

## Prolactin stress response does not predict brood desertion in a polyandrous shorebird

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### ABSTRACT

One of the fundamental principles of the life-history theory is that parents need to balance their resources between current and future offspring. Deserting the dependent young is a radical life-history decision that saves resources for future reproduction but that may cause the current brood to fail. Despite the importance of desertion for reproductive success, and thus fitness, the neuroendocrine mechanisms of brood desertion are largely unknown. We investigated two candidate hormones that may influence brood desertion in the Kentish plover *Charadrius alexandrinus*: prolactin ('parental hormone') and corticosterone ('stress hormone'). Kentish plovers exhibit an unusually diverse mating and parental care system: brood desertion occurs naturally since either parent (the male or the female) may desert the brood after the chicks hatch and mate with a new partner shortly after. We measured the hormone levels of parents at hatching using the standard capture and restraint protocol. We subsequently followed the broods to determine whether a parent deserted the chicks. We found no evidence that either baseline or stress-induced prolactin levels of male or female parents predicted brood desertion. Although stress-induced corticosterone levels were generally higher in females compared with males, individual corticosterone levels did not explain the probability of brood desertion. We suggest that, in this species, low prolactin levels do not trigger brood desertion. In general, we propose that the prolactin stress response does not reflect overall parental investment in a species where different parts of the breeding cycle are characterized by contrasting individual investment strategies.

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### Introduction

Brood desertion, i.e., when a parent stops caring and abandons its dependent young, is a radical resolution of the life-history trade-off between current and future reproduction. Desertion may entail both costs (e.g., reduced offspring survival) and benefits (e.g., increased survival or remating and successive reproduction of the parent; Houston et al., 2005; Székely et al., 1996). Understanding brood desertion is important because it often has a major influence on brood survival and, as a consequence, parental fitness. In addition, brood desertion may affect the population demography and breeding system. Research often approaches brood desertion from life-history evolution perspective to understand the trade-offs that a parent faces in terms of current versus future reproductive success (Clutton-Brock, 1991; Lessells, 1999). In addition, brood desertion is an excellent model system to understand the conflict and cooperation between

parents. While there is extensive literature on the significance of brood desertion in evolutionary biology (Arnqvist and Rowe, 2005; Maynard Smith, 1977; McGraw et al., 2010), the physiological mechanisms that trigger brood desertion remain poorly understood.

Circulating hormones are thought to be the primary physiological mediators of life-history trade-offs (Flatt and Heyland, 2011; Ketterson and Nolan, 1999; Sinervo and Svensson, 1998). For example, when individual survival prospects are compromised because of threats by predators, pathogens, food shortage, and inclement weather (commonly referred to as stressors), vertebrates respond by activating the hypothalamus–pituitary–adrenal cortex (HPA) axis, which elevates the circulating levels of glucocorticoids (reviewed by Wingfield and Sapolsky, 2003). The glucocorticoids in turn trigger behaviors that aid in immediate survival and concurrently repress those that do not (e.g., reproduction). Because of these antagonistic effects, corticosterone, the avian glucocorticoid, has been hypothesized to mediate the survival–reproduction trade-off in birds (Ricklefs and Wikelski, 2002). Recent studies have supported this hypothesis by showing that corticosterone levels are modulated according to the relative importance of the current reproduction as compared with survival (Bókony

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et al., 2009; Heidinger et al., 2006; Lendvai and Chastel, 2008; Lendvai et al., 2007).

However, corticosterone may not be the only hormonal modifier of survival–reproduction trade-offs. The vertebrate stress response is complex and involves several physiological agents. The circulating levels of prolactin also change during the stress response; for example, standard handling stress induces a significant decrease in the plasma prolactin concentrations of several avian species (Angelier et al., 2007, 2009a; Heidinger et al., 2010; Riou et al., 2010). Since prolactin is actively involved in the regulation of parental behavior in birds and facilitates incubation and brooding behaviors (Adkins-Regan, 2005; Adkins-Regan et al., 2010), it has been recently suggested to play a key role as the physiological mediator of the trade-off between current parental care and future reproduction (Angelier and Chastel, 2009; Chastel et al., 2005). Specifically, the decrease in prolactin levels in response to a standardized stress has been proposed to reflect the willingness and/or ability to maintain parental care. Therefore, the prolactin stress response may be interpreted as a proximate signal of parental investment (Angelier and Chastel, 2009).

Our aim of this study was to investigate the underlying proximate mechanisms of brood desertion in a wild bird population. We focused on individual variation in hormone levels, since its study is essential for understanding the proximate mechanisms of life-history variation (McGlothlin et al., 2007; Williams, 2008). We investigated a small shorebird, the Kentish plover *Charadrius alexandrinus*, which exhibits well-characterized brood care behavior that includes brood desertion (Amat et al., 1999; Kosztolányi et al., 2006; Lessells, 1984). After chick hatching, either the male or the female parent may desert the brood, although the frequency of brood desertion differs between sexes and populations (Amat et al., 1999; Kosztolányi et al., 2006; Lessells, 1984; Székely and Lessells, 1993). Previous studies have elucidated the various costs and benefits of brood desertion in the Kentish plover (Kosztolányi et al., 2006; Székely et al., 1999); however, the neuroendocrine mechanisms of brood desertion remain unknown.

