Postmigratory Body Condition and Ovarian Steroid Production Predict Breeding Decisions by Female Gray-Headed Albatrosses

Glenn T. Crossin[1,*]
Richard A. Phillips[2]
Katherine E. Wynne-Edwards[3]
Tony D. Williams[4]
[1]Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada; [2]British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom; [3]Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta T2N 4Z6, Canada; [4]Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Accepted 8/19/2013; Electronically Published 10/30/2013

ABSTRACT

Carryover effects have been documented in many migratory bird species, but we know little about the physiological mechanisms that mediate those effects. Here we show that the energetic, endocrine, and aerobic characteristics of postmigratory female gray-headed albatrosses (*Thalassarche chrysostoma*) can affect their decision to breed. All females in this study, whether breeding or not, were secreting ovarian steroids when they arrived at the breeding colony at Bird Island, South Georgia, which suggests that all were responding to seasonal cues. However, deferring, nonbreeding birds were characterized by a steroid profile of high progesterone (P4) and low testosterone (T), whereas breeding birds showed the opposite pattern. Deferring birds also had low body mass, hematocrit, and hemoglobin. These results suggest that postmigratory condition can influence patterns of ovarian steroidogenesis and that the maintenance of high P4 without subsequent conversion to T favors breeding deferral. Whereas breeding females normally convert P4 to T, which is a key deterministic step toward 17β-estradiol synthesis, vitellogenesis, and follicle development, deferring females did not make this conversion and instead maintained high levels of P4, perhaps due to inhibition of the hydroxylase-lyase enzyme complex, thus rendering them infertile for the current season. Results are discussed within the context of the biennial breeding system of this species, and comparisons with other biennially and annually breeding albatrosses are made.

* Corresponding author; e-mail: gtc@dal.ca.

Physiological and Biochemical Zoology 86(6):761–768. 2013. 1522-2152/2013/8606-3071$15.00. DOI: 10.1086/673755

Introduction

Migratory carryover effects have been documented in several bird species and can influence many aspects of reproduction, including the timing of breeding (Marra et al. 1998; Norris et al. 2004; Descamps et al. 2011; Harrison et al. 2011), breeding decision (Ebbinge and Spaans 1995; Crossin et al. 2012), breeding output (Ebbinge and Spaans 1995; Sorenson et al. 2009), and breeding success (Baker et al. 2004; Inger et al. 2010; Crossin et al. 2012). However, the physiological and endocrine mechanisms that mediate carryover effects are largely unknown. Recent work in birds has shown how variable individuals are in their ability to accrue and store resources in advance of reproduction, which can then have effects that are either positive for breeding (e.g., high somatic fat leading to early timing of breeding; Prop et al. 2003; Smith and Moore 2003) or negative (e.g., low fat or poor condition leading to a trade-off between current reproduction and survival). Recent work has also linked endocrine processes to carryover effects. However, the links between prebreeding condition and endocrinology remain elusive. Despite the intuitive appeal that individual condition must play a role in hormonally mediated carryover effects, very few studies that link wintering habitat, migration, breeding activity, and carryover effects include direct measures of body condition (e.g., body mass, fat mass, or mass change), either alone or in combination with endocrine measures (Ebbinge and Spaans 1995; Marra et al. 1998; Baker et al. 2004; Harrison et al. 2011). Although the mechanisms through which condition might influence breeding decisions are not well understood, this must surely involve integration with, and modulation of, the hypothalamic-pituitary-gonadal (HPG) axis (or other hormonal systems that interact with the HPG axis; e.g., Goutte et al. 2010*a*), so that reproductive readiness can be assessed before a commitment to breeding.

For the few studies that have simultaneously examined condition and endocrine effects on breeding decisions, the results have been somewhat equivocal (Harshman and Zera 2007; Williams 2012). A recent study of female black-browed albatrosses (*Thalassarche melanophrys*), for example, showed that both condition-dependent and endocrine traits were expressed at low levels in deferring (i.e., nonbreeding) birds relative to those that laid (Crossin et al. 2012). In contrast, body condition was not linked to breeding decisions by snow petrels (*Pagodroma nivea*) or by black-legged kittiwakes (*Rissa tridactyla*), although clear hormonal differences between deferring and breeding birds

were evident (e.g., glucocorticoid levels; Goutte et al. 2010*a*, 2010*b*). Here, we examine the influence of body condition and endocrine state on the breeding behavior of gray-headed albatrosses (*Thalassarche chrysostoma*) and draw comparisons with other albatrosses and with birds more generally.

