A Carryover Effect of Migration Underlies Individual Variation in Reproductive Readiness and Extreme Egg Size Dimorphism in Macaroni Penguins

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Submitted January 8, 2010; Accepted May 3, 2010; Electronically published July 16, 2010

abstract: Where life-history stages overlap, there is the potential for physiological conflicts that might be important in mediating carryover effects. However, our knowledge of the specific physiological mechanisms underlying carryover effects remains rudimentary, and specific examples remain rare. Here we show that female macaroni penguins (*Eudyptes chrysolophus*) initiate vitellogenesis and yolk formation while at sea during return migrations to breeding colonies; yolk formation takes approximately 16 days, but females lay only 7–14 days after their return. Once on land, *Eudyptes* penguins show a unique reproductive pattern of extreme egg size dimorphism in which the smaller, first-laid A-egg is 55%–75% of the size of the larger B-egg. We show that the degree of egg size dimorphism is inversely correlated with time between arrival and laying; that is, females that begin reproductive development well in advance of their return produce more dimorphic eggs. Furthermore, late-arriving females that produce the most dimorphic eggs have lower plasma levels of the yolk precursor vitellogenin on arrival; that is, they show lower reproductive "readiness." These data support the hypothesis that extreme egg size dimorphism in *Eudyptes* penguins is due to a physiological constraint imposed by a migratory carryover effect and argue against small A-eggs having a specific, adaptive function.

Keywords: carryover effect, *Eudyptes*, physiological conflict, egg development, reproductive trade-off, vitellogenin.

Introduction

It is axiomatic that an individual's fitness depends on the successful integration of multiple life-history stages, with each requiring specific physiological control mechanisms. Certain life-history events during the annual cycle appear to be organized so that direct overlaps between activities (e.g., between breeding and moult) are avoided or minimized (Dawson 2008), thereby reducing the potential for direct physiological conflicts. However, it is becoming clear that seasonal interactions among different periods of the annual cycle are common. For example, environmental conditions and/or behavior in wintering areas can influence breeding events—such as arrival time, arrival condition, and reproductive success—weeks or even months later, a phenomenon termed long-term "carryover effects" (Marra et al. 1998; Norris 2005; Norris and Marra 2007; Sorenson et al. 2009). Though it is clear that the linkages between stages of the annual cycle must have a physiological basis, our working knowledge of the specific physiological mechanisms underlying carryover effects remains rudimentary. At shorter timescales, if successive activities during the annual cycle directly overlap, this creates the potential for physiological conflicts between activities, which may also be important in mediating carryover effects. One example of this occurs in migratory birds where reproductive development can be initiated long before arrival at breeding areas. For example, during the transequatorial migrations of garden warblers (*Sylvia borin*), testosterone secretion and testes development increase in males during the latter stages of migration (Bauchinger et al. 2007). However, similar data on the onset of reproductive development during migration and the effects of short-term carryover effects are lacking for females in any avian species.

Here we show that in female macaroni penguins (*Eudyptes chrysolophus*) the physiological processes underlying egg formation begin while birds are still at sea, during return migrations to a breeding colony, and that this conflict between migration and reproduction has important implications for subsequent patterns of reproductive investment. Macaroni penguins migrate thousands of kilometers throughout the southern latitudes, and during their approximately 6-month overwintering period, they can

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Am. Nat. 2010. Vol. 176, pp. 357–366.
DOI: 10.1086/655223

forage over an area of ocean habitat greater than 3 million km^2 (Bost et al. 2009). Return migrations to breeding colonies are expeditious and direct (Bost et al. 2009), and dates of arrival are highly synchronous, varying little interannually (Williams 1995). On arrival, macaroni penguins enter the breeding colony without delay (Bost et al. 2009), promptly reoccupy previous nest sites, and begin egg laying 3–15 days after arrival (Williams 1990, 1995). Since egg production takes 16–20 days in penguins (Grau 1982; Astheimer and Grau 1990), individual birds must initiate yolk development during the return migration to varying degrees. We thus posed the question, does migration exert a carryover effect on reproduction by limiting the degree of investment in egg production? Crested penguins (*Eudyptes* spp.) are unique among birds in laying two-egg clutches but typically only ever rearing one chick and in having a smaller first-laid A-egg that is only 55%–75% the size of the second-laid B-egg (Williams 1995). Such extreme egg size dimorphism is unparalleled in birds (Slagsvold et al. 1984; Christians 2002) and, despite longstanding interest, remains unexplained (e.g., Lack 1968; Johnson et al. 1987; St. Clair 1998). Here we test a simple physiological model proposed by Williams (1990) to explain this unique pattern of intraclutch dimorphism and relate this to a carryover effect between migration and egg formation.

