

Prolactin and Parental Behavior in Adélie Penguins: Effects of Absence from Nest, Incubation Length, and Nest Failure

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Adélie penguin (*Pygoscelis adeliae*) males and females, nesting in Antarctica, alternate attendance at the nest with absences of many days to forage at sea. We investigated the importance of tactile input from egg and chicks on prolactin levels by observing nest attendance patterns and obtaining blood samples (1) during the first nest exchange of the incubation stage, (2) from birds whose incubation period was artificially increased or decreased by about 10 days, and (3) from birds whose nests had failed. Prolactin levels in females after 8 to 11 days of absence from the breeding colony did not differ from those in incubating males and did not change after females resumed incubation. Moving eggs between nests resulted in nests in which chicks hatched after about 26, 36 (normal), or 46 days. Duration of incubation did not affect prolactin levels in the parents measured during incubation, at the pip stage, hatch stage, or early brood stage. Adults first left their chicks unguarded on about the same calendar date, regardless of chick age. However, chicks from long incubation nests averaged 8 days younger when they were left unguarded than chicks from control or short-incubation nests. In females, there was no effect of nest failure on prolactin levels. In males, prolactin levels were slightly lower after nest failure than in males tending nests. Testosterone was significantly higher in males after nest failure than in males still tending nests. Prolactin is elevated in Adélie penguins as part of the program of cyclical hormonal changes that accompany the lengthy reproductive season and is relatively independent of tactile input. Sustained prolactin secretion is probably required for the maintenance of parental behavior in offshore feeding species that must be absent from the nest for many days at a time. © 2000 Academic Press

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Antarctic penguins are remarkable for the constraints on reproduction imposed by their foraging ecology (Williams, 1995). In the Adélie penguin, *Pygoscelis adeliae*, both sexes migrate to the breeding sites from offshore feeding grounds and remain on land without feeding until two eggs are laid (Ainley, LeResche, and Sladen, 1983). Incubation must begin immediately because unattended eggs will freeze or be lost to avian predators. Once the eggs hatch, chicks are brooded until they can thermoregulate effectively. Then parents guard the chicks, defending them against predators and other penguins. During incubation (35–37 days), the chick-brooding stage (about 15 days), and the guarding stage (about 18 days), females and males alternate in attendance at the nest, fasting on land and foraging at sea. The male usually takes the first and longest bout of attentiveness during incubation. Parental absences from the nest during the incubation stage vary among populations and can exceed 20 days in both males and females (Bucher and Vleck, 1998). After the guarding stage, chicks are left unattended and gather in groups (crèches). For up to a month parents return every day or so to feed them.

Prolactin influences the behavioral, physiological, and morphological attributes of the parental stage of the reproductive cycle in birds (reviewed in Buntin, 1996). Prolactin levels rise to their highest before or during incubation. In species with precocial chicks, plasma prolactin in the parents decreases rapidly during the chick phase, but in species with altricial chicks, plasma prolactin usually remains elevated as long as

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chicks remain thermally dependent on brooding adults (Goldsmith, 1991). A brood patch, which facilitates heat transfer to eggs and nestlings, develops in most incubating birds including penguins. Brood patches develop under the influence of elevated prolactin and sex steroids (Jones, 1971). Exogenous prolactin enhances the expression of parental behavior in nonbreeding birds (Wang and Buntin, 1999, and references therein). Reduction of prolactin activity via inhibition of the avian prolactin releasing hormone, vasoactive intestinal peptide, or through immunoinactivation of prolactin directly reduces parental behavior (reviewed in Sharp, 1997).

In many species incubation behavior is necessary to maintain high prolactin secretion rates, and prolactin levels decrease dramatically within 24 to 48 h if birds are prevented from incubating by removal of nests or eggs (Etches, Garbutt, and Middleton, 1979; El Hala-wani, Burke, and Dennison, 1980; Goldsmith, Burke, and Prosser, 1984; Ramsey, Goldsmith, and Silver, 1985; Hall, 1986, 1987; Sharp, Macnamee, Sterling, Lea, and Pedersen, 1988; Lea and Sharp, 1989; but see Opel and Proudman, 1988). Ring doves, *Streptopelia risoria*, separated from their nests but given exogenous prolactin for up to 10 days, maintain nest attachment and resume incubation given the opportunity (Lehrman and Brody, 1964; Janik and Buntin, 1985). In galliforms (poultry) and anseriforms (ducks) the effect of behavior on prolactin is mediated by tactile contact between the eggs or chicks and the brood patch (Opel and Proudman, 1988; Richard-Yris, Sharp, Wauters, Guémené, Richard, and Forasté, 1998). When the brood patch is anaesthetized or denervated, prolactin levels fall and incubation behavior decreases, even when the eggs are still present in the nest (Hall, 1987; Book, Millam, Guinan, and Kitchell, 1991).