The gray-headed albatross usually breeds biennially if successful and annually if failure occurs in incubation or early chick rearing. However, in any given year, there are also many deferring individuals that do not follow this pattern and instead skip the opportunity to breed (Ryan et al. 2007). Such decisions obviously have important implications for the trade-off between current reproduction and survival and affect lifetime fitness (Weimerskirch 1990; Wooller et al. 1990; Chastel et al. 1995). For females, the decision to lay may depend on their capacity to devote adequate resources to egg production without jeopardizing their own energetic needs, as documented in many annually breeding species (Descamps et al. 2011). Body condition upon arrival at the colony, after winter migration, should thus have some bearing on breeding decision, with correlated effects on prebreeding physiology. Previous studies of gray-headed albatrosses and wandering albatrosses (*Diomedea exulans*) have shown that the HPG axes and ovaries of mature but deferring females were seasonally responsive, but instead of secreting testosterone (T) and 17β-estradiol (E_2), indicative of a commitment to reproduction, the ovaries secreted progesterone (P4; Hector et al. 1986*a*, 1986*b*, 1990). Without T and E_2, the downstream activation of E_2-mediated vitellogenic pathways is not possible (Williams et al. 2004*b*), thus rendering a female functionally or physiologically sterile. Whether these patterns of steroidogenesis are mediated by variation in condition is not known, but in a study of closely related black-browed albatrosses, females that deferred breeding after winter migration had low energetic (body mass) and aerobic (hematocrit [Hct]) condition measures, as well as low P4 and T levels (Crossin et al. 2012). That P4 levels were low rather than high in the deferring black-browed albatrosses raises the intriguing possibility that the endocrine mechanisms controlling breeding decision may differ between annual (black-browed albatrosses) and biennial (gray-headed and wandering albatrosses) species.

In response to these studies, we set out to test the hypothesis that body condition affects prebreeding endocrine physiology and subsequent breeding decision. To do this, we sampled female gray-headed albatrosses as they returned from winter migrations to a large breeding colony at Bird Island, South Georgia, in order to determine a suite of morphological, hematological, and reproductive parameters. Working from a mechanistic perspective in which improved body condition should influence P4 and T levels, and therefore subsequent investment in reproduction, we hypothesized that measures of relative body condition would underlie breeding decisions. We thus predicted that prebreeding females in poor condition (e.g., low body mass, low Hct, and hemoglobin [Hb]) would defer breeding. We also predicted that body condition would influence which sex steroids were secreted by the ovary: females in poor condition should secrete high levels of P4 and thus defer breeding, whereas those in good condition should secrete T and eventually lay. Collectively, this predicts that a condition-dependent P4 signal is the mechanism that determines breeding deferral in female gray-headed albatrosses. If this prediction is supported, it would suggest that two closely related, sympatric species of albatrosses have evolved very different physiological mechanisms for the control of breeding decisions.

Material and Methods

Field Collections

Fieldwork was conducted during the austral summer beginning in September 2008–2009 at a gray-headed albatross breeding colony (colony E) on Bird Island, South Georgia (54°01′S, 38°02′W). Research was approved by the Ethics Committee of the British Antarctic Survey and carried out under permits issued by the Government of South Georgia and South Sandwich Islands; the procedures also conformed to guidelines established by the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 897B-8).

Female gray-headed albatrosses were sampled upon their return to the colony after long, pelagic migrations lasting 6–16 mo (Croxall et al. 2005). Records from a long-term banding program allowed us to generate a list of breeding-age females, which allowed us to identify newly arrived females during daily colony visits beginning in mid-September. Between October 5 and 7, we sampled 15 birds when they were first sighted in the colony, which included deferring breeders ($N = 9$) and birds that went on to lay ($N = 6$). Within this short window of time, there was no difference in the mean arrival date of deferring and breeding birds (*t*-test, $F_{1,14} = 2.23$, $P = 0.159$; mean date of egg laying for the colony was October 21). Females were captured on their nests, and 1-mL blood samples were taken from tarsal veins using heparinized syringes with 25-ga needles. Because of near freezing temperatures, it was not always possible to obtain a full 1 mL of blood, and some samples were only approximately 0.25 mL, which limited the volume available for some hormone assays. The time that it took to collect these samples, from our first approach to the nest to the end of blood sampling, was recorded to the nearest second. Blood was then transferred to heparinized 2.5-mL Eppendorf vials and centrifuged for 5 min at 10,000 *g*. Plasma was then transferred to labeled 0.6-mL vials for storage at −20°C. We recorded body mass (±10 g) and culmen and tarsus lengths (both ±1 mm). After sampling, we made weekly visits to note breeding decision and record dates of laying, hatching, failure (loss of an egg or chick), and fledging.

