidinger, Nisbet & Ketterson 2006). Age-related changes in reproductive investment are inherently difficult to measure (Clutton-Brock 1991); however consistent with the idea that reproductive investment increases with age in this population of terns, older parents are able to rear a greater number of offspring to fledging than younger parents (Nisbet, Apanius & Friar 2002) and older parents are more attentive to their nests after exposure to an experimentally simulated adult predator than younger parents (Meehan & Nisbet 2002). In this study, we extend our prior findings to examine whether: (i) the PRL stress response is also suppressed with age in this population, and (ii) PRL and CORT measures are correlated within individuals.

Materials and methods

GENERAL METHODS AND STUDY SPECIES

This research was conducted on a population of common terns breeding on Bird Island, in Buzzards Bay, MA, USA (41°40′N, 70°43′W) between May and July in 2005 (Fig. 1). All samples were collected from known-age, adult male and female terns during the middle of the incubation period. All adults had originally been banded as chicks and ages were determined based on banding records. Common terns are long-lived, colonial seabirds that typically begin breeding between 3- to 4-year-old and breed annually thereafter (Nisbet 2002). Both sexes provide extensive care for offspring (Nisbet 2002). Females typically lay 2–3 eggs and both sexes develop brood patches and incubate the eggs for an average of 22 days (Nisbet 2002). Females incubate slightly more than males during the day (32 min vs. 27 min of the hour) and > 90% of the time at night (Wiggins & Morris 1987; Nisbet 2002). In this population, incubation bout length can range from 1 min to several hours and tends to be shorter in the morning (Nisbet 2002). Reproductive success increases with age in this population of common terns. Birds that are 18 years or older lay larger clutches and are able to successfully rear a greater number of chicks to fledging than younger terns (Nisbet, Apanius & Friar 2002).

STRESS-INDUCED HORMONE LEVELS

To examine whether age influenced stress-induced levels of PRL and CORT, we identified and marked nests during the laying period and captured one known-aged adult per nest using walk-in treadle traps

Fig. 1. A pair of incubating common terns (*Sterna hirundo*) on Bird Island, MA, USA.

between days 9 and 15 of the incubation period. All adults were captured between 0600 and 1330 h. We used the following standardized capture and restraint protocol to measure stress-induced changes in PRL and CORT (Wingfield, O'Reilly & Astheimer 1995; Chastel *et al.* 2005). An initial 200 μL blood sample was collected from the jugular vein within 3 min of capture (hereafter, baseline sample) and the time required to collect the sample was recorded (hereafter, bleed time). Terns were then placed in individual holding tubes about 8 × 5 cm in cross-section, which held them loosely but prevented them from struggling or opening their wings. Additional 200 μL blood samples were collected from the alar vein at 30 and 50 min (stress-induced samples). In all cases, this serial blood sampling protocol removed ≤5% of total blood volume. A drop of blood from the initial sample was put in lysis buffer (Longmire's solution, Longmire *et al.* 1992) for sexing and terns were weighed before release. Blood samples were kept on ice for < 6 h before being centrifuged and separated and plasma was stored at −20 °C until analysis.

We determined PRL using a heterologous RIA that employs purified chicken PRL standard and a rabbit-derived antibody at the Centre d'Etudes Biologiques de Chizé (Cherel *et al.* 1994). A dose–response curve of pooled common tern plasma samples paralleled that for chicken (AFP 4444B, source: Dr Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance, CA, USA), indicating that the assay could be used to measure relative plasma PRL in common terns. The detection limit of the assay was 6 ng mL^{-1} and no samples fell below this limit. All samples were run in duplicate in a single assay and the intra-assay coefficient of variation was 9%.

We measured CORT using standard RIA techniques at Indiana University (Wingfield & Farner 1975; Ketterson *et al.* 1991). Briefly, samples were equilibrated with 2000 cpm ^{3}H-CORT overnight and extracted with diethyl ether. Samples were then assayed in duplicate using a competitive binding assay and assay values were corrected for plasma volumes and individual recoveries after extraction. The detection limit of the assay was 0·42 ng mL^{-1} and no samples fell below this limit. The intra- and inter-assay coefficients of variation were 7% and 9% respectively.

MOLECULAR SEX

To determine sex, we extracted DNA with a QIAamp® DNA Blood Mini Kit and followed the methods of Szczys, Spendelow & Nisbet (2005). Two pairs of primers were used to amplify a W-linked marker and control marker designed from an IGF cDNA sequence. Samples were run in duplicate and PCR products were resolved on 2% agarose gels, stained and scored for the presence or absence of both bands.