We tested four novel hypotheses regarding the roles of prolactin and corticosterone in brood desertion behavior. In our Kentish plover population, brood desertion by females is 11 times more frequent compared with desertion by males (Kosztolányi et al., 2006). Therefore, we first asked whether sex-biased desertion is a consequence of the lower prolactin levels in females compared with males. In precocial species such as the Kentish plover, concentrations of circulating levels of prolactin either drop sharply after the chicks hatch (Dittami, 1981; Goldsmith, 1982; Goldsmith and Williams, 1980; Hall and Goldsmith, 1983; Wentworth et al., 1983) or remain elevated after hatching and slowly decreases until the chicks are thermally independent (Boos et al., 2007; Gratto-Trevor et al., 1990; Oring et al., 1986a, 1988). Since Kentish plovers spend a significant amount of time on brooding their downy chicks (Székely and Cuthill, 1999), we expected that, in this species, the prolactin levels remain high after hatching and decline gradually as chicks grow and need less brooding. If females have lower baseline prolactin levels at hatching compared with males, their circulating prolactin may gradually decrease below a threshold level after hatching and result in brood desertion. Therefore, we predicted that baseline prolactin levels are lower in females compared with males. Second, we tested whether individual desertion decisions can be predicted by the prolactin stress response. Assuming that the decrease in prolactin in response to a standardized stressor is a surrogate measure of parental investment (Angelier and Chastel, 2009), we predicted that females deserting their brood would have a stronger prolactin stress response (i.e., lower stress-induced prolactin levels) compared with females that do not desert. Third, since the hormone corticosterone has been proposed as a mediator of life-history trade-off between reproduction and survival, we tested whether the corticosterone levels differ between females that usually desert the brood and males that rarely desert. Finally, we asked whether stress-induced corticosterone levels predict the desertion decision of individual

females. We predicted that females have higher baseline corticosterone levels compared with males and that deserting females have higher stress-induced corticosterone levels compared with non-deserting females.

## Methods

### Study area and field methods

Fieldwork was carried out at Lake Tuzla (36°43' N, 35°03' E), southern Turkey, for over two years (24 April–25 June 2009, 29 April–24 June 2010; for details about the study site and field methodology, see Kosztolányi et al., 2006; Lendvai et al., 2004). Both parents were captured using funnel traps at the nest at hatching or with the chicks after hatching. Chicks were captured either in the nest scrape immediately after hatching or at the first encounter (capture date of adults relative to hatching date of their clutch:  $0.5 \pm 0.21$  days (mean  $\pm$  SE), range:  $-2$ – $6$  days,  $n = 82$  adults). Adults were marked with a metal band and an individual combination of three color bands. Chicks were marked with two bands, one metal and one colored (the same color within a family). We measured the body mass, and the left and right tarsus length of each captured bird. Blood samples were taken from adults for hormone assays using the standard capture and restraint protocol (Wingfield, 1994):  $1.4 \pm 0.06$  min (mean  $\pm$  SE, range:  $0.8$ – $3.4$  min,  $n = 82$  adults) after capture, about  $150 \mu\text{l}$  (in 2009) or  $210 \mu\text{l}$  (in 2010) of blood was collected from the brachial vein, and the bird was then placed in a cloth bag. Neither corticosterone nor prolactin levels at the first blood sampling were significantly related to the time elapsed from installation of the trap to blood sampling (Pearson correlations, corticosterone:  $r = -0.07$ ,  $n = 39$ ,  $p = 0.676$ ; prolactin:  $r = 0.03$ ,  $n = 82$ ,  $p = 0.785$ ) or from capture to blood sampling (corticosterone:  $r = 0.15$ ,  $n = 39$ ,  $p = 0.367$ ; prolactin:  $r = 0.04$ ,  $n = 82$ ,  $p = 0.739$ ); as such, these samples are referred to as baseline. A second blood sample was collected  $30.7 \pm 0.10$  min (range:  $29.7$ – $35.8$  min) after the first blood sample. Blood samples were centrifuged, and the plasma was stored at  $-20^\circ\text{C}$  until analysis. One female was sampled in both years of the study. Only the brood from 2010 was retained in the dataset for this female. After hatching, the broods were visited regularly, and the sex and number of attending parents and the number of chicks were recorded. We attempted to follow broods until the chicks perished or until the chicks were 25 days old when considered fledged. We scored the parent as deserted if it was not present with the brood at two consecutive visits. As desertion by males was rare (see results), we analyzed the desertion decision in females only. Ten broods fledged at least one chick; all chicks died in four broods at  $0.8 \pm 0.48$  (mean  $\pm$  SE) days of age. Twenty-six broods were followed only until  $12.4 \pm 1.25$  days either because the fieldwork ended or because we were not able to locate the family; however, in five broods the female already deserted before this age.

### Hormone assays

Hormone concentrations were determined in duplicate aliquots from  $50 \mu\text{l}$  of plasma sample by radioimmunoassay at the Centre d'Études Biologiques de Chizé (CEBC), France. Prolactin measurements were available for  $n = 41$  pairs (in 2009: 21 pairs, in 2010: 20 pairs). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay at the CEBC as detailed in Cherel et al. (1994). Pooled plasma samples of Kentish plovers produced a dose–response curve that paralleled chicken prolactin standard curves ('AFP 4444B', source: Dr Parlow, NHPP Harbor-UCLA Medical Center, Torrance, CA, USA). Therefore, the cross-reactivity of the chicken prolactin antibody with prolactin was equivalent in both species and this heterologous assay could be used to assess relative concentrations of Kentish plover prolactin. The detection limit of the assay was  $19.26 \text{ ng/ml}$ , and the

lowest measurement was 194.14 ng/ml. The samples were run in two assays (intra-assay coefficient: 13%, inter-assay coefficient: 24%).

Blood for the corticosterone assay was collected from  $n = 20$  pairs in 2010; however, the plasma was not enough to run the assay in case of one male. Total corticosterone concentrations were measured in one assay as described in Lendvai et al. (2011). The intra-assay coefficient of variation was 7.07% for 10 duplicates. The minimum detectable level of corticosterone was 0.28 ng/ml (lowest measurement: 7.78 ng/ml).