Not all birds, however, display a tight relation between brood patch stimulation and elevated prolactin secretion. Prolactin levels often begin to rise before incubation begins (Buntin, 1996). In many species in which both members of the pair incubate, plasma prolactin remains high when birds are away from their nest for hours to days (Hector and Goldsmith, 1985; Hall, 1986; Lea, Vowles, and Dick, 1986; Garcia, Jouventin, and Mauget, 1996; Jouventin and Mauget, 1996; Lormée, Jouventin, Chastel, and Mauget, 1999). The manner in which prolactin and parental behavior interact varies with species, and understanding how selection has altered this relationship requires a broadly comparative approach.

In Adélie penguins prolactin levels increase from baseline values during the early courtship stage, peak

during mid-incubation and the brood stage, and do not decline significantly until about 50 days after egg laying, when chicks are no longer being brooded (Vleck, Bucher, Reed, and Kristmundsdottir, 1999). During the guard and crèche stage prolactin levels continue to decline slowly, but are still three- to four-fold higher than baseline values. Prolactin levels do not differ between males and females during the courtship phase, but after egg laying plasma prolactin is 10 to 12% higher in females than males (Vleck et al., 1999). In this article we report the levels of (1) prolactin in birds at the first nest exchange, when females return from a long absence and relieve their mate; (2) prolactin in birds in which we artificially lengthened or shortened the incubation period by about 25%; and (3) prolactin and testosterone in failed breeders after nest loss.

METHODS

Field Procedures

We studied Adélie penguins at a rookery on Torgersen Island, near the U.S. base (Palmer Station) on Anvers Island, Antarctic Peninsula (64°S45' latitude, 64°W04' longitude) during the breeding season of 1996–1997. This rookery contains up to 8000 pairs in some years. All birds in our study (approximately 500 birds) were individually marked with a metal flipper band for identification. We assigned sex using copulation behavior and nest attendance pattern (Ainley et al., 1983), condition of the cloaca and lipemic plasma in females near the time of laying, or molecular differences between sexes in the CHD gene (T. Schwartz et al., unpublished data; Griffiths, Daan, and Dijkstra, 1996). We censused daily, weather permitting, to determine which birds were present and the stage of the nesting cycle. For most birds we knew the dates on which the two eggs were laid and, for failed birds, the date on which eggs or chicks were lost. Although two-thirds of the birds in our study laid eggs within 1 week of each other, dates of first eggs ranged from November 7 to November 26. Second eggs were laid 2 to 3 days after the first egg. We characterized time for each nest relative to the day on which its first egg was laid (stage day) rather than for calendar date. Stage days between 1 and 36 represent the incubation phase, and stage days between 36 and about 50 represent the brood phase (Vleck et al., 1999).

Birds were captured by hand or with a small net. A blood sample (1 to 2 ml) was taken by jugular veni-

puncture. The mean elapsed time from first approaching the bird to obtaining the blood sample was 2.0 min (SE = 0.07, range = 1 to 8 min). All blood samples were obtained between about 08:00 and 12:00 h. Blood was kept cold, but protected from freezing until we returned to the lab, at which time the plasma was separated and frozen at -70°C for storage. All animal procedures were approved by the Animal Care and Use Committee at Iowa State University under guidelines established by the National Institutes of Health. All collecting was done under permits as required by the Antarctic Conservation Act.

Nest exchange. The length of the female's first foraging trip after egg laying varies between sites, averaging 8 to 11 days at Torgersen Island (Bucher and Vleck, 1998). We collected blood samples from eight females as they returned from this initial absence and from their mates. In four cases the male was still incubating when we sampled the pair. In the other four cases the female had sat on the eggs, but for less than 5 min. After sampling, all females began incubating while the males left the colony to take their first foraging bouts. We took a second blood sample from the females after they had incubated 1 to 3 days (mean elapsed time between first and second samples was 1.6 ± 0.3 days).