STATISTICAL ANALYSES

We measured PRL levels in serial bleeds taken at 0–3, 30, and 50 min post-capture in 52 terns that ranged in age from 3 to 29 years. We measured CORT levels at 0–3, and 30 min in 46 of these same individuals. We used repeated-measures ANOVAs to examine the influence of restraint stress on PRL and CORT levels. General Linear Models (GLM) were used to examine the influence of age, as well as other independent variables on baseline (initial samples collected within 0–3 min) and stress-induced levels of PRL and CORT. The measures of stress-induced PRL we examined were minimum PRL levels (we defined this as the lowest value at 30 or 50 min because individuals differed at which time point they reached the lowest level) and the rate of PRL decrease [(minimum PRL − baseline PRL)/time to minimum] (Chastel *et al.* 2005; Angelier *et al.* 2007b). To be consistent with our previous study, our measure of stress-induced CORT was maximum CORT at 30 min (Heidinger, Nisbet & Ketterson 2006).

We used the information-theoretic approach to compare candidate models for each measure of baseline and stress-induced PRL and CORT (Burnham & Anderson 2002). To minimize the number of potential candidate models for each stress-induced hormone measure, we examined models that included: (i) age, body mass and date because these variables have previously been shown to influence stress-induced CORT levels in this species (Heidinger, Nisbet & Ketterson 2006), (ii) all two-way combinations of these three variables known to have *a priori* importance in this system, (iii) these three variables as well as time of day, sex, and recapture history, and (iv) each independent variable separately. Time of day was included because it has previously been reported to influence stress-induced CORT levels in other bird species (Romero & Remage-Healey 2000). Recapture history (the number of years in which an individual had previously been recaptured as an adult) was included because prior exposure to a stressor (in this case capture and handling) can influence subsequent responses to that stressor (Romero 2004). Age and recapture history were never included in the same models because these two variables were highly correlated ($r = 0.747$, $P < 0.01$).

For each baseline hormone level we examined models that included: (i) bleed time (the time required to collect the initial blood sample), body mass and date because these variables have previously been shown to influence baseline CORT levels in common terns (Heidinger, Nisbet & Ketterson 2006), (ii) these three variables as well as, time of day, sex and age, and (iii) each independent variable separately. We included time of day because it has previously been reported to influence both baseline CORT and PRL levels in other bird species (Meier, Burns & Dusseau 1969; Carere *et al.* 2003).

Models were compared using Akaike's Information Criterion corrected for small sample size (AIC$_c$) and Akaike weights (Burnham & Anderson 2002). For each measure, we selected the model with minimum AIC$_c$ as the preferred model and other models with ΔAIC$_c$ < 2 (corresponding to evidence ratios > 0·4) as receiving some support. We then used model averaging to estimate regression coefficients and 95% confidence intervals (Burnham & Anderson 2002).

In addition, we examined whether baseline PRL and CORT and stress-induced measures of PRL and CORT were correlated. Baseline PRL and CORT, minimum PRL and maximum CORT were log-transformed to improve normality, but all other variables met the assumptions of the GLM. All statistical analyses were performed in R (R Development Core Team, 2008).

Results

The PRL levels significantly decreased in response to capture and restraint stress (repeated-measures ANOVA, $F_{1,51} = 122.37$, $P < 0.01$) (Fig. 2) and CORT levels significantly increased (repeated-measures ANOVA, $F_{1,45} = 178.02$, $P < 0.01$). On average, PRL levels decreased by 95 ng mL^{-1} (36%) over 50 min and CORT levels increased by 30 ng mL^{-1} (312%) over 30 min.

Variation in the rate of PRL decline was best explained by two models, one that included only age and one that included age and date (Table 1). PRL levels decreased less rapidly in older than in younger parents: the average rate of PRL decline decreased by 0·052 ng mL^{-1} min^{-1} per year of life
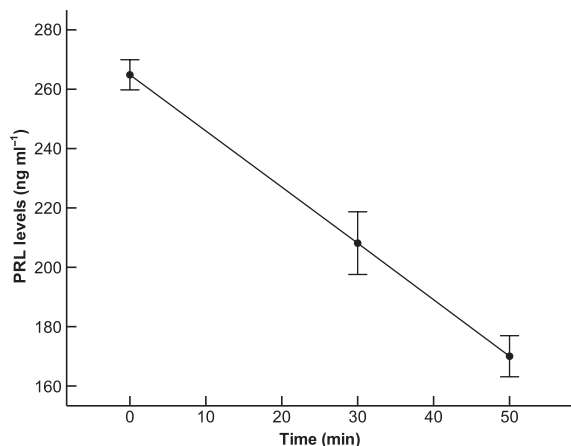
Fig. 2. The influence of capture and restraint stress on prolactin levels over 50 min ($n = 52$). Error bars $= \pm 1$SE.