### Statistical analyses

All data processing and statistical analyses were performed in the R computing environment (version: 2.14.1, R Development Core Team, 2011). First, we checked the possible effect of potential confounding variables: season (measured as days since 1 March), brood age, time of day, parental sex, tarsus length (the mean of left and right tarsus) and body condition. Body condition was estimated as residuals of standardized major axis regressions (SMAs) between body mass and tarsus length (Green, 2001; Warton et al., 2011). Since tarsus length was significantly smaller in females compared with males ( $t$ -test,  $t_{80} = 2.89$ ,  $p = 0.005$ ), body condition was estimated separately for the sexes (SMAs, males:  $b = 2.89$ ,  $n = 41$ ,  $p = 0.024$ ; females:  $b = 2.75$ ,  $n = 41$ ,  $p = 0.070$ ). Body condition was not significantly related to time of the day or season (Pearson correlations,  $n = 82$ ,  $p \geq 0.346$ ). Second, we used mixed-effects models including stress (first/second bleeding) and sex as factors, the confounding variables that had a significant effect in the preliminary analyses and all second order interactions as fixed effects. The repeated measurements of an individual were controlled for by including band ID as random structure in the models (Pinheiro and Bates, 2000). We carried out model selection using AIC values (Venables and Ripley, 2002). As several broods were not followed until fledging age (see above), the effect of hormone levels on desertion was analyzed using Cox regression. In these models, desertion was the terminal event, and non-deserted broods were censored cases. Neither baseline nor stress-induced prolactin levels differed between years ( $t$ -tests, baseline:  $t_{80} = 1.00$ ,  $p = 0.322$ ; stress-induced:  $t_{80} = 0.53$ ,  $p = 0.600$ ); therefore, we pooled and analyzed prolactin data from both years. One male exhibited an unusual corticosterone response to capture stress (see the white symbol in Fig. 3); therefore, we repeated all analyses excluding the hormone measurements of this male from the dataset. However, removal did not change our conclusions, and we only present the results based on the full data set.

## Results

### Parental care and prolactin

We observed 12 desertions in 41 broods. In 11 cases, the female deserted the family, and in one case, the male deserted the family. Median time of female desertion was 9 days post hatching (range: 2–24 days). Large females deserted the brood more often compared with small females (Cox regressions,  $n = 40$ , female tarsus length:  $b = 1.08$ ,  $p = 0.009$ ). However, neither the body size of males nor the body condition of either parent influenced the occurrence of desertion (male tarsus length:  $b = 0.43$ ,  $p = 0.145$ ; female condition:  $b = -0.12$ ,  $p = 0.202$ ; male condition:  $b = -0.12$ ,  $p = 0.274$ ). The probability of desertion tended to decrease over the breeding season (hatching date:  $b = -0.06$ ,  $p = 0.071$ ).

Baseline prolactin levels did not differ between the sexes ( $t$ -test,  $t_{80} = 0.03$ ,  $p = 0.977$ ) and were not significantly correlated with the body size or condition of the parents (Pearson correlations, sexes combined,  $n = 82$ , tarsus length:  $r = -0.12$ ,  $p = 0.289$ ; body condition:  $r = 0.10$ ,  $p = 0.362$ ). Baseline prolactin levels decreased with chick age; however, neither hatching date nor time of the day were significantly correlated with baseline prolactin levels (Pearson correlations, sexes

combined,  $n = 82$ , brood age:  $r = -0.28$ ,  $p = 0.012$ ; hatching date:  $r = 0.17$ ,  $p = 0.122$ ; time of the day:  $r = 0.16$ ,  $p = 0.159$ ).

As expected, in both sexes, circulating prolactin levels decreased in response to the capture restraint stress (Fig. 1,  $t$ -test,  $t_{162} = 14.61$ ,  $p < 0.001$ ). The sexes did not differ in their stress-induced prolactin levels ( $t$ -test,  $t_{80} = 0.62$ ,  $p = 0.540$ ). However, there was a significant positive correlation between the stress-induced prolactin level of male and female parents in a given brood (Pearson correlation,  $n = 41$ ,  $r = 0.34$ ,  $p = 0.032$ ). The mixed-effects model analysis yielded results consistent with the  $t$ -tests (Table 1).

The stress-induced prolactin levels of caring females were higher compared with deserting females (Fig. 1,  $t$ -test,  $t_{38} = 2.06$ ,  $p = 0.046$ ); however, the caring female group also included parents whose chicks died and those whose brood was not followed until fledging age. Survival analyses revealed that the stress-induced prolactin levels of females did not influence the probability of desertion in females (Cox regression controlling for female size,  $n = 40$ , stress-induced prolactin:  $b = 0.001$ ,  $p = 0.712$ ; tarsus:  $b = 1.12$ ,  $p = 0.009$ ). Adding the stress-induced prolactin level of males into the model did not significantly increase the model fit (Likelihood ratio test,  $\chi^2 = 0.65$ ,  $df = 1$ ,  $p = 0.422$ ). The plot of observed caring history of broods against female stress-induced prolactin levels revealed that although we did not observe desertion among females with the highest stress-induced prolactin levels, several long-caring females had relatively low stress-induced prolactin levels at sampling (Fig. 2).

### Corticosterone

Baseline corticosterone levels did not differ between male and female parents ( $t$ -test,  $t_{37} = 1.15$ ,  $p = 0.259$ ). Corticosterone levels did not correlate significantly with parent's body size or condition, age of the brood, hatching date or time of the day (Pearson correlations, sexes combined,  $n = 39$ , all  $p \geq 0.173$ ). As expected, capture stress induced a significant increase in corticosterone levels (Fig. 3, Table 1,  $t$ -test,  $t_{76} = 11.76$ ,  $p < 0.001$ ), and females had higher stress-induced corticosterone levels compared with males ( $t_{37} = 2.24$ ,  $p = 0.031$ ). There was no significant correlation between stress-induced corticosterone levels between the male and the female of a given pair (Pearson correlation,  $n = 19$ ,  $r = 0.17$ ,  $p = 0.495$ ).