Incubation period manipulation. We marked eggs of 13 pairs of penguins that laid eggs relatively early (between Nov. 7 and Nov. 12) and 13 pairs that laid relatively late (between Nov. 17 and Nov. 22). We then exchanged eggs from the late nests on approximately their 13th day of incubation with eggs from the early nests which were near their 23rd day of incubation. This exchange resulted in two experimental groups: pairs that incubated eggs for only about 26 days before hatch (short-incubation group) and pairs that incubated eggs for about 46 days before hatch (long-incubation group). In addition, in 15 nests we exchanged eggs laid between Nov. 12 and Nov. 18 with eggs laid on the same day as a control for the effects of moving eggs (control-incubation group). Duration of incubation in control pairs was not altered. After egg exchange we did not disturb a nest until 2 days prior to the predicted pip date of the first egg, after which it was checked daily. We obtained blood samples from both the male and female of each pair at the following times: before moving eggs (incubation sample); at the pip stage (pip sample); at the hatch stage, usually 2 days after pipping (hatch sample); and during the brood stage, 5 to 6 days after hatch (brood sample). The incubation sample for males was collected during the 1st week of incubation during his first incubation

bout, and the sample from females was collected during the 2nd week of incubation, after the female had returned from her first foraging bout. We also took a sample from birds on the day that their own eggs hatched (own-hatch sample), unless another sample was scheduled within 1 day of the own-hatch date. In such cases we also used that scheduled sample as an own-hatch sample. We do not have every sample from every bird because one member of a pair was sometimes absent at a designated sampling time and because some nests failed.

Failed birds. When penguins lose their eggs or chicks to predators they will often remain in the colony for a day or more, and many of them return intermittently to their former nest site throughout the breeding season. We obtained blood samples from 28 males and 25 females for which we knew the date on which their eggs had been laid and the date on which the nest was lost. These samples were categorized according to three time measures: (1) whether the nest was lost early in the nesting cycle (≤ 17 days of incubation) or late in the nesting cycle (> 18 days of incubation), (2) the number of days between nest failure and sampling, and (3) the date on which the sample was taken. Because we know that prolactin values begin to drop naturally in successful Adélie penguins after the end of the brooding stage (Vleck *et al.*, 1999), we only used samples obtained from failed birds taken within 50 days of the egg lay date, and we included only one sample per bird (the first obtained) to avoid pseudoreplication.

Hormone Measurements

We assayed plasma hormone levels of testosterone and prolactin in triplicate by radioimmunoassay (RIA). For the prolactin RIA we used purified chicken prolactin (reference preparation AFP-10328B) as a standard and a rabbit antiserum (AFP-151040789) raised against prolactin (both obtained from Dr. A. F. Parlow, Director of the Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, California). We radiolabeled chicken prolactin (AFP-4444B) with I^{125} using chloramine-T ($0.64 \mu\text{g}$ chloramine-T/ μg prolactin and 8-min exposure followed by purification on a Sephadex G-75 column). Assays were run with $20 \mu\text{l}$ of plasma. The least detectable concentration was about 2 ng/ml. Dilutions of Adélie penguin plasma bind to the antibody in a manner parallel to the standard curve (Fig. 1A). For testosterone we used kits obtained from Diagnostics Systems Laboratories, Inc. (Webster, TX). This RIA kit measures tes-

