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ORIGINAL PAPER

Maternal investment in eggs is affected by male feet colour and breeding conditions in the blue-footed booby, *Sula nebouxii*

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Abstract Females are expected to vary investment in offspring according to variables that may influence the offspring fitness in a way that optimises her inclusive fitness for a particular context. Thus, when sexual ornaments signal the quality of the male, females might invest in reproduction as a function of the attractiveness of their mate. We tested whether breeding conditions and male feet colour influence reproductive decisions of blue-footed booby females. In the blue-footed booby, male feet colour is a dynamic condition-dependent sexually selected trait that is related to paternal effort. During two consecutive years, an El Niño year (poor breeding conditions) and a year with good breeding conditions, we experimentally reduced male attractiveness by modifying their feet colour after the first egg was laid and recorded female investment in the second egg. We found that, relative to the first egg in the clutch, females laid heavier second eggs during the poor year than during the good year. Females paired with males with duller feet colour reduced second-egg mass and volume and delayed the laying of the second egg, independently of the year. Absolute yolk androstenedione (A4) concentration (but not testosterone, T) in second eggs was higher during a poor year than during a good year. Only during a year with poor breeding conditions, females paired with experimental males decreased the relative A4 concentration (but not T) in the second egg compared to control females. Thus, blue-footed booby females probably favour brood reduction by decreasing egg quality and increasing size asymmetry between chicks when the breeding and the mate conditions are poor.

Keywords Sexual traits · Egg quality · Laying asynchrony · Yolk androgens · *Sula nebouxii* · Maternal effects

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Introduction

Females are expected to vary investment in offspring according to variables that may influence the offspring fitness in a way that optimises her inclusive fitness for a particular context (Mousseau and Fox 1998a, b; Christians 2002; Verboven et al. 2003; Sockman et al. 2006; Sheldon 2000). Accordingly, in some bird species, females allocate resources in relation to variables that may influence both the offspring and the mother fitness, such as environmental conditions (Mousseau and Fox 1998a, b; Gil 2003, Gil et al. 2004a), breeding density (Mazuc et al. 2003; Müller et al. 2004) and chick sex (Müller et al. 2003; Saino et al. 2003). Furthermore, when sexual ornaments signal the quality of the male, females might adjust the level of their investment in reproduction as a function of the attractiveness of their mate (differential allocation hypothesis, Burley

1986, 1988). Accordingly, in some bird species, females paired with attractive males modify investment in eggs producing larger eggs (Cunningham and Russell 2000; Uller et al. 2005) or vary the levels of yolk testosterone (Gil et al. 1999, 2004b; Tanvez et al. 2004), antibodies (Saino et al. 2002a) and antioxidants (Saino et al. 2002b; Williamson et al. 2006).

The amount and quality of resources allocated to eggs by mothers often has a strong influence on the behaviour, growth and survival of the progeny (Schwabl 1993; Williams 1994; Christians 2002; Groothuis et al. 2005). In birds, egg size is positively correlated with hatching probability, and early growth and survival of the young (Williams 1994; Christians 2002). In addition, increasing evidence indicates that variations in maternally derived yolk androgens during early development can have positive effects on chick development (Schwabl 1996; Eising et al. 2001; Eising and Groothuis 2003; Navara et al. 2006), though negative effects have also been reported (Sockman and Schwabl 2000; Müller et al. 2005; Navara et al. 2005; Rubolini et al. 2006; Von Engelhardt et al. 2006; Tobler et al. 2007). For instance, high yolk testosterone concentrations have been shown to enhance begging behaviour and thereby the amount of food received by black-headed gull chicks (*Larus ridibundus*; Eising and Groothuis 2003) and the social rank after fledging of canary chicks (*Serinus canaria*, Schwabl 1993). Furthermore, in asynchronously hatching clutches, increased androgens in the last egg could compensate for competitive asymmetries among brood mates (Schwabl 1993, 1997; Sockman and Schwabl 2000; Eising et al. 2001; Sockman et al. 2006; Sandell et al. 2007).

Besides modifying egg quality, females may influence offspring fitness by varying the laying interval between eggs and consequently the degree of asynchrony at hatching, a key trait for sibling competition (Lack 1954; Mock and Parker 1997; Drummond 2006). Hatching asynchrony determines initial differences in size and development among brood mates which can be amplified by sibling competition affecting the nutritional, physiological and social asymmetries and survival between chicks (Mock and Parker 1997; Drummond 2006). Furthermore, parental adjustment of the degree of hatching asynchrony to the abundance or predictability of food has been interpreted as an adaptation to optimise the number and quality of offspring under variable breeding conditions (Lack 1954; Wiebe and Bortolotti 1995; Wiebe et al. 1998). Thus, by modifying the laying interval and consequently the degree of hatching asynchrony, females may facilitate brood reduction.