Fig. 3. The relationship between age and the rate of PRL decline ($n = 52$).

(i.e. 2·6 ng mL^{-1} over a 50-min period) (unstandardized coefficient $b = -0.052$, $r^2 = 0.076$, SE $\pm$ 0·024, CI $= -0.003, -0.100$, Fig. 3). This age-related rate of decline in PRL reflected a tendency for older birds to reach minimum PRL at 50 min rather than at 30 min (binary logistic regression of time of minimum vs. age, Wald statistic $= 3.4$, $P = 0.065$). PRL levels also decreased less rapidly with date, although confidence limits on the regression coefficient included zero. On average, the rate of PRL decline decreased by 0·029 ng mL^{-1} min^{-1} day^{-1} (unstandardized coefficent $b = -0.029$, $r^2 = 0.003$, SE $\pm$ 0·026, CI $= -0.0801, +0.021$).

Variation in minimum PRL was harder to account for, but was best explained by two models, one that included only time of day and one that included only recapture history (Table 1). Minimum PRL levels increased with time of day (unstandardized coefficient on log-transformed variable $b = 0.0004$, $r^2 = 0.058$, SE $\pm$ 0·0002, CI $= -0.00005, +0.0008$) and decreased with recapture history (unstandardized coefficient on log-transformed variable $b = -0.037$, $r^2 = 0.051$, SE $\pm$ 0·023, CI $= -0.081, +0.007$). On average, minimum PRL levels increased by about 3·2 ng mL^{-1} h^{-1} and declined by about 4·9 ng mL^{-1} year^{-1} in which a bird had previously been recaptured as an adult. No terns included in this study had been recaptured in more than 6 prior years, and many terns (42%) had not been recaptured since they were originally banded as chicks. However, these models did not provide strong evidence for the reported relationships, because confidence limits on the regression coefficients included zero in both cases.

Variation in baseline PRL levels was best explained by a model that included only time of day (Table 1). Baseline PRL levels increased throughout the morning and early afternoon. On average, baseline PRL increased by about 3·9 ng mL^{-1} h^{-1} between 0600 and 1330 h (unstandardized coefficient on log-transformed variable $b = 0.0003$, $r^2 = 0.121$, SE $\pm$ < 0·0000, CI $= 0.00006, 0.0004$).

Variation in maximum CORT levels was best explained by four models: a model that included age and body mass, a model that included age and date, a model that included age, body mass and date, and a model that included only age
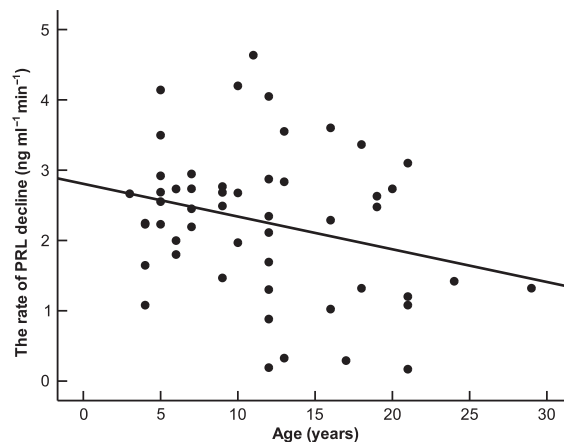
(Table 1). Consistent with our previous findings in this species, older parents had lower maximum CORT levels than younger parents: on average maximum CORT levels decreased by about 0·87 ng mL^{-1} year^{-1} of life (unstandardized coefficient on log-transformed variable $b = -0.027$, $r^2 = 0.115$, SE $\pm$ 0·010, CI $= -0.047, -0.007$). Variation in baseline CORT levels was best explained by body mass (Table 1). Heavier individuals had lower baseline CORT: on average baseline CORT levels decreased by about 0·17 ng mL^{-1} g^{-1} (unstandardized coefficient on log-transformed variable $b = -0.023$, $r^2 = 0.085$, SE $\pm$ 0·012, CI $= -0.046, -0.001$); this is also consistent with our previous study (Heidinger, Nisbet & Ketterson 2006).