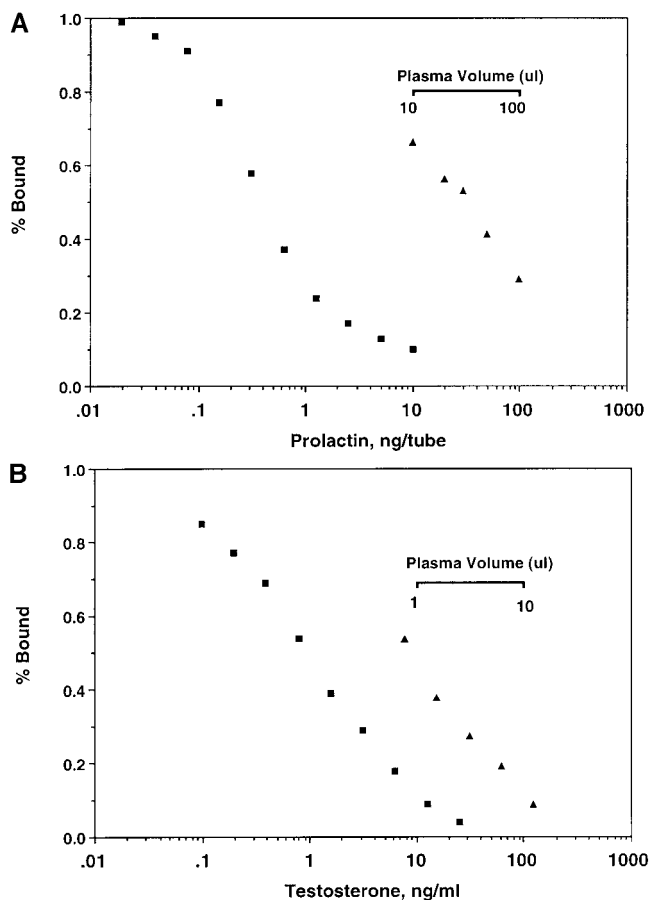


FIG. 1. Representative standard curves from an avian prolactin (A) and a testosterone (B) radioimmunoassay. The graphs show the relationships between percentage binding of radiolabeled reference hormone to the antiserum (squares) and the amount of chicken prolactin (A) or concentration of testosterone (B). Also shown are the results of binding in competition with various dilutions of Adélie penguin plasma (triangles).

tosterone concentrations in plasma directly using an I^{125} -labeled hormone. The kit was modified in the following ways. We ran all volumes at 25% of the suggested kit volume to conserve plasma for other purposes, and standards were made by adding known amounts of testosterone to charcoal-stripped chicken plasma. Least detectable concentration in the four assays used was about 0.1 ng/ml. Serial dilutions of penguin plasma produced curves that were parallel to the standard curves (Fig. 1B). The intraassay coefficient of variation (CV) from the triplicate values for each sample and interassay CV from a penguin pool sample run in each assay were 10.8 and 11.7% for testosterone. The intra-assay CV for prolactin was 7.0% and the interassay variability for the two prolactin assays was 9.0%.

Statistical Analysis

Statistical analyses were carried out using regression, analysis of variance, or Student's *t* tests with JMP 3.1 software (SAS Institute, 1995). Prolactin values were normally distributed. Testosterone values were log-transformed to compensate for their nonnormal distribution. In the incubation manipulation experiment we used a repeated-measures design by nesting individual within treatment as a random effect. For multiple comparisons in this experiment we used the Tukey-Kramer honestly significant difference as our criterion for significance. To test for an effect of nest loss on prolactin and testosterone we used an analysis of covariance with early or late nest loss, days after loss, and sample date as main effects and tested for heterogeneity of slopes using interaction terms. All interaction terms that were not significant were excluded from the model. We considered differences significant if $P < 0.05$ and report values as mean \pm SE.

RESULTS

Nest Exchange Birds

Mean prolactin levels in eight females returning to their nest (31.4 ± 1.3 ng/ml) after an 8- to 11-day absence from the colony did not differ from mean prolactin levels in their mates (30.9 ± 1.3 ng/ml), which had been incubating during the females' absence. Prolactin levels did not change in the females over the first 1 to 3 days of their first bout of incubation.

Incubation Period Manipulation

Our incubation manipulation protocol resulted in groups that had significantly different incubation periods, defined as the days elapsed from the laying of the first egg until the first hatching ($F_{2,33} = 560$, $P < 0.0001$). Incubation periods were 26.3 ± 0.4 days for the short-incubation group, 35.6 ± 0.4 days for the control group, and 45.5 ± 0.4 days for the long-incubation group. Within a stage prolactin did not vary with the incubation period treatment in either females or males (Table 1). Prolactin levels in females were significantly higher than in males ($F_{1,171} = 69.34$, $P < 0.0001$).

To examine whether prolactin changed in individuals across the sampling stages, we averaged the pip and hatch samples for each individual because for

TABLE 1
Mean Prolactin \pm SE in Nanograms per Milliliter (sample size) at Various Nest Stages in Female and Male Adélie Penguins in Which Incubation Period (IP) Was Altered by Moving Eggs between Nests^a

Sampling stage	Female incubation treatment			Male incubation treatment		
	Short IP	Control IP	Long IP	Short IP	Control IP	Long IP
Incubation	34.1 \pm 1.3 (13)	32.2 \pm 0.7 (9)	33.4 \pm 0.7 (9)	29.6 \pm 1.0 (12)	27.2 \pm 0.9 (12)	27.4 \pm 1.9 (8)
Pip	32.3 \pm 1.2 (9)	32.5 \pm 0.7 (7)	32.3 \pm 0.9 (8)	30.6 \pm 1.4 (9)	27.2 \pm 0.9 (6)	27.2 \pm 1.1 (6)
Hatch	33.4 \pm 1.2 (12)	33.2 \pm 2.0 (6)	33.2 \pm 1.3 (9)	30.2 \pm 1.0 (12)	28.0 \pm 0.8 (12)	28.0 \pm 0.9 (7)
Brood	32.6 \pm 0.9 (12)	32.9 \pm 1.1 (6)	30.3 \pm 1.5 (7)	28.6 \pm 1.1 (10)	27.2 \pm 1.0 (9)	27.2 \pm 0.6 (7)
Own-hatch	33.3 \pm 0.7 (10)	33.2 \pm 1.7 (7)	33.2 \pm 0.9 (7)	26.7 \pm 1.2 (9)	27.1 \pm 0.9 (11)	28.5 \pm 0.8 (8)