In *Eudyptes* penguins, the smaller A-egg begins yolk development about 4 days before the larger B-egg (Grau 1982). Williams (1990) suggested that if reproductive development were constrained by return migrations, the rate of yolk deposition might be submaximal for a greater proportion of the total rapid yolk development phase for A-eggs compared with later-developing B-eggs. This predicts that the interindividual variation in time between arrival and laying—or, conversely, the interindividual variation in the amount of time spent migrating while producing eggs—should underlie patterns of yolk precursor production and A-egg : B-egg size dimorphism. We thus tested the hypothesis that "reproductive readiness," as measured by plasma yolk precursor levels (Deeley et al. 1975; Walzem et al. 1999; Challenger et al. 2001; Caro et al. 2009), is subject to a constraint imposed by a migratory carryover effect and that, once free of this constraint, yolk precursor production increases with time after colony arrival. We recorded dates of colony arrival and time to A-egg laying in female macaroni penguins and related this to A- and B-egg size and the extent of egg size dimorphism. We then measured interindividual and temporal variation in yolk precursor levels in female blood sampled at arrival and after laying their A- and B-eggs. We tested three specific predictions: (1) egg size dimorphism will be positively correlated with length of time spent in the colony between arrival and laying, such that those laying only a few days after arrival would have the most dimorphic eggs (as per Williams 1990); (2) plasma yolk precursor concentrations would be lower in penguins that begin laying shortly after their arrival, indicative of lower "reproductive readiness" due to the constraint imposed by migration (see "Discussion"); and (3) yolk precursor levels at arrival would be positively correlated with egg size dimorphism (as per Williams 1990).

Methods

Study Site and Field Sampling Protocol

Fieldwork was conducted between October and November 2008 at a large breeding colony of approximately 40,000 pairs of macaroni penguins on Goldcrest Point, Bird Island, South Georgia (54°01′S, 38°02′W). Research was conducted through permits issued by the British Antarctic Survey and conformed to guidelines established by the Canadian Committee on Animal Care (Simon Fraser University Animal Care Permit 897B-8).

Macaroni penguins are protandrous, with males arriving 1–2 weeks before females (Williams 1995). We identified a suitable area of the colony for study before females arrived, marked lone males at nest sites using water-based paint, and then identified newly arrived females as birds paired with males who had been marked and alone on the previous day. All newly arrived females ($N = 48$, sampled over 9 days) were captured on the nest, within 24 h of arrival, and 2-mL blood samples were taken from the brachial veins using heparinized syringes fitted with 25-g needles. Blood was transferred to heparinized 2.5-mL Eppendorf vials and centrifuged for 5 min at 10,000 *g*, and plasma was then transferred to labeled 0.6-mL vials and frozen at −20°C until analysis. We recorded the time (± 1 s) that it took to collect a blood sample from first approach to the end of blood collection. We recorded body mass (± 10 g) and measured bill length (± 1 mm) to confirm sex (<22 mm = female; Williams 1995). Before being released, each female received a PIT tag bearing a unique identifying number, and to facilitate easy identification and serial sampling, we painted a large number on the female's breast with black hair dye. This sampling procedure, from first approach to release, averaged 7.4 min. Within 24 h of the A- and B-eggs being laid, females were blood sampled again, and body mass and fresh egg mass were recorded (± 1 g for the latter). Of the 48 newly arrived females sampled, 46 were sampled at all three sampling periods or stages (colony arrival, A-egg, and B-egg), and all laid the full two-egg clutch (two females abandoned the study area after arrival sampling, but both were later spotted in different parts of the colony). As is typical for the species, all A-eggs were lost, via predation and/or the intentional

ejection from the nest, on either the day before or the day of B-egg laying. Two B-eggs were lost to skua (*Stercorarius antarctica*) predation but not as a result of our handling, and all other B-eggs hatched successfully ($N = 44$). We also obtained blood samples from a separate group of female penguins ($N = 17$) approximately 2.5 months after laying, during the brood-guard stage, when females were making 1–5-day foraging trips at sea to provision their growing chicks. We obtained data on mass-specific egg composition from Grau (1982) and Gwynn (1993) to aid the analysis and interpretation of our egg mass data.

Blood and Plasma Analysis

Plasma samples were assayed for vitellogenic zinc (Zn; zinc kit, Wako Chemicals) and total triglycerides (glycerol reagents A and B; Sigma) as indexes of the yolk precursors vitellogenin (VTG) and yolk-targeted very-low-density lipoprotein (VLDLy), respectively, following Mitchell and Carlisle (1991) and as previously described (e.g., Challenger et al. 2001; Caro et al. 2009; Gorman et al. 2009). VTG and VLDLy are the two main yolk precursors in birds and are transported from the circulation into developing yolky follicles by receptor-mediated uptake (Walzem 1996); VTG is generally regarded the more reliable plasma indicator of follicle development in birds (Challenger et al. 2001). We assayed plasma samples from females during brood rearing for total triglycerides to determine baseline levels of generic rather than yolk-targeted VLDL in non-egg-producing females. All assays were measured using a Biotek 340i microplate reader. Intraassay coefficients of variation for VTG, using a domestic laying hen (*Gallus domesticus*) plasma pool, and for total VLDLy, using a 19-week domestic hen plasma pool, ranged from 5.4% to 7.1% and from 5.5% to 6.9%, respectively. Interassay coefficients of variation were 6.13% for VTG and 5.05% for total VLDLy.

Although not directly related to our main hypothesis and predictions, we also measured hematocrit and plasma hemoglobin to determine whether the physiological cost of egg production in macaroni penguins was associated with "reproductive anemia" (sensu Williams et al. 2004; Wagner et al. 2008*a*). Hematocrit (Hct) was measured on fresh whole blood at the time of blood sampling as packed cell volume (%) following centrifugation of whole blood in microhematocrit tubes for 5 min at 10,000 *g*. Hemoglobin (Hb; g dL^{-1} whole blood) was measured with the cyanomethemoglobin method (Drabkin and Austin 1932) modified for use with a microplate spectrophotometer, using 5 μL whole blood diluted in 1.25 mL Drabkin's reagent (D5941 Sigma-Aldrich, Oakville, Ontario) and with absorbance measured at 540 nm.