In the blue-footed booby, male feet colour is a dynamic condition-dependent sexually selected trait (Torres and Velando 2003; Velando et al. 2006). Preferred males, those with bright green–blue feet, have better nutritional and health condition compared to males with duller blue feet, and feet coloration can change in less than 48 h if no food is provided (Torres and Velando 2003; Velando et al. 2006). A cross-fostering experiment showed that blue-footed booby offspring condition correlates with the feet colour of the foster father, and to a lesser degree, with the feet colour of the genetic father (Velando et al. 2005). Furthermore, females decreased second-egg volume when the male feet colour was manipulated to a duller blue after the first egg was laid (Velando et al. 2006), and smaller eggs have lower hatching success and produce lighter chicks at hatching (D'Alba and Torres 2007). In this experiment, we further investigated how females adjust their investment in eggs after a sudden deterioration of male feet colour. Less than 24 h after the first egg was laid, feet colour of experimental males was modified to duller blue, mimicking males in low condition, and female investment in second egg's mass, volume and yolk androgen concentration and laying interval was measured. Reproductive success of the blue-footed booby is strongly influenced by climatic variation related to El Niño Southern Oscillation, which may affect the onset of breeding, the growth of chicks and the rate of nest abandonment (Drummond et al. 1986; Wingfield et al. 1999). To evaluate the effects of annual environmental variability on maternal investment in eggs, the experiment was repeated during two consecutive years with contrasting environmental breeding conditions: 2005, an El Niño year with poor breeding conditions (mean reproductive success of the colony 0.18 fledglings produced per total nests) and 2006, a year with good breeding conditions (1.43 fledglings produced per total nests; Drummond H. and Torres R., unpublished data). If females adjust investment within the clutch according to expected breeding conditions, we predicted that, to mitigate the asymmetries between brood mates, during a poor year females should increase investment in second eggs (egg volume, mass and yolk androgen concentration) compared to investment in second eggs during a good year. Yet, if the mate feet colour deteriorates indicating a decrease in male condition, compared to females in the control group, females paired with males with duller feet colour should decrease second-egg mass, volume and yolk androgen concentration and may delay the laying of the second egg, particularly in years with poor breeding conditions.

Materials and methods

The study was carried out in the breeding colony of blue-footed boobies at Isla Isabel, off the Pacific cost of Mexico (25° 52′ N, 105° 54′ W), from March to April 2005 and from January to May 2006.

The blue-footed booby

The blue-footed booby is a long-lived bird with a modal clutch size of two eggs (Drummond et al. 1986), laid with an average interval of 3.9±1.5 days (D'Alba and Torres 2007); and the laying interval is positively related to the hatching interval between chicks (D'Alba and Torres 2007). Both parents incubate the clutch for roughly 41 days and care for the characteristic brood of two chicks during approximately 4 months (Drummond et al. 1986; Torres and Drummond 1999). Chicks hatch on average at an interval of 4 days and the resulting asymmetry in size and development between them facilitates the establishment of a dominant–subordinate relationship (Drummond et al. 1991; Osorno and Drummond 1995). Typically, the senior outcompete the younger chick, resulting in differential mortality of the subordinate nestling (in 136 two-chick broods, 20.56% of junior chicks died while 5.88% of senior chicks died, Drummond et al. 1986). Male contribution to parental effort seems to be important for female breeding success: experimental reduction of paternal effort had a negative effect on the condition and probably future reproduction of females (Velando and Alonso-Alvarez 2003).

Experimental modification of male feet colour

Courting pairs were monitored daily to determine laying date. We manipulated feet colour of 26 males during a poor year (2005) and 64 males during a good year (2006) less than 24 h after the first egg was laid. Males were captured, banded with a numbered metal ring and randomly assigned to either the experimental or the control group. In the experimental group, feet colour was modified to a duller blue with a non-toxic intensive makeup to mimic a male in low condition (Torres and Velando 2003). In the control group, we simulated the manipulation (using a crayon in a plastic bag) without changing the original male feet colour (Torres and Velando 2003). This method of colour modification has been used before with no effects on bird behaviour and the artificial colour on experimental males lasts for 5–6 days (Torres and Velando 2003). Total handling time per bird was less than 5 min. Feet colour was measured with a spectrophotometer (MINOLTA 2600d) before and after the manipulation. Before the manipulation, feet colour of control and experimental males did not differ (peak of maximum reflectance: control 514.8±2.80 nm, experimental 518.4±2.98 nm; Mann–Whitney test, N=48, U=214.5, P=0.09; total reflectance: control 1,296.90±20.93, experimental 1,335.26±34.56; $t_{1,\ 46}$=0.93, P=0.35). Also, previous to manipulation, feet colour of males during a poor and a good year did not differ (peak of maximum reflectance Mann–Whitney test, N=48, U=205.5, P=0.10; total reflectance $t_{1,\ 46}$=2.14, P=0.38). After the manipulation, the peak of maximum reflectance on the feet colour of experimental males decreased 11.18% (460.4±0.40 nm), and total reflectance decreased 46.4% (715.11±39.99); nevertheless, the lower values remained within the natural range of variation reported for courting males in the same population (Velando et al. 2006).