^a Within a nest stage there were no significant differences between incubation treatment groups for either females or males ($P > 0.05$, Tukey–Kramer honest significant differences).

many birds we had one or the other, but not both samples. When we had both samples, they did not differ. There were no effects of treatment or sampling stage on prolactin, but there was a significant bird effect for both males ($F_{29,85} = 2.81$, $P = 0.0001$) and females ($F_{29,80} = 3.73$, $P < 0.0001$). Some birds had consistently higher prolactin than others.

Although there were no differences in prolactin levels within the treatment groups over the nest stages we sampled, there were differences in parental attendance patterns. The age of chicks when their foster parents stopped brooding and started guarding them did not differ among groups (overall mean age = 15.7 ± 0.7 days, $N = 18$ nests). The age at which the chicks were first left unguarded by the parents (age at first crèching) did, however, vary among groups ($F_{2,15} = 7.21$, $P = 0.006$). The chicks from the control ($N = 6$ nests) and short-incubation ($N = 7$ nests) nests did not differ in age at first crèching (33.7 ± 1.1 days), but the chicks from the long-incubation nests ($N = 5$ nests) first crèched at a significantly younger age (25.0 ± 1.9 days) (Tukey–Kramer HSD, $q = 2.60$, $P < 0.05$). This occurred because the mean calendar date (January 20 ± 1 day) on which the parents no longer remained in the colony to guard the chicks did not differ between groups. The adults in the long-incubation group, which stopped guarding their chicks at an earlier chick age, were actually at the same stage day of their nesting cycle (70 days past egg lay) as the control birds (69 days past egg lay). In contrast, the adults in the short-incubation group, which stopped guarding their chicks at a more advanced chick age, were themselves at a significantly earlier stage day (60 days past egg lay). Because we did not band chicks, we could not determine whether there were differences between groups in chick survival.

Failed Birds

The effect of nest failure on plasma prolactin differed between males and females. Our samples came from birds that had lost their eggs or chicks from 2 through 49 days after the eggs were laid. About half the nest losses occurred early, during the first half of incubation, and the other half occurred later in the nesting cycle. In failed males, prolactin levels were lower in birds that lost their nests late compared to those that lost their nests early ($F_{1,25} = 8.63$, $P = 0.007$), and prolactin levels were lower the later after nest loss that birds were sampled ($F_{1,25} = 8.62$, $P = 0.008$) (Fig. 2). There was no significant interaction

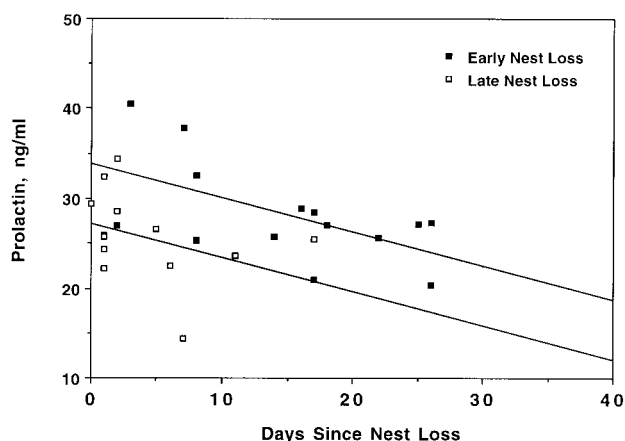


FIG. 2. Prolactin levels in male Adélie penguins that have lost their eggs or chicks as a function of days since nest loss. Solid symbols are birds that lost their nests during the first half of incubation and open symbols are birds that lost their nests later than this. Lines fit to the data for each group were obtained from an analysis of covariance. Slopes do not differ between groups. The common slope, which gives the rate of decline in prolactin after nest loss, is -0.38 ng/ml/day.

between these variables. There was also no significant effect of sampling date on prolactin in males. In females, nest loss did not affect prolactin levels. The mean prolactin level for failed females was 29.5 ± 1.28 ng/ml ($N = 25$), which did not differ from that in females caring for eggs or young in the incubation manipulation experiment (Table 1). The mean prolactin level for failed males, 26.9 ± 1.0 ng/ml ($N = 28$) was significantly lower ($t_{148} = 2.13$, $P = 0.03$) than that in males caring for eggs or young in the incubation manipulation experiment.