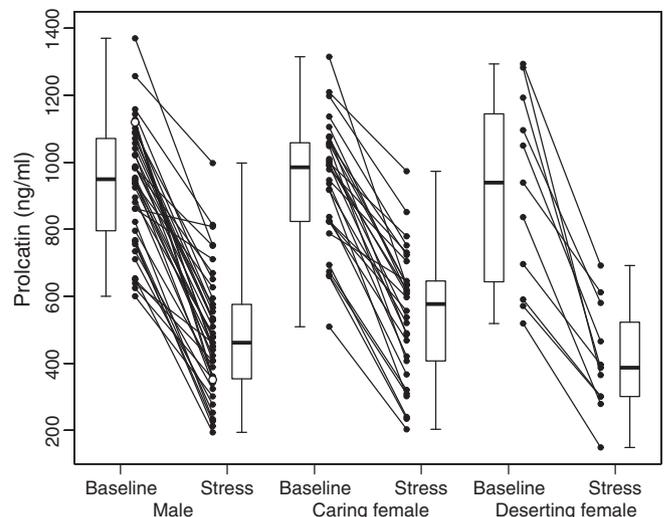


Fig. 1. Individual baseline and stress-induced prolactin levels and boxplots of hormone levels in the Kentish plover. For each box, the central line represents the median, and the bottom and the top of the box are the lower and upper quartiles, respectively. The lower and upper whiskers extend to the lowest and highest observations, respectively. The open circles denote a male with an unusual change in corticosterone levels (see Methods section).

**Table 1**

Parameter estimates from the minimal mixed-effects models for prolactin (ng/ml) and corticosterone (ng/ml) plasma levels (response variables) in the Kentish plover after AIC-based model selection.

	Prolactin					Corticosterone				
	Value	SE	df	t	p	Value	SE	df	t	p
Brood age	−32.53	9.81	80	3.32	0.001					
Sex						−8.96	4.05	37	2.21	0.033
Stress	−438.84	17.80	81	24.65	<0.001	44.72	3.29	38	13.59	<0.001

The initial model for prolactin contained brood age, sex and stress and all second order interactions and for the corticosterone sex, stress and sex × stress interaction.

Stress-induced corticosterone levels did not predict desertion behavior (Cox regression controlling for female size,  $n = 19$ , stress-induced corticosterone:  $b = -0.003$ ,  $p = 0.892$ ; tarsus:  $b = 1.32$ ,  $p = 0.036$ ). Adding the stress-induced corticosterone level of males did not significantly improve model fit (Likelihood ratio test,  $\chi^2 = 2.24$ ,  $df = 1$ ,  $p = 0.134$ ).

**Discussion**

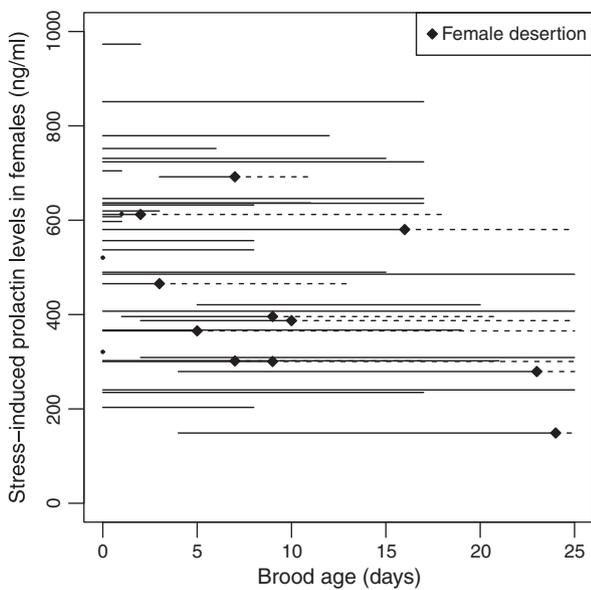
Prolactin levels are associated with parental care in many species, including birds, mammals and fish (reviewed by Adkins-Regan et al., 2010; Angelier and Chastel, 2009; Rall et al., 2004; Ziegler et al., 2009), although in our study, prolactin levels at (or near) hatching did not predict brood desertion by Kentish plovers. We postulate that prolactin is unrelated to the deserting decision in this species and that this behavior may be regulated by alternative pathways. Conversely, prolactin may influence the probability of desertion behavior; however, we did not detect this relationship since the decrease in prolactin levels occurs immediately prior to desertion.

Brood desertion is an adaptive strategy if the benefits associated with desertion outweigh the costs (Clutton-Brock, 1991; McGraw et al., 2010; Székely et al., 1996). Such benefits may be either the increased survival or the possibility to rapidly remate and increase reproductive success. Although prolactin has been shown to correlate with clutch abandonment, brood desertion or temporary egg neglect (Angelier et al., 2007; Chastel and Lormée, 2002; Groscolas et al., 2008; Spee et al., 2010), desertion in these species occurred when the parents were at a severe energy deficit. As such, this behavior is the