Statistical Analyses

Analyses were run with either the JMP 7.0 or the SAS 9.0 software package. All variables were tested for normal distribution via plots of residuals against predicted values followed by Shapiro-Wilk tests for normality. All variables were normally distributed, so data transformation was not necessary. A repeated-measures mixed linear model (SAS PROC MIXED) was used to compare temporal, intra-individual changes in VTG, VLDLy, Hct, and Hb levels at different reproductive stages (i.e., within 24 h after colony arrival, A-egg laying, and B-egg laying). Tukey-Kramer post hoc tests were run to identify significant contrasts between stages. Linear regression and multiple linear regression models were used to explore the influence of independent continuous variables (e.g., date of colony arrival, time between colony arrival and egg laying, body mass) on A-egg : B-egg mass ratios, individual egg mass, and total clutch mass. All values presented in figures are least squares means ± SEM unless otherwise noted. To further explore the correlations among the various independent and dependent variables and identify potential causal relationships, three a priori path models were constructed and tested using path analysis (Shipley 1997, using SAS PROC CALIS). Each model was structured to address our three main predictions: (1) that the interval between arrival and laying influences egg size dimorphism, (2) that a shorter interval between arrival and laying (or high migratory overlap) would be associated with lower plasma yolk precursor concentrations, and (3) that lower plasma yolk precursor concentrations at arrival underlie extreme egg size dimorphism.

Results

Female macaroni penguins in this study returned to the breeding colony between November 2 and November 10, 2008 (median date November 6; table 1), which is consistent with long-term monitoring records kept at Bird Island (Williams 1995; British Antarctic Survey, unpublished data). Egg laying commenced 7–14 days after arrival, with the mean date of A-egg laying occurring on November 18 and of B-egg laying on November 21. Date of colony arrival was negatively correlated with body mass at arrival ($r = -0.357$, $P = .013$, $N = 48$); that is, early-arriving females were heavier than later arriving females. However, there was no effect of arrival date on total clutch mass (A-egg + B-egg mass; $r = -0.1915$, $P > .22$, $N = 42$) or individual A- and B-egg masses (A-egg, $r = -0.146$, $P > .36$, $N = 42$; B-egg, $r = -0.209$, $P > .15$, $N = 46$). We could not examine correlations between arrival date and the time interval between arrival and laying since both traits contain a common variable (i.e., interval = laying

Table 1: Mean mass of macaroni penguin (*Eudyptes chrysolophus*) A- and B-eggs measured at Bird Island, South Georgia, in 2008

Egg component	A-egg	B-egg	Difference	Contribution to egg mass dimorphism
Mass (g)	95.8	147.5	51.7	51.7
Yolk wet mass (lipid + protein; g)	28.8	35.5	6.7	6.7
Albumen wet mass (protein; g)	55.6	96.4	40.8	40.8
Yolk + albumen (g)	…	…	…	47.5
Egg mass difference from yolk + albumen wet mass total (g)	…	…	…	4.0
Yolk dry matter (lipid + protein; g)	14.1	17.1	3.0	3.0
Albumen dry matter (protein; g)	5.7	10.5	4.8	4.8
Yolk dry matter (%)	…	…	…	38
Albumen dry matter (%)	…	…	…	62

Note: Also presented are the wet and dry masses of yolk and albumen and the dry matter contributions to egg mass dimorphism (wet mass data from Gwynn 1993 and dry matter calculations derived from Grau 1982).

date − arrival date). Since there is no reasonable proxy for interval between arrival and laying, we excluded arrival date from all subsequent models. Independent variables in subsequent models therefore included time interval between arrival and egg laying, body mass corrected for variation in arrival date (i.e., residual body mass), and clutch mass.

Between colony arrival and A-egg laying, females lost an average of 0.63 kg (13%) of their arrival mass, and by B-egg laying, females had lost 1.21 kg (23%) of their arrival mass (table 1). Mean A-egg mass was 95.8 ± 9.1 g (SD), and mean B-egg mass was 147.5 ± 11.8 g (SD; table 1); however, there was marked interindividual variation in egg size (fig. 1). Total clutch mass (A-egg + B-egg mass), A-egg mass, and B-egg mass were all highly correlated (total clutch vs. A-egg, $r = 0.87$, $P < .0001$; total clutch vs. B-egg, $r = 0.94$, $P < .0001$; A-egg vs. B-egg, $r = 0.67$, $P < .0001$). Through multiple regression analysis, intraclutch egg size dimorphism (A-egg : B-egg mass ratio, or "egg size dimorphism") was significantly related to the amount of time spent in the colony before egg laying ($P = .0016$, $N = 42$; fig. 1*A*), independent of arrival-date-corrected body mass ($P > .51$), and total clutch mass ($P > .60$), supporting our first prediction. However, individual variation in the mass of A- or B-eggs was independent of time spent in the colony before laying and was independent of arrival-date-corrected body mass (i.e., residual body mass; all $P > .10$; fig. 1*B*, 1*C*).