Blood and Plasma Analyses

Hct was measured in fresh whole blood by centrifugation in microhematocrit tubes for 5 min at 10,000 *g* and is reported as packed cell volume (%). Hb (g dL^{-1} whole blood) was measured with the cyanomethemoglobin method modified for use with a microplate spectrophotometer, using 5 μL whole blood diluted in 1.25 mL Drabkin's reagent (D5941 Sigma-Aldrich

Canada, Oakville, Ontario). Absorbance was measured at 540 nm. P4 and T were assayed in duplicate by liquid chromatography–tandem mass spectrometry (LC-MS/MS) based on the method of Koren et al. (2012). Both steroids were assayed in a single injection, starting from a sample volume between 50 and 100 mL. All samples received a bioidentical deuterated internal standard representing a final concentration of 5 ng mL^{-1} P4-d9 and 1 ng mL^{-1} T5-d2 and were diluted to 500 mL with water. Sample preparation consisted of solid-phase extraction over C18, with elution in 1 mL of ethyl acetate. Samples were dried under nitrogen gas and reconstituted in 50% MeOH. Liquid chromatography (Agilent 1200 SL system) used an injection volume of 40 mL, a 100 × 300-mm Kinetex C18 Column (Phenomenex), and water/methanol as mobile phases. Mass spectrometry (AB Sciex Q-trap 5500) used APCI +ve mode, with the following multiple reaction monitoring transitions: Progesterone 315/97, Progesterone-d9 324/100, Testosterone 289/97, Testosterone-d2 291/99. Quantitation was by area ratio against the deuterated internal sample that had gone through sample preparation with the serum sample. Simultaneous assay for E_2 was not possible because the sample concentrations were too low for quantitation by this method and the available sample volume was insufficient for a second, dedicated LC-MS/MS run that would have been above the limit of detection.

Statistical Analyses

Analyses were run with JMP 9.0 or SAS 9.0 software packages. All variables were tested for normality via plots of residuals against predicted values followed by Shapiro-Wilk tests. Data transformations were applied when residual distributions were nonnormal. Correlations among all variables (date of colony arrival, body mass, Hct, Hb, P4, T) were examined through a Pearson's correlation matrix. Bonferroni corrections for multiple comparisons were applied when assessing significance levels ($0.05/15 = 0.0033$). ANOVA tests were used to compare Hct between breeding and deferring birds (no covariates were included because Hct did not correlate with any other variable). Using long-term demographic data from our study colony, we also compared Hct in females according to whether they bred in the previous year. Because arrival date was correlated with both body mass and P4, we accounted for this covariation (ANCOVA) when comparing body mass and P4 levels between status groups (breeding vs. deferring). Because Hb and T were correlated with both arrival date and body mass, ANCOVA models must account for covariation from both sources when comparing Hb and between status groups when comparing T. Ideally, both date and mass would be used as covariates, but because of loss of degrees of freedom, we could not do so. We thus used residuals from a regression of body mass on arrival date as a single covariate in the Hb and T models. All ANCOVA models included interaction terms (e.g., main effect × covariate effect), but when these were nonsignificant, they were removed from final models to preserve degrees of freedom and increase statistical power (see "Results"). All values presented in figures are untransformed, least squares means ± SEM.

Results

We sampled 15 female gray-headed albatrosses upon first arrival at nests after winter migration (9 deferring, 6 breeding). Among the variables (date of arrival, arrival body mass, plasma P4, plasma T, Hct, Hb, tarsus length, culmen length), body mass was significantly correlated with date of arrival ($r = 0.606$, $P = 0.022$), plasma T levels ($r = 0.822$, $P = 0.002$), and blood Hb levels ($r = 0.666$, $P = 0.009$). Date of arrival was also significantly correlated with plasma P4 levels ($r = 0.632$, $P = 0.021$). No other significant correlations were observed.

When comparing breeding and deferring birds, significant differences were observed in all endocrine and condition-related traits. Deferring females had significantly higher P4 levels (1.24 ± 0.19 ng mL^{-1}) relative to breeding females (0.44 ± 0.31 ng mL^{-1}; ANCOVA: whole model, $F_{2,13} = 8.811$, $P = 0.006$; main effect, $F = 6.591$, $P = 0.028$; date of arrival covariate, $F = 15.156$, $P = 0.003$; fig. 1*A*). Conversely, plasma T was higher in breeding females (6.52 ± 1.05 ng mL^{-1}) than in deferring females (1.11 ± 0.66 ng mL^{-1}; ANCOVA: whole model, $F_{2,13} = 19.671$, $P < 0.001$; main effect, $F = 17.866$, $P = 0.002$; residual body mass covariate, $F = 1.573$, $P = 0.241$; fig. 1*B*). There was a negative relationship between P4 and T ($P = 0.013$, $N = 15$): T decreased exponentially as P4 increased (fig. 2).

Regarding condition traits, deferring females were significantly lighter than breeding females (3.44 ± 0.06 kg vs. 3.72 ± 0.09 kg; ANCOVA, $F_{2,13} = 9.708$, $P = 0.003$; main effect, $F = 6.398$, $P = 0.026$; date of arrival covariate, $F = 8.763$, $P = 0.012$; fig. 3*A*). Deferring females also had lower Hct (38.3% ± 1.3% vs. 45.0% ± 1.7%; ANOVA, $F_{1,14} = 6.700$, $P = 0.027$) and lower Hb (14.7 ± 0.5 g dL^{-1} whole blood vs. 17.5 ± 0.9 g dL^{-1} whole blood; ANCOVA, $F_{2,13} = 6.796$, $P = 0.012$; main effect, $F = 5.920$, $P = 0.033$; residual body mass covariate, $F = 0.010$, $P = 0.922$; fig. 3*B*, 3*C*) levels.