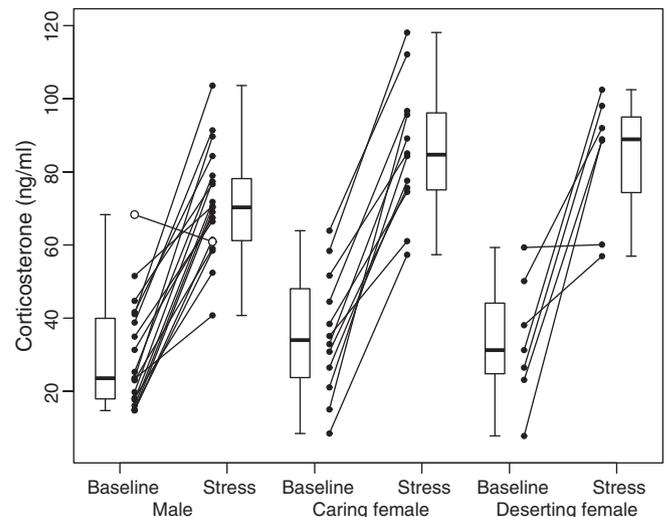
result of an emergency reaction and serves to increased survival. In one study, clutch desertion was related to reduced prolactin levels; however, both the desertion decision and the prolactin decrease may have been triggered by the partial clutch loss, i.e., cues of nest predation (Hall, 1987). In contrast, brood desertion in Kentish and snowy plovers *Charadrius nivosus*, formerly considered *C. alexandrinus nivosus*, is part of the natural breeding system, and deserting parents often remate and lay new clutches (Székely and Williams, 1995; Warriner et al., 1986). Therefore, different neuroendocrine pathways may regulate these two types of desertion, and the decision of desertion may be independent of influence from prolactin levels in plovers.

Elevated prolactin levels are known to induce parental behaviors such as persistent incubation and brooding (Adkins-Regan et al., 2010; Angelier and Chastel, 2009; Buntin, 1996). Since prolactin also has antagonist effects on luteinizing hormone (LH) and gonadal steroids, both of which are necessary for rebreeding (Sharp et al., 1998), specific adaptations would be required if birds were to leave their brood and initiate a new clutch with elevated prolactin levels. Such a scenario is plausible, as it has already been reported in the multi-brooded song sparrow *Melospiza melodia* whose prolactin levels do not decline between subsequent nests or after nest failure (Wingfield and Goldsmith, 1990).

Behavioral effects of prolactin may be achieved by modulating cellular receptors rather than circulating hormone concentrations per se. For instance, although red-winged blackbirds *Agelaius phoeniceus*, European starlings *Sturnus vulgaris* and brown-headed cowbirds *Molothrus ater* show elevated prolactin levels during reproduction, the nest-parasitic brown-headed cowbird, which provides no parental care, has reduced prolactin binding in the preoptic area of the brain compared with the two care giving species, the red-winged blackbird and the European starling (Ball, 1991). Since female plovers are able to initiate and incubate a new clutch a few days after desertion



**Fig. 2.** Stress-induced prolactin levels in female Kentish plovers and care type of 40 biparental or female deserted broods. Each brood is represented by a horizontal line over the brood ages that it was observed (or a dot if it was observed only on one day); a solid line indicates biparental whereas a broken line indicates male-only care.



**Fig. 3.** Individual baseline and stress-induced corticosterone levels and boxplots of hormone levels in the Kentish plover. Boxplots as in Fig. 1. The open circles denote a male with unusual corticosterone levels (see Methods section).

(Székely et al., 1999), it seems reasonable to suggest that, instead of shutting down and restarting prolactin production, they modulate cellular sensitivity during times the physiological effects of prolactin are inappropriate.

If, however, prolactin plays a role in the regulation of brood desertion, it must be temporarily decoupled from the hormone levels that we measured close to hatching. Consistent with the idea that prolactin reflects the amount of active parental care, we found that, similar to other polygamous shorebirds, baseline prolactin decreased after hatching when the chicks became more independent (e.g., Wilson's phalaropes *Phalaropus tricolor*, Oring et al., 1988; red-necked phalaropes *Phalaropus lobatus*, Gratto-Trevor et al., 1990). These results suggest that the need for brooding decreases rapidly in the first few days after hatching as the thermoregulation of chicks develops (Visser and Ricklefs, 1993). Female Kentish plovers did not have lower baseline prolactin levels compared with males. This result is consistent with the idea that prolactin generally reflects the amount of parental activity (incubation or brooding), since both sexes of this species participate in incubation (Kosztolányi and Székely, 2002). For instance, the baseline prolactin levels reflect the sex differences in incubation in other shorebirds: there is no difference in the biparental ruddy turnstones *Arenaria interpres* (Perkins, 2004), but males have higher prolactin levels in three polyandrous sandpiper species with predominant or exclusive male care (Gratto-Trevor et al., 1990; Oring et al., 1986b, 1988).

Our result shows that the different desertion patterns between the sexes are not the consequence of the lower prolactin levels of females at hatching. Furthermore, the slope of the decrease in prolactin after hatching was similar in males and females (results not shown), which suggests that, if plummeting prolactin levels are responsible for the sex-biased desertion pattern, then the fall of prolactin levels should occur later, potentially only shortly before desertion. For example, in Eurasian penduline tits *Remiz pendulinus*, another bird species where desertion by either sex is part of the natural breeding system, desertion is a rapid process, and the behavior of the parents shortly before they leave does not predict their decision (van Dijk et al., 2007). In species such as the penduline tit or the Kentish plover, where there is an intensive sexual conflict over parental care and both sexes may desert (Székely et al., 2006), selection may favor physiological mechanisms that allow for rapid shifts in behavior. In contrast, in species where desertion occurs as a response to an energetic crisis, clutch or brood abandonment seems to be slow. For instance, an association of low prolactin levels, a progressive decrease in nest attendance and a transitory neglect of the eggs preceding definitive desertion or clutch failure have been observed in king penguins *Aptenodytes patagonicus* (Groscolas et al., 2008), red-footed boobies *Sula sula* (Chastel and Lormée, 2002), snow petrels *Pagodroma nivea* (Angelier et al., 2007) and black-legged kittiwakes *Rissa tridactyla* (Angelier et al., 2009b).

The rapid transition between care and desertion may explain why the results did not support our second prediction, namely, that deserting females will respond more strongly to a standardized stressor compared with females that continue to provide care. By measuring the prolactin stress response, we may have measured the parental investment of the parents at the day of sampling (i.e., the end of the incubation or the beginning of chick rearing), which may not have been different for females that later decided to desert or to care.