Mean plasma VTG varied significantly with stage of egg laying (repeated-measures ANOVA, full model: $F = 77.93$, df = 2, $P < .0001$; table 1). Plasma VTG levels were significantly higher at the A-egg stage than at colony arrival (repeated measure, $P < .0001$) or when the B-egg was laid (repeated measure, $P < .0001$), supporting our second prediction. Plasma VLDLy also varied significantly with stage (repeated-measures ANOVA, full model: $F = 8.33$, df = 2, $P < .0001$; table 1) but with a different pattern than VTG: VLDLy levels did not vary significantly between time of arrival and the A-egg stage (repeated measure, $P = .657$) but decreased significantly after the B-egg was laid (repeated measure, $P < .0001$). In females with young chicks, generic VLDL levels, measured as plasma triglyceride, were significantly lower than during the prelaying and laying periods (0.97 ± 0.43 vs. 8.05–9.70 mmol mL^{-1}, respectively; $P < .0001$, $N = 150$).

Plasma VTG levels in females at arrival were positively correlated with time between arrival and A-egg laying ($P = .030$, $N = 46$); that is, females that laid sooner after arrival had lower plasma VTG levels at arrival, again supporting our second prediction (fig. 2*A*). Furthermore, plasma VTG at arrival was significantly and positively related to A-egg : B-egg size dimorphism ($P = .0449$, $N = 42$); that is, females with lower plasma VTG at arrival laid more dimorphic eggs, supporting our third prediction (fig. 2*B*).

Although we tested three path models to examine the influence of various exogenous (arrival date, interval between arrival and laying) and endogenous (body mass, clutch mass, VTG, and VLDLy) variables on egg size dimorphism, we present only the most parsimonious a priori path model (fig. 3). This model had the best overall fit (i.e., goodness of fit index = 0.911) and successfully explained the observed covariance among the multiple variables ($\chi^2 = 15.293$, df = 9, $P = .088$). Path coefficients with $t > 1.96$ are deemed significant (Mueller 1996), and we indicate significant coefficients with bold and asterisks in figure 3. Path analysis confirmed results from multiple regression analysis: variation in egg size dimorphism was explained by a path linking the interval between arrival and laying (the inverse of migratory overlap) and plasma VTG levels at arrival, supporting our main predictions (fig. 3).

Mean hematocrit varied significantly with stage of egg laying (repeated-measures ANOVA, full model: $F =$

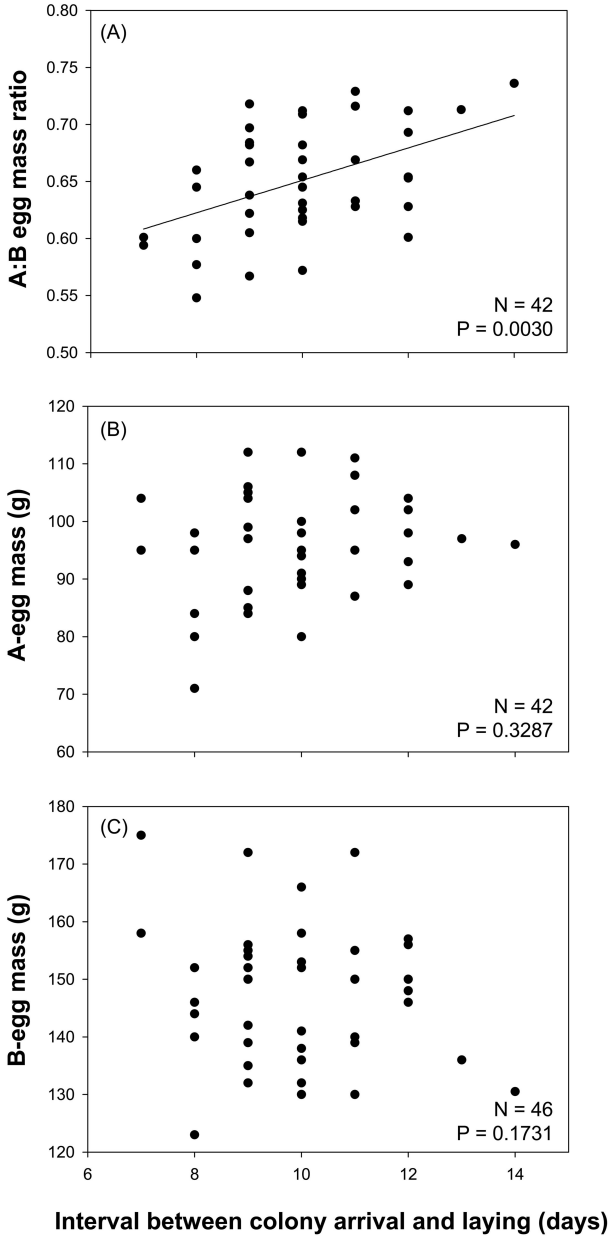

Figure 1: Relationship between egg size dimorphism (*A*), A-egg mass (*B*), and B-egg mass (*C*) with the time interval between colony arrival and laying in female macaroni penguins breeding at Bird Island, South Georgia. Line in *A* is best linear fit.

48.56, df = 2, $P < .0001$; table 2). Hematocrit was higher at arrival than at either the A-egg or the B-egg stage ($P < .0001$ in both cases). Hb also varied significantly with stage (repeated-measures ANOVA, full model: $F = 12.7$, df = 2, $P < .0001$; table 2), decreasing between arrival and the A-egg stage ($P < .001$) but not between then and laying of the B-egg ($P = .911$; table 2).