Testosterone levels were significantly elevated in males after nest loss compared to males that were caring for eggs and young ($t_{224} = 6.80$, $P = <0.0001$: breeding bird data in Vleck et al., 1999), and many of the failed birds displayed both courtship and aggressive behavior toward other penguins. Testosterone in failed males averaged 1.35 ± 0.45 ng/ml (range = 0.1 to 9.8 ng/ml), whereas the average for males with active nests is 0.16 ± 0.06 ng/ml (range = 0.1 to 1.7 ng/ml). Failed males also had significantly higher testosterone levels than failed females ($t_{50} = -2.46$, $P = 0.018$), whose plasma testosterone averaged 0.19 ± 0.05 ng/ml (range = 0.1 to 1.0 ng/ml). There was no significant correlation between an individual's levels of prolactin and testosterone in these failed males, nor did testosterone vary with time of nest loss, days since loss, or date in either males or females.

Reproduction can also fail when eggs are infertile or inviable. We identified seven pairs of penguins in our banded populations that were incubating eggs that never hatched. Both parents continued to incubate the eggs well past the expected hatch day at about stage day 35. The mean length of time these birds continued incubating was 52 ± 7 days (range = 45 to 61 days).

DISCUSSION

In Adélie penguins, as in other pelagic seabirds, biparental care is required for successful reproduction, and individuals must take long absences from the nest because foraging grounds are remote from nesting colonies. The constant threat of nest predators and inclement weather select for persistent nest attentiveness. The switch from courtship and aggressive behaviors to parental behaviors is abrupt, occurring as soon as the first egg is laid. This switch occurs as testosterone levels in males drop to less than 2% of their pre-egg values and prolactin levels are nearing their peak (Vleck et al., 1999). Unless eggs or chicks are lost to predation, nest abandonment commonly occurs

only when a fasting birds runs out of energy before its mate returns (Ainley et al., 1983; Davis and McCaffrey, 1986; Trivelpiece and Trivelpiece, 1990; Watanuki, Kato, Mori, and Naito, 1993).

Given these aspects of Adélie penguin biology, it would be surprising if prolactin secretion was influenced primarily by tactile feedback from the eggs. Rather, prolactin levels are elevated as part of a program of cyclical hormonal changes that accompany the reproductive season, and they remain elevated throughout the lengthy nesting season. This assertion is supported by the following points. Prolactin levels in the eight females sampled after return from long foraging bouts were not different from those in incubating males, although in comparisons with larger sample sizes (see incubation manipulation and Vleck et al., 1999) females do have higher prolactin levels than males. Artificial manipulation of the incubation period and thus alteration of the timing of tactile input from eggs and chicks did not alter the pattern of prolactin secretion through the brood stage (Table 1), but adults tended to leave the chicks unattended at about the same calendar date, regardless of the age of the chicks. Prolactin levels did not decrease in females that had lost their nest, and in males the decrease was very modest (Fig. 2), with levels remaining well above baseline values of 5 to 8 ng/ml (Vleck et al., 1999) for many weeks after loss.

Prolactin and Nest Absences during Incubation

Despite lengthy absences from their nests, penguins foraging at sea during the breeding season probably don't experience a decrease in prolactin level. Prolactin levels in females returning after their first foraging bout were higher than when those females left the colony after egg laying (Vleck et al., 1999). Continuously elevated prolactin may be necessary to stimulate foraging birds to return to the nest when their own energy needs are met. We found no change in prolactin levels of females after they returned to their nests and began incubating. Similar results have been reported for other pelagic species, including *Diomedea* albatrosses (Hector and Goldsmith, 1985) and cape gannets, *Sula capensis* (Hall, 1986). In incubating king penguins, *Aptenodytes patagonicus*, prolactin levels also remain elevated during foraging bouts. Garcia et al. (1996) reported the prolactin levels tend to increase about 5% after 7 days of incubation, but Cherel, Mauget, Lacroix, and Gilles (1994) found no significant change over an incubation bout in that species. In the winter-breeding emperor penguin, *Aptenodytes for-*

steri, males incubate while females forage until about the time of hatching. Female prolactin levels were higher when they returned to feed and brood the chicks than when they left approximately 2 months earlier (Lormée *et al.*, 1999). In pelagic seabirds with long absences from the nest, tactile feedback from the brood patch plays no apparent role in adjusting or maintaining prolactin secretion.