The decision of a parent to desert may depend on the willingness of the mate to continue care (Houston et al., 2005). Prolactin stress response of the male did not explain female desertion in our analyses, but we did find that stress-induced prolactin levels were significantly correlated between pair members. This intriguing result may indicate assortative mating. For instance, similar parental personality traits within breeding pairs have positive effects on offspring fitness in the zebra finch *Taenopygia guttata* (Schuett et al., 2011). Similarity in behavioral traits may enhance reproductive success if they can reduce

sexual conflict over parental care in a stage when parents are bound to cooperate (Kosztolányi et al., 2009). Alternatively, this correlation between parents may result from some spatial or temporal variation in the breeding conditions that may have affected both parents. The assortative pattern in prolactin levels also explains why male prolactin levels did not explain female desertion.

Desertion was influenced by female size but not body condition. Larger females deserted more often; however, seasonal effects may confound this relationship. Females breeding earlier in the season had significantly larger tarsi (results not shown), and desertion early in the season is expected to be more beneficial, since remating probabilities decrease over the breeding season (Székely et al., 1999). The most likely explanation for the lack of an effect of condition on desertion behavior is that body mass (and hence condition) of female Kentish plovers may change considerably over the day (Szentirmai et al., 2001).

Corticosterone is considered an important mediator of the life-history between reproduction and survival, and therefore, the levels of this hormone may affect fitness (Blas et al., 2007; Bonier et al., 2009; Ricklefs and Wikelski, 2002; Wingfield et al., 1995). The biological effect of baseline and stress-induced levels is different, with baseline levels having essentially metabolic effects, whereas corticosterone may trigger significant shifts in behavior in concert with other hormones at the higher stress-induced levels (Angelier et al., 2009b; Hau et al., 2010; Wingfield and Sapolsky, 2003). We found that baseline corticosterone levels did not differ between the sexes but that stress-induced corticosterone levels were higher in females compared with males. The similarity of baseline levels between the two sexes suggests that female desertion in Kentish plovers is not driven by an energy deficit but that it reflects a reproductive strategy. On the other hand, the higher stress-induced corticosterone levels in females compared with males indicate that the value of a given reproduction is lower for the potentially multibrooded polyandrous females compared with the males. However, individual corticosterone levels in females were not related to the desertion probability. Recent studies have shown that natural variation in corticosterone levels is negatively related to post-stress parental care (Lendvai and Chastel, 2010; Miller et al., 2009). We found an eightfold variation in baseline and a threefold variation in stress-induced corticosterone levels, but this extensive variation contributed little to explaining the probability of desertion in individual females.

## Conclusions

In this study, we investigated whether hormone levels predict desertion in a species where brood desertion is part of the natural breeding system. We tested the specific hypothesis of Angelier and Chastel (2009) that the prolactin stress response may be regarded as a surrogate measure of parental investment. We studied the natural individual variation in response to a standardized experimental stressor, and we found that neither the 'stress hormone' corticosterone nor the 'parental hormone' prolactin measured near hatching of the young was related to brood desertion. While our results corroborate the idea that the magnitude of prolactin stress response may indicate parental investment at the moment of sampling, this study also refutes the interpretation that prolactin stress response may indicate an overall parental investment. This conclusion may be valid for any species where different parts of the breeding cycle may represent varying parental investment strategies. Brood desertion by female Kentish plovers cannot be the consequence of their low overall parental commitment or their energy deficit. We propose that brood desertion is a rapid process in this species and that it may not be under hormonal control. The decisive test for this hypothesis would require manipulating prolactin secretion or its binding to receptors, or inducing desertion behavior by manipulating mating opportunities in natural habitats of plovers.