Discussion

The extreme intraclutch egg size dimorphism seen in crested penguins (*Eudyptes*) represents a unique pattern in avian life histories that, despite more than 60 years of research effort (see "Introduction"), remains unresolved (Lack 1968; Johnson et al. 1987; Lamey 1990; St. Clair 1998). Our study provides novel insight to the physiological basis of *Eudyptes* egg size dimorphism, supports the hypothesis put forward by Williams (1990), and links this unique reproductive pattern to a carryover effect involving direct physiological conflict between migration and reproduction. Our results suggest that migration can impose a direct carryover effect on reproduction by limiting the "reproductive readiness" of arriving females, as indicated by lower circulating levels of plasma vitellogenin. We in-

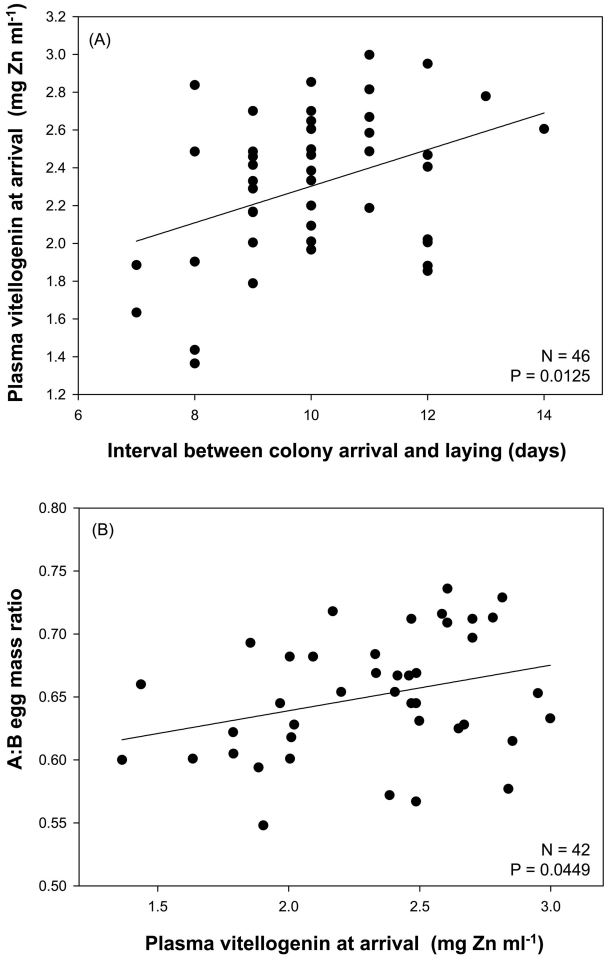

Figure 2: Vitellogenin concentrations in newly arrived female macaroni penguins as a function of time spent in the breeding colony before egg laying (*A*) and A- to B-egg size dimorphism as a function of arrival vitellogenin concentrations (*B*). Lines are best linear fit.

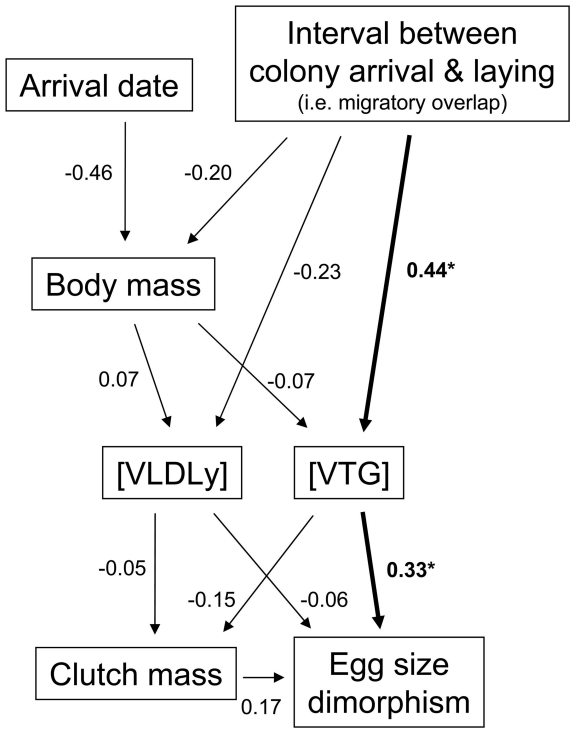

Figure 3: Results of a path analysis examining the causal relationships between the multiple exogenous and endogenous variables underlying variation in egg size dimorphism in macaroni penguins. Exogenous variables include arrival date and the interval between arrival and laying. Endogenous variables include the plasma yolk precursors vitellogenin (VTG) and yolk-targeted very-low-density lipoprotein (VLDLy) as well as total clutch mass (A-egg + B-egg mass). Standardized β coefficients linking the variables through various pathways are presented, and those marked with asterisks are significant ($t > 1.96$; see "Methods"). The bold arrows linking interval–vitellogenin–egg size dimorphism indicate the most heavily weighted path, that is, the one that best explains the observed variation in egg size dimorphism.