Alteration of Incubation Period

Increasing or decreasing the incubation period by about 10 days did not affect the temporal pattern of prolactin secretion in male or female penguins during incubation and the early brood stage. Plasma prolactin remained elevated over all sampling stages in all birds. This result was not unexpected given the observation that average prolactin levels in Adélie penguins do not drop until after stage day 50 (Vleck *et al.*, 1999), and the latest samples we took (in birds with lengthened incubation periods at the brood stage) were taken between stage days 48 and 53. Penguins in general have elevated prolactin levels well into the chick-rearing stage (Williams and Sharp, 1993; Mauget, Garcia, and Jouventin, 1995; Lormée *et al.*, 1999). In king penguins prolactin levels remain elevated throughout the entire chick-rearing period, which includes about 4 months over the winter when chicks remain in crèches, and parents may return only infrequently (Cherel *et al.*, 1994; Jouventin and Mauget, 1996). Cherel *et al.* (1994) suggested that breeding king penguins must maintain elevated prolactin levels throughout the winter to ensure periodic return to the breeding colony to care for dependent young.

Incubation manipulation experiments in other species indicate variation in the extent to which prolactin secretion patterns can be altered by hatching events. In *Diomedea* albatrosses and the pied flycatcher, *Ficedula hypoleuca*, lengthening the incubation period did not alter the timing of the posthatching decline in prolactin levels (Hector and Goldsmith, 1985; Silverin and Goldsmith, 1984), although pied flycatchers with shortened incubation periods did show an early decline (Silverin and Goldsmith, 1984). In Wilson's phalaropes, *Phalaropus tricolor*, extending the incubation period lengthened the period of prolactin elevation and shortening it had the opposite effect (Oring, Fivizzani, Colwell, and El Halawani, 1988). Oring *et al.* (1988) suggested prolactin secretion should be cued by hatching when the natural incubation period is variable, as it is in Wilson's phalaropes. Conversely, en-

dogenous control should be adaptive in species with little variation in the incubation period, such as the pied flycatcher, to decrease time spent incubating inviable eggs. In penguins an extended interval of prolactin elevation after hatching may lead some birds to incubate inviable eggs as we observed, but also ensures that care of young continues for many weeks posthatching, despite long commutes to foraging grounds.

Adélie penguins in our incubation manipulation experiment first left their chicks unguarded on about January 20, regardless of the age of the chicks. Thus, chicks from long-incubation nests joined crèches at an age about 8 days younger than birds in the control or short-incubation nests. Hector and Goldsmith (1985) reported a similar result in gray-headed albatrosses, *Diomedea chrysostoma*. Albatrosses whose incubation periods were lengthened about 9 days left their chicks unattended about 10 days earlier than controls. A similar effect was recently reported for chinstrap penguins, *Pygoscelis antarctica* (Moreno, Barbosa, Potti, and Merino, 1997). These workers moved chicks between nests in the 1st week after hatching to separate effects of parental quality and calendar time on crèche age. As in our study, late-hatching chicks were left unguarded at an earlier age than early-hatching chicks, regardless of the parent's actual lay dates. In high-latitude penguins like Adélie penguins, decrease in attentiveness to chicks at the crèche stage, which is correlated with a decline in plasma prolactin (Vleck *et al.*, 1999), may be linked to a seasonal cue like photoperiod. This would ensure that adults switch from chick guarding to foraging and fat deposition in time to prepare their own fat stores adequately for the postbreeding molt and the 2- to 4-week fast (Adams and Brown, 1990) that molt entails.

Nest Failure, Prolactin, and Testosterone

Nest failure in Adélie penguins did not result in the abrupt cessation of prolactin secretion to nonbreeding levels seen in nonsphenisciform birds (reviewed in Buntin, 1996). At 2 weeks after nest loss, prolactin levels in males were only about 20% lower than levels in males still caring for eggs or young, and there was no drop in failed females. Nest loss later in the season was associated with a greater prolactin decrease in males than early nest loss, although the reasons for this are not clear since there is no drop in prolactin over the same time in birds that are showing parental behavior (Table 1). We do not know whether the difference in hormonal response to nest loss between

males and females has any behavioral correlates. In ring doves, males will not incubate infertile eggs beyond the normal incubation period, whereas females, in the presence of a male, will do so (Ramsey *et al.*, 1985, but see Buntin, 1977).