Whether a bird bred in the present year depended on its breeding status in the previous year. Birds breeding in year x were significantly more likely to defer in year $x + 1$, whereas birds deferring in year x were more likely to breed in year $x + 1$ (contingency analysis, χ^2 likelihood ratio = 4.58, $P = 0.032$, $N = 15$). Those same breeding birds in year x had significantly lower Hct levels upon their arrival at the breeding colony in year $x + 1$, relative to those that deferred in year x but bred in year $x + 1$ (ANOVA, $F_{1,12} = 5.486$, $P = 0.037$; fig. 4).

Discussion

In this study we assessed the effects of body condition on patterns of ovarian steroidogenesis and subsequent breeding decisions by postmigratory female gray-headed albatrosses. Our results suggest that after a pelagic migration lasting 6–16 mo (Croxall et al. 2005), breeding decision is the cumulative effect of individual variation in prebreeding state, which reflects var-

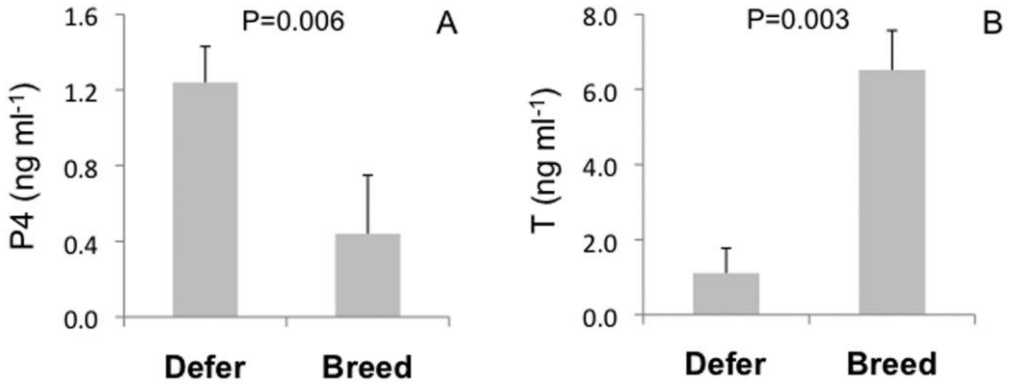

Figure 1. Endocrine differences (progesterone [P4], *A*; testosterone [T], *B*) between breeding and deferring gray-headed albatrosses, measured upon their return to a breeding colony at Bird Island, South Georgia, after winter migration. Columns represent least squares means (ANCOVA) + SEM. Fifteen females were sampled (9 deferring, 6 breeding).

iation in both body condition and hormonal status at time of arrival at the breeding colony (e.g., circulating sex steroid levels). Specifically, the condition measures of low body mass, Hct, and Hb concentrations were associated with high ovarian P4 secretion and with breeding deferral. By favoring the secretion of P4 instead of T at this early stage of the breeding season, deferring females had presumably decided, long before their arrival at the breeding colony and reunion with their mates, to physiologically preempt E_2 synthesis and vitellogenesis and thus their commitment to reproduction. These results support the idea that a migratory carryover effect on prebreeding body condition constrained the allocation of resources to egg production via a condition-dependent modulation of ovarian steroidogenesis. We also identified significant links between aerobic capacity and breeding decision, which we discuss in the context of costs of reproduction and migratory carryover effects. Collectively, we provide new physiological insights to the mechanisms through which migratory carryover effects might constrain breeding activity (reviewed in Harrison et al. 2011).

Seasonality, Ovarian Steroidogenesis, and Breeding Decisions

Breeding and deferring female gray-headed albatrosses had elevated levels of ovarian sex steroids upon arrival at the colony, which contrasts with the basal levels that occur toward the end of the breeding season (Hector et al. 1986*a*). This suggests that all the individual birds in our study were responding to seasonal cues via HPG upregulation, which presumably involved the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and of gonadotropins from the pituitary (luteinizing hormone [LH], follicle-stimulating hormone [FSH]; Dawson 2008). Our results are supported by an earlier study of gray-headed albatrosses in which breeding and deferring birds exhibited hormonal profiles indicative of an active HPG axis and that, despite great interindividual variation, all birds had higher LH levels when they arrived at the colony than later in the breeding season (Hector et al. 1986*b*). Together with our data, this is consistent with the hypothesis that day length or photoperiod is the principal zeitgeber of the annual cycle, controlling seasonal events such as the timing of the spring migrations back to the breeding areas and the regulation of the initial recrudescence and development of the reproductive axis before breeding (Dawson 2008).