terpret lower plasma vitellogenin levels at arrival as indicating lower reproductive readiness on the basis of previous studies of yolk precursor dynamics during the egg-laying cycle. Studies of seabirds (Vanderkist et al. 2000), sea ducks (Gorman et al. 2009), and passerines (Challenger et al. 2001; Salvante and Williams 2002) have shown that plasma VTG concentrations are very tightly coupled to follicle development. More importantly, the relationship between yolk precursor concentrations and follicle mass follows a hyperbolic function, with a rapid increase in plasma VTG immediately after the onset of vitellogenesis and yolk uptake to "maximal" values typical of those of females with a full follicular hierarchy (e.g., see fig. 1 in Challenger et al. 2001); that is, there is no gradual increase in plasma VTG as yolk development proceeds. As we predicted, females with the shortest interval between arrival and the onset of egg laying, and thus the highest degree of overlap between migration and egg production, had the lowest plasma VTG levels at arrival and laid the most dimorphic eggs. Females laying shortly after arrival would have thus had to complete a greater proportion of rapid yolk development while still actively migrating at sea. We suggest that this overlap results in a submaximal rate of yolk deposition or yolk precursor uptake by developing eggs and that this is greater in earlier-developing A-eggs compared with later-developing B-eggs (see fig. 4). We showed that this effect of migration-reproduction overlap was independent of measures of female "quality" (arrival date, body mass, clutch mass); that is, the physiological conflict affects egg size dimorphism per se (allocation of resources between A- and B-eggs) in females with very different levels of total reproductive investment. Although not directly related to our main hypothesis and predictions, we also show that macaroni penguins undergo reproductive anemia during egg laying, with significant decreases in hematocrit and hemoglobin concentrations between arrival and egg laying. Thus, the negative effects of egg production on hematological processes (Wagner et al. 2008*a*, 2008*b*) may represent a key component of future costs associated with egg production in this species.

In *Eudyptes* penguins, complete development of a single follicle takes approximately 16 days, yolks begin development 4 days apart, and there is a 7-day lag between completion of follicle development and oviposition (Grau 1982). Thus, clutch formation takes a total of 23–24 days on average, but the macaroni penguins in our study arrived in the breeding colony only 7–14 days before onset of egg laying. This means that in females showing a high degree of overlap between migration and follicle development—that is, those arriving 7 days before laying, with 9 days of follicle development at sea—as much as 100% of A-egg yolks were formed at sea (see fig. 4). In contrast, females arriving 14 days before laying and developing A-egg follicles for only 2 days at sea have little overlap between migration and egg formation and therefore produced as little as 25% of A-egg yolk mass at sea (see fig. 4). Using data from Grau (1982) and Gwynn (1993), we calculated that yolk contributes about 40% to total egg size dimorphism in macaroni penguins, on the basis of dry lipid and protein content (table 1), and albumen contributes 60% (almost entirely protein for dry mass). Follicle size is thought to be an important mechanistic determinant of albumen deposition, since both the physical or mechanical stimulus of a developing follicle moving down the oviduct and the size of the follicle regulate the function of albumen-secreting cells (Gilbert 1979; Lavelin et al. 2002). Thus, the effects of the migration-reproduction conflict on yolk formation could itself carry over to mediate al-

Table 2: Summary of the biological attributes of female macaroni penguins (*Eudyptes chrysolophus*) breeding at Bird Island, South Georgia, in 2008

Mean dates and biological attributes	Colony arrival	*N*	A-egg	*N*	B-egg	*N*
Mean date	November 6	…	November 18	…	November 21	…
Body mass (kg)	4.97 ± .30	48	4.34 ± .29	46	3.85 ± .27	46
Egg mass (g)	NA	…	95.8 ± 9.1	42	147.5 ± 11.8	46
Plasma VTG (mg Zn mL^{-1})	2.30 ± .09	46	2.88 ± .10	39	1.44 ± .09	44
Plasma VLDLy (triglyceride; mmol L^{-1})	9.70 ± .30	46	9.32 ± .32	42	8.05 ± .31	45
Hct (PCV; %)	50.35 ± .56	48	44.33 ± .63	38	45.91 ± .58	45
Hb (g dL^{-1})	24.36 ± .35	48	22.75 ± .40	48	22.57 ± .37	44

Note: Values are means ± SEM except for dates, which are medians. Hematocrit (Hct) and hemoglobin (Hb) were measured to assess potential future costs of reproduction and are not used to address our main hypothesis (see "Methods"). NA, not applicable; VTG, vitellogenin; VLDLy, yolk-targeted very-low-density lipoprotein; PCV, packed cell volume.

bumen deposition, or there might be additional albumen-dependent physiological mechanisms that contribute to egg size dimorphism. Nevertheless, yolk formation is clearly an important determinant of egg size dimorphism in *Eudyptes* penguins, which, in turn, is strongly correlated with the degree of migratory/reproductive overlap experienced by individual females (as per Williams 1990).