Nest monitoring and yolk sampling

Nests were checked daily until the complete clutch had been laid, and all freshly laid eggs were marked with a non-toxic pen. Egg mass was determined with an electronic balance (±0.1 g), and their maximum length and width were measured with a calliper (±0.1 mm) to calculate egg volume in cubic centimeter (length×width^2×0.51/1,000; Hoyt 1979). Less than 24 h after laying, a yolk sample (15–20 mg) was obtained by introducing a syringe (needle type 21G) into the egg through a small hole in the shell (Schwabl 1993). The hole was sealed using a tiny drop of dentistal cement (VIARDEN, Mexico) and immediately after the egg was placed back in the nest. Yolk samples were stored in liquid nitrogen until laboratory analyses were performed. Overall, hatching success for manipulated eggs was low: 21.6% of the eggs that were yolk-sampled hatched. For the analysis, clutches of two and three eggs were used: 12 two-egg clutches (five from the experimental group and seven from the control group) from 2005 and 26 two-egg clutches (13 in the experimental group and 13 in the control group) and 14 three-egg clutches (five in the experimental group and nine in the control group) from 2006. In 2006, clutch size did not differ between treatments (Mann–Whitney test, N=52, U=964.5, P=0.63), and the number of eggs in a clutch did not have an effect on the second-egg volume (general linear model (GLM), $F_{2,\ 50}$=0.39, P=0.67), mass (GLM, $F_{2,\ 50}$=0.17, P=0.86) or androgen concentration (GLM, A4, $F_{2,\ 48}$=0.40, P=0.52; T, $F_{2,\ 49}$=0.08, P=0.77). Hence, the first two eggs from these three-egg clutches were included in the analyses. Females that failed to lay a second egg were not included in the analysis (14 in 2005 and 18 in 2006).

Hormone assays

Yolk androgen concentrations were determined by radio immune assay (RIA, Schwabl 1993). In order to extract androgens, 10–15 mg of yolk were homogenised in 1 ml of distilled water; 0.5 ml of this homogenised solution was mixed with 5 ml of diethyl ether and vortexed during 1 min. The ether phase was decanted after snap freezing in an alcohol bath at −30°C and evaporated. The dried extract was redissolved in 1 ml of isooctane. This wet extract passed through celite chromatography columns under

nitrogen flux in order to separate androstenedione (A4), dihydrotestosterone (DHT), and finally testosterone (T), using 3.5 ml of isooctane (100%), 3.5 ml of isooctane and ethyl acetate (95%:5%) and 3.5 ml of isooctane and ethyl acetate (85%:15%), respectively. Each extract was evaporated and redissolved in 1 ml of phosphate buffer (0.1 M with 1% of gelatin). The rest of the method followed the standard RIA technique (Wingfield and Farner 1975; Wingfield et al. 1999).

All androgens were determined in duplicate and were incubated overnight at 4°C with 5,000 cpm with its respective [H^3] androgen before the quantification. Duplicate values of each sample were compared to a standard curve that ranged in concentration from 12.5 to 400 pg for A4, from 9.9 to 316.8 pg for T and from 6.25 to 200 pg for DHT. The mean recovery values were 64% for A4, 57% for DHT and 58% for T. The coefficient of variation inter-assay was 6.87% for A4 and 8.38% for T, while intra-assay variation was 3.07% for A4 and 3.24% for T. Specific A4 and DHT antibodies were provided by MP Biomedicals, LLC, OH, USA (catalogue number; A4: 61320 and DHT: 61340). T antibodies were provided by World Health Organisation RIA Reagent Programme (catalogue number: K200710). The cross-reactivities of the antibodies were A4: T=4.5%, T:A4=3.5%, T:DHT=1.3%, DHT:A4=2.4% and DHT:T=22.7%.