Despite their apparent seasonal responsiveness in terms of gonadotropin release, breeding and deferring females in our study differed markedly at the downstream end of the HPG axis, at the level of the ovary. Patterns of hormonal secretion differed between birds according to their eventual breeding fate, with the ovaries of deferring females favoring P4 secretion (as

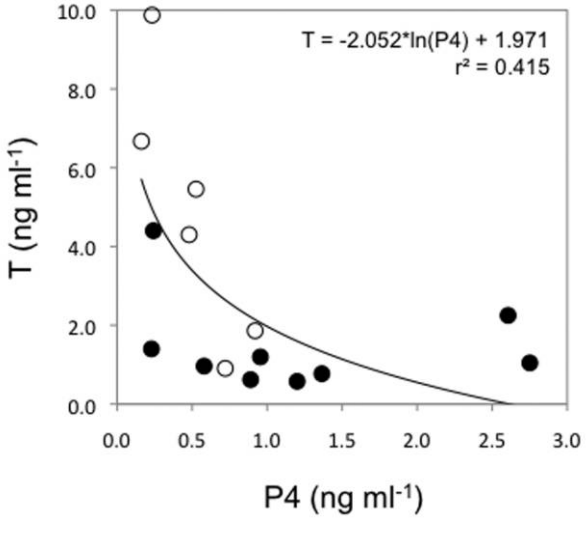

Figure 2. The relationship between prebreeding progesterone (P4) and testosterone (T) concentrations is negatively exponential in female gray-headed albatrosses. Open circles indicate breeding individuals, filled circles deferring individuals. Albatrosses were sampled at the end of migration upon first arrival at the colony.

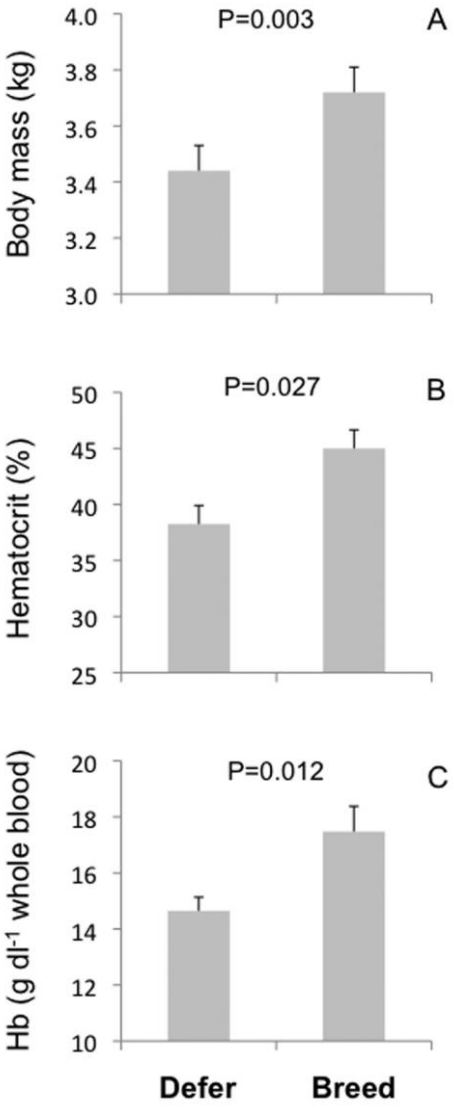

Figure 3. Energetic (*A*) and aerobic (*B*, *C*) traits differ significantly between breeding and deferring gray-headed albatrosses. Individual females were sampled upon arrival at the breeding colony. Columns represent least squares means (ANCOVA) + SEM.

previously observed; Hector et al. 1986*a*, 1986*b*, 1990). For example, Hector et al. (1986*b*) suggested that ovarian P4 secretion by mature females represents a functional block to vitellogenesis and follicle development. By secreting P4 instead of converting this to T and then to E_2, the female is left functionally sterile for the current annual cycle (Hector et al. 1986*a*, 1990). This occurs in the ovarian theca cells surrounding the developing follicles, where P4 is synthesized. Although we did not measure E_2 in this study (because of a lack of plasma), our interpretation of the condition and hormonal data provide a logical explanation for the breeding patterns that we observed. Having said that, estrogen synthesis can also occur via the androstenedione (A4) pathway, which involves neither P4 nor T, and hence measurement of A4, and indeed E_2, would be worthwhile in future studies.

Working through the P4 pathway of ovarian development places this all in context. P4 is the first sex steroid produced by the ovary in response to LH and is itself a precursor for other steroids, including T and E_2. Under normal circumstances for birds about to breed, P4 is then converted to the androgens in the granulosa cells, which are then converted to the estrogens. As our data show, an absence of T when P4 is high suggests a low activity of 17α-hydroxylase and 17,20 lyase (known collectively as hydroxylase-lyase) in the theca. Because LH is generally accepted as the activating agent for the enzymes controlling P4 synthesis, and FSH as the activating agent for enzymes controlling androgen synthesis, we postulate a potential inhibition at the level of FSH, although this would need to be confirmed with controlled experimental studies. Whatever the mechanism, this endocrine tactic, where P4 secretion by deferring females favors self-maintenance at the expense of reproductive investment, has been previously observed in biennially breeding albatrosses, notably in the wandering albatross (Hector et al. 1986*a*, 1990) and previously in the gray-headed albatross (Hector et al. 1986*b*). What is intriguing is that this pattern differs markedly from a closely related but annual breeder, the black-browed albatross, in which both deferring and breeding females arrive at the colony with upregulated T levels (Crossin et al. 2012). This suggests that all female black-browed albatrosses arrive at the colony physiologically ready to breed but that the decision to defer is made subsequently. This raises the possibility that two very different regulatory mechanisms have evolved to control breeding in sister species of the genus *Thalassarche*.