We found strong evidence in support of the "reproductive readiness" hypothesis in terms of lower plasma VTG levels, but there were no similar differences for the second yolk precursor very-low-density lipoprotein. We have shown previously that VTG, measured as plasma zinc, is a more reliable indicator of reproductive state than VLDLy, measured as triglyceride (e.g., Vanderkist et al. 2000; Gorman et al. 2009). However, there could be other explanations for this result. Vitellogenin's singular function is as the primary source of yolk protein, and vitellogenin production alone comprises approximately 50% of the daily hepatic protein synthesis of the laying hen (*Gallus gallus domesticus*) and may triple the amount of protein secreted into the blood (Gruber 1972). In contrast, very-low-density lipoprotein is expressed constitutively in nonbreeding females as generic VLDL and functions to transport triacylglycerides throughout the body for storage in adipose tissue or metabolism, primarily in muscle tissue. In laying females, there is an estrogen-dependent shift in VLDL synthesis from the production of larger "generic" VLDL particles to smaller yolk-targeted VLDL particles (VLDLy), with associated changes in apolipoprotein composition (Walzem 1996). In domesticated species such as the hen, there is an almost complete shift to VLDLy synthesis. However, in free-living birds, which produce eggs under less favorable and far more variable environmental conditions, it seems unlikely (and perhaps even maladaptive) that a total shift from generic to yolk-targeted VLDL would occur as in the domesticated hen (Salvante et al. 2007). For example, free-living female macaroni penguins would presumably still require access to generic VLDL to meet their own metabolic demands during the nonbreeding period in general and especially during the period between arrival and laying, when females are fasting on land and lose approximately 80–110 g in weight per day. In other words, there must be a resource allocation trade-off for VLDL as females partition triglycerides to self-maintenance and activity versus yolk formation once in the colony. Our assay for VLDL, measuring total triglyceride, cannot distinguish between generic VLDL and VLDLy. One possibility, therefore, is that macaroni penguins have higher baseline generic VLDL levels, that is, that this forms a greater proportion of the total 8–10 mmol mL^{-1} we see in arriving females. Our data from females sampled during the brood-guard stage, long after clutch completion, show plasma triglyceride levels in a range common to many nonbreeding birds, which suggests that only generic VLDL was being expressed, although direct measures of generic VLDL and VLDLy in laying females would be needed to confirm this.

Despite the apparent conflict between migration and reproduction and the resulting carryover constraining A-egg yolk formation, it is tempting to conclude that there is no overall fitness consequence of small A-egg size per se, since macaroni penguins are obligate brood reducers, A-eggs are almost invariably lost before or just as B-eggs are laid, and pairs therefore never produce more than a single chick (Williams and Croxall 1991; Williams 1995). However, production of two eggs when only one chick is produced is clearly maladaptive, and we argue that this in itself can be considered a direct fitness consequence of a migratory constraint on egg production. While our study has added a novel physiological perspective to our understanding of this unique pattern of egg size dimorphism, we did not provide answers to many long-standing and unresolved questions; specifically, (1) why is this reproductive pattern restricted to *Eudyptes* penguins? and (2) why has there not been an evolutionary response to selection against investment in A-eggs? or, alternatively, why does egg size dimorphism persist? With regard to the first question, *Eudyptes* penguins, which show the most extreme

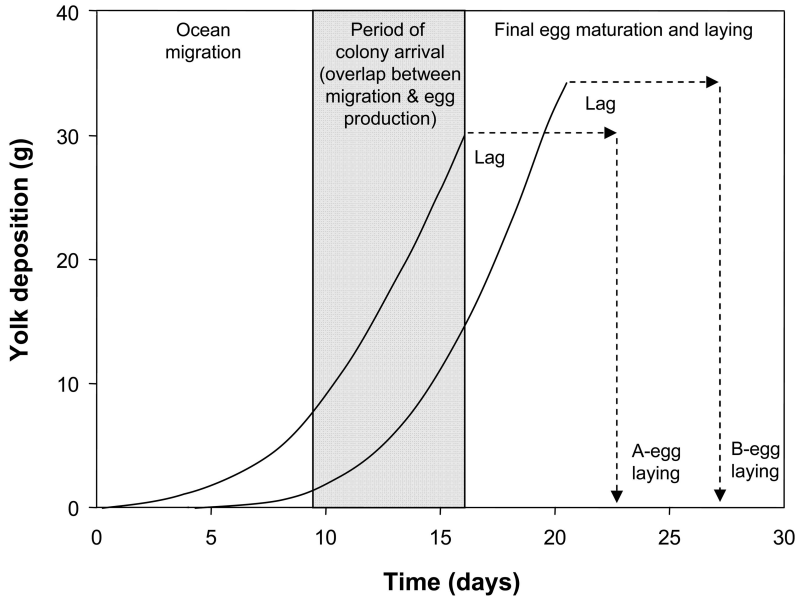

Figure 4: Rates of yolk deposition in macaroni penguin A- and B-eggs. Yolk masses were calculated using egg constituent percentages measured in macaroni penguin eggs by Gwynn (1993). Second-order polynomial curves were fitted to estimates of yolk mass backcalculated from final yolk mass (see Astheimer and Grau 1990, fig. 6, model 2). Mean interval from initiation of egg formation to A-egg laying is 23.4 days, assuming 16.4 days for yolk formation (as per Grau 1982) plus an additional 7-day lag during which albumin and shell constituents are deposited before laying.

intraclutch egg size dimorphism, appear to have relatively inflexible, time-constrained breeding cycles compared with other sympatric species (e.g., *Pygoscelis* spp.; Williams 1995). Bost et al.'s (2009) study also suggests that macaroni penguins have a very extreme migratory cycle during the nonbreeding season, certainly compared with *Pygoscelis* penguins, which are typically sedentary where they are sympatric with macaroni penguins. These factors might preclude macaroni penguins from either arriving earlier or delaying egg laying for longer after arrival in an effort to reduce the overlap between migration and reproduction and avoid carryover effects. The second question is much harder to address in the context of this study, but a forthcoming comparative phylogenetic analysis of life-history evolution in penguins suggests that while *Eudyptes* might be evolving toward a single-egg clutch, this transition appears to be constrained, perhaps because of the inability to eliminate the first ovulation giving rise to A-eggs (R. W. Stein and T. D. Williams, in preparation). The possibility of a constraint on clutch size reduction is also supported by the evolution of two traits that are unique to *Eudyptes* and that ensure the production of a functional, single-egg clutch: reversed hatching asynchrony and egg ejection behavior (St. Clair et al. 1995; St. Clair 1996). Many studies have sought to identify the adaptive value of egg size dimorphism in *Eudyptes* penguins, focusing on the adaptive value of producing a small first-laid A-egg, but without success. Our data show that, in any given year, when females are released from the migration-dependent physiological constraint, thus providing a longer interval between arrival and laying, they actually invest more equally to A- and B-eggs, thereby reducing the degree of egg size dimorphism. This would appear to be even more counterproductive, given that even larger A-eggs rarely, if ever, give rise to offspring in macaroni penguins (Williams and Croxall 1991), and further supports the idea that egg size dimorphism in *Eudyptes* penguins reflects a physiological constraint rather than the idea that small A-eggs have some specific adaptive function (Johnson et al. 1987; St. Clair 1998).