No measures of PRL and CORT were significantly correlated with one another (baseline PRL and CORT: $r = -0.169$, $n = 46$, $P = 0.262$; minimum PRL and maximum CORT: $r = 0.229$, $n = 46$, $P = 0.126$; the rate of PRL decline and maximum CORT: $r = -0.155$, $n = 46$, $P = 0.304$).

Discussion

We found that in common terns, PRL decreased and CORT increased in response to capture and restraint stress, as has been reported in other bird species (Chastel et al. 2005; Angelier et al. 2007b; Verreault et al. 2008). Importantly, PRL declined more slowly and maximum CORT levels were also lower in older than in younger parents. While the age-related decrease in stress-induced maximum CORT has been shown before in common terns (Heidinger, Nisbet & Ketterson 2006), this is the first study to show that age attenuates the stress response with respect to both CORT and PRL when measured simultaneously in the same individuals. These results suggest that the commonly observed increase in reproductive success with parental age in many taxa may occur at least in part because older parents are less responsive to stressors than younger parents and thus less likely to respond to an environmental challenge by reducing parental effort in favour of self-maintenance (Heidinger, Nisbet & Ketterson 2006; Angelier et al. 2007b; Angelier & Chastel 2009).

Table 1. General linear models that examined the influence of independent variables on baseline and stress-induced measures of PRL and CORT. Age and recapture history (recapture) were never included in the same model. The best models ($\Delta AIC_c < 2$) are shown in bold

Models	K	AIC_c	ΔAIC_c	Akaike weight
Stress induced measures of PRL and CORT				
Minimum PRL				
Time of day	**3**	**26·608**	**0·000**	**0·303**
Recapture	**3**	**26·948**	**0·340**	**0·256**
Date	3	28·619	2·011	0·111
Age	3	29·308	2·700	0·079
Body mass	3	29·640	3·032	0·067
Sex	3	30·250	3·642	0·049
Age + date	4	30·915	4·307	0·035
Body mass + date	4	30·970	4·362	0·034
Recapture + body mass + date	5	31·420	4·812	0·027
Age + body mass	4	31·631	5·023	0·025
Age + body mass + date	5	33·368	6·760	0·010
Recapture + body mass + date + time of day + sex	7	35·262	8·654	0·004
Age + body mass + date + time of day + sex	7	37·543	10·935	0·001
The rate of PRL decline				
Age	**3**	**153·229**	**0·000**	**0·302**
Age + date	**4**	**153·763**	**0·535**	**0·231**
Recapture	3	155·233	2·004	0·111
Age + body mass	4	155·579	2·350	0·093
Age + body mass + date	5	156·138	2·909	0·071
Date	3	157·182	3·953	0·042
Body mass	3	156·791	4·062	0·040
Time of day	3	157·342	4·113	0·039
Sex	3	157·438	4·209	0·037
Recapture + body mass + date	5	159·173	5·944	0·015
Age + body mass + date + time of day + sex	7	161·307	8·078	0·005
Recapture + body mass + date + time of day + sex	7	165·216	11·987	0·001
Maximum CORT				
Age + body mass	**4**	**49·961**	**0·000**	**0·282**
Age + date	**4**	**50·685**	**0·724**	**0·196**
Age + body mass + date	**5**	**50·729**	**0·767**	**0·192**
Age	**3**	**51·059**	**1·097**	**0·163**
Age + body mass + date + time of day + sex	7	53·323	3·361	0·052
Body mass	3	54·262	4·301	0·033
Recapture	3	54·726	4·765	0·026
Recapture + body mass + date	3	55·825	5·864	0·015
Date	3	56·095	6·134	0·013
Body mass + date	4	56·461	6·500	0·011
Time of day	4	56·501	6·539	0·011
Sex	3	58·439	8·478	0·004
Recapture + mass + date + time of day + sex	7	59·751	9·790	0·002
Baseline measures of PRL and CORT				
Baseline PRL				
Time of day	**3**	**−62·406**	**0·000**	**0·721**
Age	3	−59·522	2·883	0·171
Date	3	−57·133	5·273	0·052
Body mass	3	−55·848	6·557	0·027
Sex	3	−55·843	6·563	0·027
Bleed time + age + body mass + date + time of day	7	−50·232	12·174	0·002
Bleed time + age + body mass + date + time of day + sex	8	−47·455	14·951	0·000
Bleed time	3	−45·568	16·837	0·000
Baseline CORT				
Body mass	**3**	**101·252**	**0·000**	**0·554**
Bleed time + body mass + date	5	104·165	2·913	0·129
Time of day	3	104·365	3·114	0·117
Age	3	105·140	3·889	0·079
Date	3	105·311	4·060	0·073
Sex	3	106·286	5·035	0·045
Bleed time + age + body mass + date + time of day + sex	8	111·816	10·565	0·003
Bleed time	3	114·275	13·023	0·001