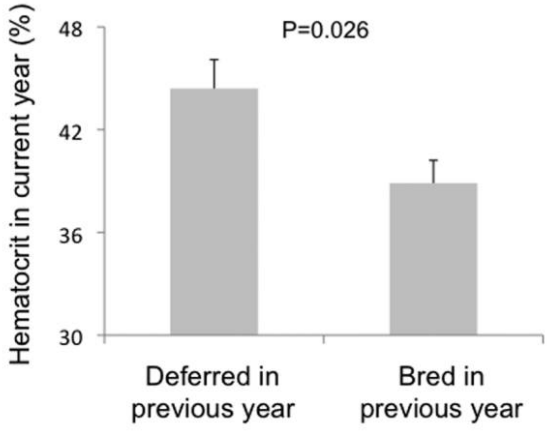

Figure 4. Hematocrit levels in postmigratory female gray-headed albatrosses were significantly influenced by their breeding status in the previous year. Females that bred in year x had significantly reduced Hct before breeding in year $x + 1$. Columns represent least squares means (ANCOVA) + SEM.

Role of Body Condition

Although the relationship is not universal, many studies show that low body condition tends to be associated with deferred breeding activity, which suggests an energetic threshold to breeding (e.g., the "prudent parent" hypothesis; Drent and Daan 1980; Descamps et al. 2011). Consistent with several (but not all) seabird studies, our data clearly show that low body mass characterized deferred breeding, with deferring females weighing nearly 500 g less than breeding females. However, unlike Hector et al. (1986*a*, 1986*b*), we also provide a link between condition and steroidogenesis in gray-headed albatrosses, which suggests a condition-dependent mechanism of breeding decision. How condition might influence patterns of ovarian steroidogenesis is not known at present. One possibility is that increased glucocorticoid secretion (e.g., corticosterone), due to prebreeding nutritional and/or other stressors encountered before the return to the breeding colony, could suppress ovarian function as observed in other seabirds (Goutte et al. 2010*a*). From this perspective, P4 secretion could be viewed as an indirect signal of a stress response. By forgoing conversion to T and a commitment to reproduction, P4 is made available as a substrate for glucocorticoid synthesis, an important component of the acute stress response and emergency energy mobilization (via 21-hydroxylase and 11β-hydroxylase activity). P4 is thus an important precursor at a crossroads between the androgenic and glucocorticoid biosynthetic pathways and represents a key deterministic step in the regulation of breeding decisions in female gray-headed albatrosses. Whether this process is mediated by or simply correlated with elevated plasma glucocorticoid levels requires further study (Goutte et al. 2010*a*, 2010*b*).

Aerobic Condition and Implications for Carryover Effects in Breeding Decisions

In addition to poor body condition (e.g., low body mass), deferred breeding by female gray-headed albatrosses was also associated with low Hct and Hb concentrations, two traits that reflect aerobic performance and oxygen transport capacity (Wagner 1996) and that are key for sustaining the high energetic costs of flight. An interesting avenue for future study is the role that phenotypic variation in the aerobic capacity and oxygen transport capacities of blood plays in breeding decisions (Calbet et al. 2006; Williams 2012). We have shown a similar pattern previously in black-browed albatrosses, where breeding deferral was also associated with reduced Hct and Hb concentration (Crossin et al. 2012). We suggest two possible explanations for reduced Hct at arrival: either it reflects foraging conditions and success during the latter part of the nonbreeding period and, in particular, potentially the short-term cost of the final few days spent in flight back to the colony or it indicates a longer-term "cost of reproduction" in the form of reproductive anemia stemming from the previous breeding season. Although there is an intuitive appeal to the idea that long-distance flight could exact a cost in the form of reduced Hct, this is not consistent with studies that identify Hct upregulation as an adaptation to increase oxygen-carrying capacity in migrating birds (Bairlein and Totzke 1992; Piersma et al. 1996; Landys-Ciannelli et al. 2002).