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## References

- Adkins-Regan, E., 2005. *Hormones and Animal Social Behavior*. Princeton University Press, Princeton.
- Adkins-Regan, E., DeVoogd, T.J., Moore, J.M., 2010. Social behaviour and bird song from a neural and endocrine perspective. In: Székely, T., Moore, A.J., Komdeur, J. (Eds.), *Social Behaviour: Genes, Ecology and Evolution*. Cambridge University Press, Cambridge, pp. 59–84.
- Amat, J.A., Fraga, R.M., Arroyo, G.M., 1999. Brood desertion and polygamous breeding in the Kentish plover *Charadrius alexandrinus*. *IBIS* 141, 596–607.
- Angelier, F., Chastel, O., 2009. Stress, prolactin and parental investment in birds: a review. *Gen. Comp. Endocrinol.* 163, 142–148.
- Angelier, F., Moe, B., Blanc, S., Chastel, O., 2009a. What factors drive prolactin and corticosterone responses to stress in a long-lived bird species (snow petrel *Pagodroma nivea*)? *Physiol. Biochem. Zool.* 82, 590–602.
- Angelier, F., Clément-Chastel, C., Weckler, J., Gabrielsen, G.W., Chastel, O., 2009b. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Funct. Ecol.* 23, 784–793.
- Angelier, F., Moe, B., Weimerskirch, H., Chastel, O., 2007. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? *J. Anim. Ecol.* 76, 1181–1191.
- Arnqvist, G., Rowe, L., 2005. *Sexual Conflict*. Princeton University Press, Princeton.
- Ball, G.F., 1991. Endocrine mechanisms and the evolution of avian parental care. *Acta XX Congressus Int. Ornithologici* 199, 984–991.
- Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. *PNAS* 104, 8880–8884.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642.
- Boos, M., Zimmer, C., Carriere, A., Robin, J., Petit, O., 2007. Post-hatching parental care behaviour and hormonal status in a precocial bird. *Behav. Processes* 76, 206–214.
- Buntin, J.D., 1996. Neural and hormonal control of parental behaviour in birds. In: Rosenblatt, J.S., Snowdon, C.T. (Eds.), *Advances in the Study of Behavior*, vol. 25. Academic Press, New York, pp. 161–213.
- Bókony, V., Lendvai, Á.Z., Líker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* 173, 589–598.
- Chastel, O., Lacroix, A., Weimerskirch, H., Gabrielsen, G., 2005. Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. *Horm. Behav.* 47, 459–466.
- Chastel, O., Lormée, H., 2002. Patterns of prolactin secretion in relation to incubation failure in a tropical seabird, the red-footed booby. *Condor* 104, 873–876.
- Cherel, Y., Maugé, R., Lacroix, A., Gilles, J., 1994. Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. *Physiol. Zool.* 67, 1154–1173.
- Clutton-Brock, T.H., 1991. *The Evolution of Parental Care*. Princeton University Press, Princeton, New Jersey.
- Dittami, J.P., 1981. Seasonal changes in the behavior and plasma titers of various hormones in barheaded geese, *Anser indicus*. *Z. Tierpsychol. J. Comp. Ethol.* 55, 289–324.
- Flatt, T., Heyland, A., 2011. *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press, New York.
- Goldsmith, A.R., 1982. The Australian black swan (*Cygnus atratus*): prolactin and gonadotropin secretion during breeding including incubation. *Gen. Comp. Endocrinol.* 46, 458–462.
- Goldsmith, A.R., Williams, D.M., 1980. Incubation in mallards (*Anas platyrhynchos*): changes in plasma-levels of prolactin and luteinizing hormone. *J. Endocrinol.* 86, 371–379.
- Gratto-Trevor, C.L., Oring, L.W., Fivizzani, A.J., Elhalawani, M.E., Cooke, F., 1990. The role of prolactin in parental care in a monogamous and a polyandrous shorebird. *Auk* 107, 718–729.
- Green, A.J., 2001. Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82, 1473–1483.
- Groscolas, R., Lacroix, A., Robin, J., 2008. Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? *Horm. Behav.* 53, 51–60.
- Hall, M., 1987. Nesting success in mallards after partial clutch loss by predators. *J. Wildl. Manage.* 51, 530–533.
- Hall, M.R., Goldsmith, A.R., 1983. Factors affecting prolactin secretion during breeding and incubation in the domestic duck (*Anas platyrhynchos*). *Gen. Comp. Endocrinol.* 49, 270–276.
- Hau, M., Ricklefs, R.E., Wikelski, M., Lee, K.A., Brawn, J.D., 2010. Corticosterone, testosterone and life-history strategies of birds. *Proc. R. Soc. Lond. B* 277, 3203–3212.
- Heidinger, B., Chastel, O., Nisbet, I., Ketterson, E., 2010. Mellowing with age: older parents are less responsive to a stressor in a long-lived seabird. *Funct. Ecol.* 24, 1037–1044.
- Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2006. Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Proc. R. Soc. Lond. B* 273, 2227–2231.
- Houston, A.I., Székely, T., McNamara, J.M., 2005. Conflict between parents over care. *Trends Ecol. Evol.* 20, 33–38.
- Ketterson, E.D., Nolan Jr., V., 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* 154, S4–S25.
- Kosztolányi, A., Székely, T., 2002. Using a transponder system to monitor incubation routines of snowy plovers. *J. Field Ornithol.* 73, 199–205.
- Kosztolányi, A., Cuthill, I.C., Székely, T., 2009. Negotiation between parents over care: reversible compensation during incubation. *Behav. Ecol.* 20, 446–452.
- Kosztolányi, A., Székely, T., Cuthill, I.C., Yılmaz, K.T., Berberoğlu, S., 2006. Ecological constraints on breeding system evolution: the influence of habitat on brood desertion in Kentish plover. *J. Anim. Ecol.* 75, 257–265.
- Lendvai, Á.Z., Bókony, V., Chastel, O., 2011. Coping with novelty and stress in free-living house sparrows. *J. Exp. Biol.* 214, 821–828.
- Lendvai, Á.Z., Chastel, O., 2008. Experimental mate-removal increases the stress response of female house sparrows: the effects of offspring value? *Horm. Behav.* 53, 395–401.
- Lendvai, Á.Z., Chastel, O., 2010. Natural variation in stress response is related to post-stress parental effort in male house sparrows. *Horm. Behav.* 58, 936–942.
- Lendvai, Á.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. *Proc. R. Soc. Lond. B* 274, 391–397.
- Lendvai, Á.Z., Kis, J., Székely, T., Cuthill, I.C., 2004. An investigation of mate choice based on manipulation of multiple ornaments in Kentish plovers. *Anim. Behav.* 67, 703–709.
- Lessells, C.M., 1984. The mating system of Kentish plovers *Charadrius alexandrinus*. *IBIS* 126, 474–483.
- Lessells, C.M., 1999. Sexual conflict in animals. In: Keller, L. (Ed.), *Levels of Selection in Evolution*. Princeton University Press, Princeton, pp. 75–99.
- Maynard Smith, J., 1977. Parental investment: a prospective analysis. *Anim. Behav.* 25, 1–9.
- McCloughlin, J.W., Jawor, J.M., Ketterson, E.D., 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *Am. Nat.* 170, 864–875.
- McGraw, L., Székely, T., Young, L.J., 2010. Pair bonds and parental behaviour. In: Székely, T., Moore, A.J., Komdeur, J. (Eds.), *Social Behaviour: Genes, Ecology and Evolution*. Cambridge University Press, Cambridge, pp. 271–301.
- Miller, D.A., Vleck, C.M., Otis, D.L., 2009. Individual variation in baseline and stress-induced corticosterone and prolactin levels predicts parental effort by nesting mourning doves. *Horm. Behav.* 56, 457–464.
- Oring, L.W., Fivizzani, A.J., Colwell, M.A., Elhalawani, M.E., 1988. Hormonal changes associated with natural and manipulated incubation in the sex-role reversed Wilson's phalarope. *Gen. Comp. Endocrinol.* 72, 247–256.
- Oring, L.W., Fivizzani, A.J., Elhalawani, M.E., 1986a. Changes in plasma prolactin associated with laying and hatch in the spotted sandpiper. *Auk* 103, 820–822.
- Oring, L.W., Fivizzani, A.J., Elhalawani, M.E., Goldsmith, A.R., 1986b. Seasonal changes in prolactin and luteinizing hormone in the polyandrous spotted sandpiper, *Actitis macularia*. *Gen. Comp. Endocrinol.* 62, 394–403.
- Perkins, D.E., 2004. The breeding ecology and behavioral endocrinology of ruddy turnstones (*Arenaria interpres*) in the eastern Canadian Arctic. Master Thesis, University of Maine.
- Pinheiro, J.C., Bates, D.M., 2000. *Mixed-effects Models in S and S-PLUS*. Springer-Verlag, New York, NY.
- R Development Core Team, 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria3-900051-07-0. URL <http://www.R-project.org>.
- Rall, M.K., Liljander, M., Borg, B., 2004. Prolactin diminishes courtship behaviour and stimulates fanning in nesting male three-spined sticklebacks, *Gasterosteus aculeatus*. *Behaviour* 141, 1511–1519.
- Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. *Trends Ecol. Evol.* 17, 462–468.
- Riou, S., Chastel, O., Lacroix, A., Hamer, K.C., 2010. Stress and parental care: prolactin responses to acute stress throughout the breeding cycle in a long-lived bird. *Gen. Comp. Endocrinol.* 168, 8–13.
- Schuett, W., Dall, S.R.X., Royle, N.J., 2011. Pairs of zebra finches with similar 'personalities' make better parents. *Anim. Behav.* 81, 609–618.
- Sharp, P.J., Dawson, A., Lea, R.W., 1998. Control of luteinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. C* 119, 275–282.
- Sinervo, B., Svensson, E., 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83, 432–442.
- Spee, M., Beaulieu, M., Dervaux, A., Chastel, O., Le Maho, Y., Raclot, T., 2010. Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the adelie penguin. *Horm. Behav.* 58, 762–768.
- Székely, T., Cuthill, I.C., 1999. Brood desertion in Kentish plover: the value of parental care. *Behav. Ecol.* 10, 191–197.