Acknowledgments

Many thanks are extended to S. Adlard at the Bird Island Research Station for field assistance. Financial support for this work was provided by the British Antarctic Survey through an Antarctic Funding Initiative Collaborative Gearing Scheme awarded to A.D., P.N.T., and R.A.P. Additional support was provided through a National Science and Engineering Research Council of Canada (NSERC) Postdoctoral Fellowship and NSERC E-BIRD funding to G.T.C. and an NSERC Discovery Grant to T.D.W.

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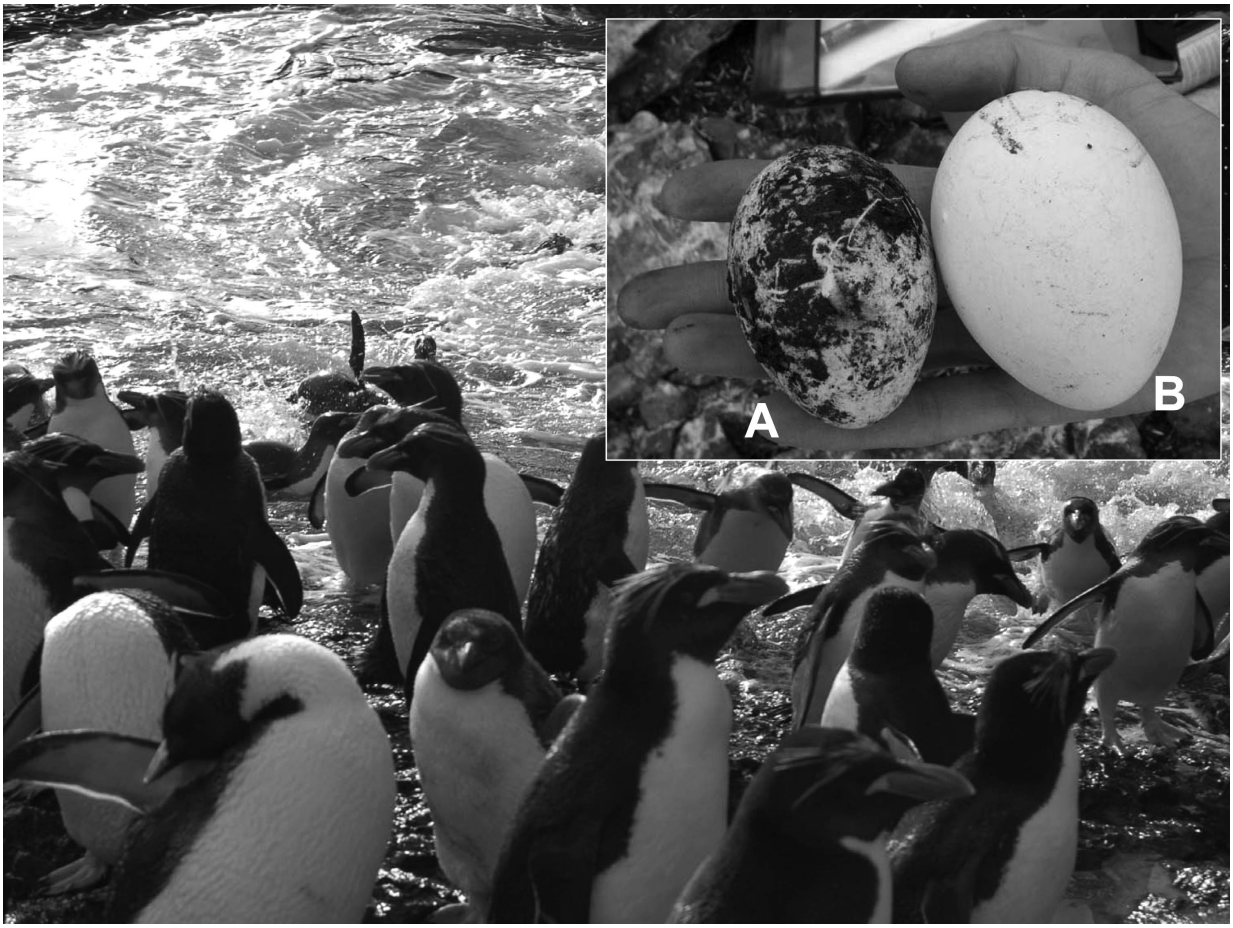

After migrating throughout the southern latitudes for more than 6 months, macaroni penguins arrive each spring at a breeding colony in Bird Island, South Georgia. The inset shows the dimorphism of A-eggs and B-eggs, which is the result of a physiological conflict imposed by migration. Photograph by Glenn T. Crossin.