Conversely, there is evidence for long-term costs of reproduction via E_2-mediated reproductive anemia (Williams et al. 2004*a*). By experimentally increasing the cost of current reproduction in great skuas (*Stercorarius skua*), Kalmbach et al. (2004) show that increased levels of egg production via egg removals increased E_2-mediated vitellogenesis, which had the consequence of reduced Hct and red blood cell numbers that persisted for more than a year, spanning winter migration and parts of the next breeding season. This suggests that Hct reductions and reproductive anemia might be proportional to reproductive effort, such that females laying more (or any) eggs may incur higher costs of reproduction relative to those laying fewer (or none). Although we did not measure premigratory Hct levels in the albatrosses in this study, we do know the breeding histories for each individual female that we sampled. As we have shown in figure 3, deferring females had significantly lower postmigratory (prelaying) Hct levels than breeding females (fig. 3*B*). But when we group females according to their previous year's breeding outcome, current postmigratory Hct levels were significantly lower in those females that bred in the previous year than in those that did not breed (fig. 4). This suggests a potential cost of reproduction on Hct levels (i.e., reproductive anemia) that carried over to the next breeding season and led in part to deferred breeding, but to confirm this we would need systematic measurements of Hct levels in individuals before and after migration (repeated measures). Nevertheless, this seems an intriguing possibility that could explain the low body mass of deferring females in this study. If females did suffer from reproductive anemia in the previous year, then this cost of reproduction could have persisted through the winter to influence future breeding activity, as observed by Kalmbach et al. (2004). If this is the case, then reduced Hct would limit aerobic capacity and thus migratory efficiency in terms of energy use and foraging efficiency, resulting in the lower prebreeding body masses that we measured in deferring birds upon arrival at the breeding colony. Certainly though, reductions in Hct could occur as a result of events experienced during winter migrations independent from reproductive anemia. Albatrosses migrating for 6–16 mo throughout the Southern Ocean experience frequent storms and other challenges, so it is feasible that anemia could develop in some individuals that have difficulty coping or that such environmental conditions could exacerbate a preexisting reproductive anemia. Whatever the case, the important point is that previous studies have linked Hct to aerobic capacity and flight performance (Hammond et al. 2000). Some suggest that Hct can be adaptively regulated to match the aerobic demands associated with specific life-history events such as migration (Bairlein and Totzke 1992; Piersma et al. 1996; Landys-Ciannelli et al. 2002), which could form the basis for potential trade-offs. Our results are consistent with this idea, but experimental work is still needed to firmly establish the relationships between

hematological status, aerobic capacity, workload, individual quality, and trade-offs, including costs of reproduction and carryover effects.

Acknowledgments

We extend thanks to Derren Fox for field support, to Lea Bond for laboratory support, and to Andy Wood for data support. Financial support was provided by the British Antarctic Survey through an Antarctic Funding Initiative Collaborative Gearing Scheme. Additional support was provided by a National Science and Engineering Research Council of Canada (NSERC) postdoctoral fellowship and NSERC E-BIRD funding to G.T.C. and by NSERC Discovery Grants to T.D.W. and K.E.W.-E. This study represents a contribution to the British Antarctic Survey Ecosystem Programme.

Literature Cited

Bairlein F. and U. Totzke. 1992. New aspects on migratory physiology of trans-Saharan passerine migrants. Ornis Scand 23:244–250.

Baker A.J., P.M. González, T. Piersma, L.J. Niles, I.L.S. do Nascimento, P.W. Atkinson, N.A. Clark, C.D.T. Minton, M.K. Peck, and G. Aarts. 2004. Rapid population decline in red knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. Proc R Soc B 271:875–882.

Calbet J.A.L., C. Lundby, M. Koskolou, and R. Boushel. 2006. Importance of hemoglobin concentration to exercise: acute manipulations. Respir Physiol Neurobiol 151:132–140.

Chastel O., H. Weimerskirch, and P. Jouventin. 1995. Influence of body condition on reproductive decision and reproductive success in the blue petrel. Auk 112:964–972.

Crossin G.T., R.A. Phillips, P.N. Trathan, D.S. Fox, A. Dawson, K.E. Wynne-Edwards, and T.D. Williams. 2012. Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses. Gen Comp Endocrinol 176:151–157.

Croxall J.P., J.R.D. Silk, R.A. Phillips, V. Afanasyev, and D.R. Briggs. 2005. Global circumnavigations: tracking year-round ranges of nonbreeding albatrosses. Science 307:249–250.

Dawson A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variables. Phil Trans R Soc B 363:1621–1633.

Descamps S., J. Bêty, O.P. Love, and G.H. Gilchrist. 2011. Individual optimization of reproduction in a long-lived migratory bird: a test of the condition-dependent model of laying date and clutch size. Funct Ecol 25:671–681.

Drent R.H. and S. Daan. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68:225–252.

Ebbinge B.S. and B. Spaans. 1995. The importance of body reserves accumulated in spring staging areas in the temperate zone for breeding dark-bellied Brent geese *Brante b. bernicla* in the high Arctic. J Avian Biol 26:105–113.

Goutte A., F. Angelier, C. Clément Chastel, C. Trouvé, B. Moe, C. Bech, G.W. Gabrielsen, and O. Chastel. 2010*a*. Stress and the timing of breeding: glucocorticoid-luteinizing hormones relationships in an arctic seabird. Gen Comp Endocrinol 169: 108–116.