- Székely, T., Cuthill, I.C., Kis, J., 1999. Brood desertion in Kentish plover: sex differences in remating opportunities. *Behav. Ecol.* 10, 185–190.
- Székely, T., Lessells, C.M., 1993. Mate change by Kentish plovers *Charadrius alexandrinus*. *Ornis Scand.* 24, 317–322.
- Székely, T., Thomas, G.H., Cuthill, I.C., 2006. Sexual conflict, ecology, and breeding systems in shorebirds. *Bioscience* 56, 801–808.
- Székely, T., Webb, J.N., Houston, A.I., McNamara, J.M., 1996. An evolutionary approach to offspring desertion in birds. In: Nolan Jr., V., Ketterson, E.D. (Eds.), *Current Ornithology*. Plenum Publisher, New York, pp. 271–330.
- Székely, T., Williams, T.D., 1995. Costs and benefits of brood desertion in female Kentish plovers, *Charadrius alexandrinus*. *Behav. Ecol. Sociobiol.* 37, 155–161.
- Szentirmai, I., Kosztolányi, A., Székely, T., 2001. Daily changes in body mass of incubating Kentish plovers. *Ornis Hung.* 11, 27–32.
- van Dijk, R.E., Szentirmai, I., Komdeur, J., Székely, T., 2007. Sexual conflict over parental care in penduline tits *Remiz pendulinus*: the process of clutch desertion. *IBIS* 149, 530–534.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S*. Springer, New York, NY.
- Warriner, J.S., Warriner, J.C., Page, G.W., Stenzel, L.E., 1986. Mating system and reproductive success of a small population of polygamous snowy plovers. *Wilson Bull.* 98, 15–37.
- Warton, D.I., Duursma, R.A., Falster, D.S., Taskinen, S., 2011. SMATR 3 — an R package for estimation and inference about allometric lines. *Methods Ecol. Evol.* doi:10.1111/j.2041-210X.2011.00153.x
- Wentworth, B.C., Proudman, J.A., Opel, H., Wineland, M.J., Zimmermann, N.G., Lapp, A., 1983. Endocrine changes in the incubating and brooding turkey hen. *Biol. Reprod.* 29, 87–92.
- Williams, T.D., 2008. Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean'. *Philos. Trans. R. Soc.* 363, 1687–1698.
- Wingfield, J.C., Goldsmith, A.R., 1990. Plasma levels of prolactin and gonadal steroids in relation to multi-brooding and re-nesting in free-living populations of the song sparrow, *Melospiza melodia*. *Horm. Behav.* 24, 89–103.
- Wingfield, J.C., 1994. Modulation of the adrenocortical response to stress in birds. In: Davey, K., Peter, R., Tobe, S. (Eds.), *Perspectives in Comparative Endocrinology*. National Research Council of Canada, Ottawa, Canada, pp. 520–528.
- Wingfield, J.C., O'Reilly, K.M., Astheimer, L.B., 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *Am. Zool.* 35, 285–294.
- Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* 15, 711–724.
- Visser, G.H., Ricklefs, R.E., 1993. Development of temperature regulation in shorebirds. *Physiol. Zool.* 66, 771–792.
- Ziegler, T.E., Prudom, S.L., Zahed, S.R., Parlow, A.F., Wegner, F., 2009. Prolactin's mediative role in male parenting in parentally experienced marmosets (*Callithrix jacchus*). *Horm. Behav.* 56, 436–443.