PRL, CORT AND PARENTAL BEHAVIOUR

Parental behaviour is often positively correlated with PRL (reviewed in Buntin 1996; Freeman *et al.* 2000) and negatively correlated with CORT (Silverin 1986; Kitaysky, Wingfield & Piatt 2001; Wingfield & Sapolsky 2003). In birds, individuals with higher endogenous PRL spend more time incubating (Schoech 2001; Van Roo, Ketterson & Sharp 2003), incubate more consistently (Buntin *et al.* 1996) and feed chicks more frequently (Schoech, Mumme & Wingfield 1996; Duckworth, Badyaev & Parlow 2003). Further, in contrast to controls, experimentally elevated PRL levels have been reported to maintain incubation behaviour in nest-deprived hens (Sharp *et al.* 1988) and to cause incubating female willow ptarmigan (*Lagopus l. lagopus*) to sit more tightly when approached, and give more distraction displays when flushed (Pedersen 1989). In contrast, experimentally elevated CORT generally reduces incubation behaviour (Criscoulo *et al.*, 2005, B.J. Heidinger, I.C.T. Nisbet, & E.D. Ketterson, unpublished data), brooding behaviour (Kitaysky, Wingfield & Piatt 2001; Angelier & Chastel 2009), and feeding of offspring (Silverin 1986; although see Koch, Wingfield & Buntin 2004), while increasing the probability of nest abandonment in birds (Silverin 1986; Criscoulo *et al.*, 2005). Hence, our finding that PRL decreases and CORT increases in response to standardized capture and restraint stress suggests that common terns would respond to stressors by reducing parental behaviour.

AGE AND STRESS-INDUCED CHANGES IN PRL AND CORT

Of particular interest were the relationships between age and stress-induced changes in PRL and CORT. Although minimum PRL levels did not vary with age, PRL levels declined less rapidly in older than in younger parents. This occurred because older parents tended to reach minimum PRL levels later than younger parents. Relationships between hormones and behaviour are often attributed to changes in the strength of a hormonal signal, including the rate of change in signal strength. For example, in gestating rats, the rate of change in luteinizing hormone influences the maintenance of progesterone secretion by luteal cells (Nulsen, Kavel & Peluso 1991), and in humans, the rate of change in growth hormone is known to influence growth (Hindmarsh *et al.* 1992). Our results indicate that the PRL levels of younger parents would change more rapidly in response to the same stressors than those of older parents, which is expected to lead to less consistent incubation behaviour in younger than in older parents (Buntin *et al.* 1996).

Only one study has investigated the relationship between natural variation in stress-induced PRL levels and parental behaviour. In snow petrels (*Pagodroma nivea*), another long-lived seabird, Angelier *et al.* (2007b) observed a negative relationship between stress-induced PRL levels and the frequency with which the petrels left their eggs unattended. Furthermore, stress-induced minimum PRL levels declined with age,

and in females PRL levels decreased more rapidly in younger than in older parents, which suggests that a more rapid decline in PRL may have an important influence on parental behaviour, at least in females.

In addition to an attenuation of the PRL stress response with age, we also found that stress-induced maximum CORT levels were lower in older than in younger parents, as we have previously reported in this species (Heidinger, Nisbet & Ketterson 2006), but this was not observed in snow petrels (Angelier *et al.* 2007b). Previously we have found that a transient, experimental increase in CORT suppresses incubation behaviour in this population of common terns (Heidinger, Nisbet, & Ketterson, unpublished data). A slower decline in PRL coupled with a smaller rise in CORT, as reported here, would be expected to make older terns less likely to reduce parental care in the face of stressors than younger terns – at least in the short-term – and may contribute to an increase in reproductive success with age without compromising the ability of older terns to respond robustly to long-term stressors. However, the mechanisms by which stress-induced changes in PRL and CORT influence parental behaviour are currently unknown and would be an exciting area of future study.