Other penguins show the same response to nest failure. Plasma prolactin does not change in king penguins within 48 h of nest loss, although after more than 30 days it decreases from about 45 to 28 ng/ml (Jouventin and Mauget, 1996). In another report on king penguins, prolactin levels in failed breeders were elevated over basal levels, but lower than that in incubating birds, but the time since failure was not reported (Garcia *et al.*, 1996). In the emperor penguins, prolactin levels are elevated for up to 3 months after failure (Lormée *et al.*, 1999). A feedback loop between tactile input from eggs or chicks and continued prolactin secretion should be adaptive in birds that can renest after premature loss of eggs or chicks. If the nest is lost in such species, continued prolactin secretion might hinder the rise in gonadotropins and sex steroids necessary for reinitiation of another nest cycle after nest failure (reviewed in Sharp, Dawson, and Lea, 1998). With the exception of some lower latitude species (Mauget *et al.*, 1995), however, penguins cannot renest after egg loss. Some populations of Gentoo penguins (*Pygoscelis papua*) can lay a replacement clutch and do display a decline in prolactin levels between losing eggs and laying a replacement clutch (Mauget *et al.*, 1995). In high-latitude populations of penguins, continued prolactin secretion after nest loss may contribute to the propensity for failed penguins to remain in the nesting colony when not foraging and for some failed Adélie penguins to attempt to steal and incubate the eggs of other birds (personal observation). This lack of a strong response to nest failure may not characterize all pelagic seabirds, however. In cape gannets (order Procellariiformes), which also make long (~2-day) foraging trips during incubation, prolactin levels of breeders drop by more than 50% within 24 h of egg loss (Hall, 1986).

Adélie penguins will incubate infertile eggs for nearly 4 weeks past the normal hatch date. Within limits such behavior may be adaptive since the incubation period for individual eggs in these birds may vary from 31 to 40 days (Bucher, unpublished data), presumably due to variations in weather, nest site, and parental incubation behavior. Persistence in incubating well past a normal hatch date probably reflects the prolonged elevation in prolactin. This feature in turn, may be the result of selection to maintain a hormone milieu conducive to promoting return of

foraging birds to the colony to care for chicks during the 6 weeks or more that chicks depend upon adults for survival.

The most obvious difference between male and female penguins after nest loss was the rise in testosterone in males. Increase in testosterone after nest failure has also been observed in Magellanic penguins, *Spheniscus magellanicus* (Fowler, Wingfield, Boersma, and Sosa, 1994). Testosterone secretion is controlled by LH, and there is evidence in incubating poultry species that high plasma prolactin contributes to the suppression of LH secretion during incubation (Sharp *et al.*, 1998). In canaries, *Serinus canarius* (Goldsmith *et al.*, 1984), and ring doves (Ramsey *et al.*, 1985) plasma prolactin falls and LH rises within a few hours of nest loss. Hall (1986) found increases in both plasma LH and testosterone and a drop in prolactin level in cape gannets after egg removal. King penguins with pharmacologically depressed prolactin levels show increases in plasma LH and testosterone (Jouventin and Mauget, 1996). We do not know whether LH rises in male Adélie penguins as prolactin levels fall after nest failure, nor do we know whether there is any cause-and-effect relationship between the decrease in prolactin and rise in testosterone. In other bird species elevation of testosterone via exogenous sources does not affect prolactin levels (Oring, Fivizzani, and El Halawani, 1989; Schoech, Ketterson, Nolan, Sharp, and Buntin, 1998) or may even cause an increase (Silverin and Goldsmith, 1997). We found no correlation between testosterone and prolactin levels in individual male penguins. However, the pulsatile nature of testosterone release, especially after agonistic encounters (Wingfield and Wada, 1989), may hamper attempts to identify such a relationship in individuals.

Adélie penguins and other penguin species have a profile of prolactin secretion that differs from most lower latitude species that have been studied, but that is consistent with prolactin profiles found in other Sphenisciformes. Prolactin levels increase early in the breeding season and remain high for many weeks throughout the entire incubation, brood, and guard stages (Vleck *et al.*, 1999). Alteration of hatching events, egg loss, and long absences from the nest do not alter this program. This sustained prolactin secretion is likely required for the maintenance of parental behavior in offshore feeding species that must be absent from the nest for many days at a time. At the end of the breeding season in Adélie penguins, a decline in prolactin occurs, associated with declining investment in chicks. This decline may be important in accommo-

dating the trade-off between self-maintenance and further chick investment.

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