Goutte A., E. Antoine, H. Weimerskirch, and O. Chastel. 2010*b*. Age and the timing of breeding in a long-lived bird: a role for stress hormones? Funct Ecol 24:1007–1016.

Hammond K.A., M.A. Chappell, R.A. Cardullo, R.S. Lin, and T.S. Johnsen. 2000. The mechanistic basis of aerobic performance variation in red junglefowl. J Exp Biol 203:2053–2064.

Harrison X.A., J.D. Blount, R. Inger, D.R. Norris, and S. Bearhop. 2011. Carryover effects as drivers of fitness differences in animals. J Anim Ecol 80:4–18.

Harshman L.G. and A.J. Zera. 2007. The cost of reproduction: the devil in the details. Trends Ecol Evol 22:80–86.

Hector J.A.L., J.P. Croxall, and B.K. Follett. 1986*a*. Reproductive endocrinology of the wandering albatross *Diomedea exulans* in relation to biennial breeding and deferred sexual maturity. Ibis 128:9–22.

Hector J.A.L., B.K. Follett, and P.A. Prince. 1986*b*. Reproductive endocrinology of the black-browed albatross *Diomedea melanophris* and the gray-headed albatross *D. chrysostoma*. J Zool (Lond) 208:237–253.

Hector J.A.L., S.P.C. Pickering, J.P. Croxall, and B.K. Follett. 1990. The endocrine basis of deferred sexual maturity in the wandering albatross, *Diomedea exulans* L. Funct Ecol 4:59–66.

Inger R., X.A. Harrison, G.D. Ruxton, J. Newton, K. Colhoun, G.A. Gudmundsson, G. McElwaine, M. Pickford, D. Hodgson, and S. Bearhop. 2010. Carry-over effects reveal reproductive costs in a long-distance migrant. J Anim Ecol 79: 974–982.

Kalmbach E., R. Griffiths, J.E. Crane, and R.W. Furness. 2004. Effects of experimentally increased egg production on female body condition and laying dates in the great skua *Stercorarius skua*. J Avian Biol 35:501–514.

Koren L.Z., E.S.M. Ng, K.K. Soma, and K.E. Wynne-Edwards. 2012. Sample preparation and liquid chromatography–tandem mass spectrometry for multiple steroids in mammalian and avian serum. PLoS ONE 7:e32496, doi:10.1371/journal.pone.0032496.

Landys-Ciannelli M.M., J. Jukema, and T. Piersma. 2002. Blood parameter changes during stopover in a long-distance migratory shorebird, the bar-tailed godwit *Limosa lapponica taymyrensis*. J Avian Biol 33:451–455.

Marra P.P., K.A. Hobson, and R.T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable carbon isotopes. Science 282:1884–1886.

Norris D.R., P.P. Marra, K. Kyser, T.W. Sherry, and L.M. Ratcliffe. 2004. Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proc R Soc B 271:59–64.

Piersma T., J.M. Everaarts, and J. Jukema. 1996. Build-up of red blood cells in refueling bar-tailed godwits in relation to individual migratory quality. Condor 98:363–370.

Prop J., J.M. Black, and P. Shimmings. 2003. Travel schedules to the high arctic: barnacle geese trade-off the timing of migration with accumulation of fat deposits. Oikos 103:403–414.

Ryan P.G., R.A. Phillips, D.C. Nel, and A.G. Wood. 2007. Breeding frequency in grey-headed albatrosses. Ibis 149:45–52.

Smith R.J. and F.R. Moore. 2003. Arrival fat and reproductive performance in a long-distance passerine migrant. Oecologia 134:325–331.

Sorenson M.C., J.M. Hipfner, T.K. Kyser, and D.R. Norris. 2009. Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. J Anim Ecol 78:460–467.

Wagner P.D. 1996. Determinants of maximal oxygen transport and utilization. Annu Rev Physiol 58:21–50.

Weimerskirch H. 1990. The influence of age and experience on breeding performance of the Antarctic fulmar *Fulmarus glacialoides*. J Anim Ecol 59:867–875.

Williams T.D. 2012. Physiological adaptations for breeding in birds. Princeton University Press, Princeton, NJ.

Williams T.D., W.O. Challenger, J.K. Christians, M. Evanson, O.P. Love, and F. Vézina. 2004*a*. What causes the decrease in hematocrit during egg production? Funct Ecol 18:330–336.

Williams T.D., A.S. Kitaysky, and F. Vézina. 2004*b*. Individual variation in plasma estradiol-17b and androgen levels during egg formation in the European starling *Sturnus vulgaris*: implications for regulation of yolk steroids. Gen Comp Endocrinol 136:346–352.

Wooller R.D., J.S. Bradley, I.J. Skira, and D.L. Serventy. 1990. Short-tailed shearwater. Pp. 387–404 in I. Newton, ed. Lifetime reproduction in birds. Academic Press, London.