Age-related attenuations of the PRL and CORT stress responses may be two distinct mechanisms that permit an increase in reproductive performance in older parents but whose relative implementation varies among individuals. Alternatively, they may be co-regulated mechanisms. In common terns, stress-induced measures of PRL and CORT were not correlated within individuals. Similarly, stress-induced measures of PRL and CORT were not correlated in either breeding snow petrels (*Pagodroma nivea*) (Angelier *et al.* 2007b) or black-legged kittiwakes (*Rissa tridactyla*) (Chastel *et al.* 2005). Although these studies suggest that the PRL and CORT stress responses may be independent mechanisms, an experimental approach will be necessary to determine whether or not they are casually linked.

HORMONES, AGE AND REPRODUCTIVE PERFORMANCE

Reproductive investment, while difficult to quantify, appears to increase with age in this population of common terns. As one example, older parents maintained higher nest attentiveness than younger parents after exposure to an experimentally simulated predator (Meehan & Nisbet 2002). Findings reported here suggest that modulations of the PRL and CORT stress responses may be important among the mechanisms that allow parents to flexibly modify reproductive investment with age. There are however, alternative explanations: age-related changes in the stress response might be attributable to previous capture or reproductive experience, senescence or prior selection events (reviewed in Forslund & Pärt 1995). For example, previous capture experience with a stressor may lead to an attenuation of the stress response on subsequent exposures because of habituation (Yelvington, Weiss & Ratner 1985; Sapolsky, Romero & Munck 2000). However, contrary to what would be expected if this were the case, we found limited evidence that minimum PRL levels

decreased rather than increased with recapture history. Further, consistent with what we have reported previously in this species, we did not find any evidence that stress-induced CORT was related to recapture history (Heidinger, Nisbet & Ketterson 2006).

Prior reproductive experience may also be important, as it has been reported to influence parental behaviour (Fleming & Sarker 1990; Wang & Buntin 1999), basal PRL and CORT levels (Wang & Buntin 1999; Fleming *et al.* 2002; Angelier *et al.* 2006, 2007a; Delahunty *et al.* 2007), and PRL receptor densities (Anderson *et al.* 2006). Most of the studies that have addressed the effects of age on parental behaviour and hormone levels have focused on changes early in life, i.e. when comparing nulliparous to multiparous breeders or seeking age-related effects during the first few breeding attempts. It is currently unclear whether experience continues to influence physiology later in life, particularly in long-lived species (Forslund & Pärt 1995).

Experienced breeders might also be expected to respond less strongly to stressors if they are in better condition or have access to higher quality territories or resources that make them relatively more buffered against stressors than inexperienced breeders (Angelier *et al.* 2007a). There is no evidence that condition increases with age in common terns (Heidinger, Nisbet & Ketterson 2006). However, it is not possible to tease apart the relative effects of age and breeding experience on residual reproductive value, reproductive performance and the stress response in this species because adults typically breed every year once they have reached reproductive maturity (Nisbet 2002). It would be interesting to address these questions in a captive population of a shorter-lived species in which breeding experience could be more easily manipulated.

It is also possible that the age-related declines in the stress response that we observed are due to senescence. Senescent individuals are often characterized by elevated baseline CORT as a result of impaired negative feedback regulation (Stein-Behrens & Sapolsky 1992). Baseline CORT is not related to age in this species, which suggests that there is no overall decline in pituitary or adrenal function (Heidinger, Nisbet & Ketterson 2006). However, we have previously reported that older terns have reduced adrenal capacity (ability to produce or secrete CORT in response to exogenous levels of ACTH), which may contribute to an adaptive attenuation of the stress response with age or be indicative of adrenal deterioration (Heidinger, Nisbet & Ketterson 2008). There was also a weak negative relationship between age and baseline PRL levels, which may indicate that the capacity of the pituitary to synthesize or produce PRL decreases with age. However, age-related declines in baseline PRL are not uncommon and are often accompanied by an increase in PRL receptor density and hence sensitivity to PRL (Anderson *et al.* 2006).

In summary, this is the first study to report that in addition to the CORT stress response, the PRL stress response is also suppressed with age in the same breeding adults. These results are consistent with the hypothesis that modulations of both the PRL and CORT stress responses are flexible mechanisms that contribute to age-related changes in reproductive success. These results also highlight the need to consider the potential interactive effects of stress-induced changes in PRL and CORT on parental behaviour. Future longitudinal studies will be necessary to determine the relative roles of reproductive investment, previous experience, senescence, and prior selection events on age-related changes in the stress response. In addition, future research should investigate the mechanisms by which the PRL and CORT stress responses influence parental behaviour and are modulated with age (Heidinger, Nisbet & Ketterson 2008).

Acknowledgements

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