Statistical analysis

The mass, volume and absolute concentration of yolk androgens of second eggs were analysed using general mixed models in PROC MIXED in SAS with normal error distribution (SAS Institute 1999) and the Satterthwaite approximation for the denominator degrees of freedom (Littell et al. 1996). The models included clutch identity as a random factor and the treatment, year and either mass, volume or yolk androgens of first eggs as fixed factors. As variation in yolk androgen concentrations between females is big and may have an intrinsic component that determine to some extent hormones deposition (Sandell et al. 2007; Tobler et al. 2007), yolk androgen concentrations of the first egg were included in the models. Because our experimental manipulation was performed after the first egg in the clutch was laid, and egg allocation adjustment within the clutch is probably relevant in the blue-footed booby, we also analysed whether females adjust androgens transferred to the second egg relative to the first egg in the clutch. Relative concentrations were calculated as (A4 or T concentration in the second egg × 100 / A4 or T in the first egg) − 100. Positive values indicate that second eggs received more A4 or T than the first egg and negative values indicate the contrary. After a significant interaction post hoc comparisons were performed using *t* test. Laying date was initially included in all models but was not significant ($P>0.05$ in all cases); we therefore excluded this variable from analysis. Concentrations of yolk DHT could not be detected in both eggs in 29 out of the 52 clutches; then, this androgen was not analysed. Laying intervals of control and experimental clutches were compared with a generalised linear model with Poisson error distribution. Mean ± SE are shown throughout the manuscript and $P<0.05$ was considered significant.

Results

First-laid eggs (that is, eggs laid before the experimental manipulation) from control and experimental pairs did not differ in mass (treatment, $F_{1, 48}=1.15$, $P=0.28$; treatment × year $F_{1, 48}=0.23$, $P=0.63$), volume (treatment, $F_{1, 48}=0.70$, $P=0.41$; treatment × year $F_{1, 48}=0.44$, $P=0.10$) and concentration of T (treatment, $F_{1, 47}=0.44$, $P=0.51$; treatment × year $F_{1, 47}=3.25$, $P=0.08$; Table 1); although during the good year, first eggs in the control group had greater concentrations of A4 than fist eggs in the experimental group (treatment, $F_{1, 46}=0.01$, $P=0.94$; treatment × year $F_{1, 46}=7.93$, $P=0.007$, Table 1, post hoc: $t_{1, 39}=2.48$, $P=0.01$). When comparing between years, first eggs in a good year were heavier (61.32±0.67 g in 2006 and 56.91±1.37 g in 2005; $F_{1, 48}=6.82$, $P=0.01$) and had more T (20.52±1.65 pg/mg of yolk in 2006 and 14.08± 1.68 pg/mg of yolk in 2005; $F_{1, 49}=4.26$, $P=0.044$) than first eggs in the El Niño year of 2005, but they did not differ in size (56.83±0.74 cm^3 in 2006 and 54.04±1.12 cm^3

Table 1 Mean ± SE concentrations of yolk androstenedione (A4) and testosterone (in picogram per milligram of yolk) of first and second eggs from clutches in the control and experimental groups during a year with poor breeding conditions (2005) and a year with good breeding conditions (2006)

Androgens	Poor year (2005)		Good year (2006)	
	Control (N=7)	Experimental (N=5)	Control (N=22)	Experimental (N=18)
A4				
First eggs	307.43±74.15	586.31±134.45	704.31±83.05	466.11±36.78
Second eggs	541.55±138.35	645.45±139.99	586.83±53.74	503.40±35.75
Testosterone				
First eggs	10.75±1.07	18.74±2.67	22.39±2.33	18.36±2.27
Second eggs	16.68±1.80	19.74±2.21	17.48±2.28	18.31±1.50

in 2005; $F_{1, 48}=3.61$, $P=0.06$) nor in yolk concentrations of A4 (602.01±53.06 pg/mg of yolk in 2006 and 423.64± 78.78 pg/mg of yolk in 2005; $F_{1, 48}=3.45$, $P=0.069$).

Effects of male feet colour modification on egg investment

Egg mass and volume

As predicted, females paired with males with duller feet laid lighter second eggs than females in the control group (Table 2; Fig. 1a). The mass of second eggs differed between years and was positively related to the mass of the first egg in the clutch (Table 2), but the interaction between treatment and year was not significant ($P=0.92$). Second eggs were slightly heavier in the poor year than in the good year, when the mass of the first egg was controlled for (60.11±0.89 g in 2005 and 59.35±0.40 g in 2006; Table 2). The experimental manipulation of male feet colour had a similar effect on egg volume. Females in the experimental group laid smaller second eggs than control females (Table 2; Fig. 1b). The volume of second eggs was positively related to the volume of the first egg in the clutch, but there were no differences in the volume of second eggs between years (Table 2). After controlling for the volume of the first egg in the clutch, the volume of second eggs in the experimental group was 2.97% smaller than in the control group (Table 2).

Laying asynchrony

Females paired with experimental males deferred laying of the second egg in the clutch. Irrespective of year, experimental females delayed on average 0.77 days more than control females the laying of the second egg (treatment $F_{1, 48}=6.37$, $P=0.01$; year $F_{1, 48}=0.14$, $P=0.70$; treatment × year $F_{1, 48}=0.93$, $P=0.33$; Fig. 2).

Table 2 Comparison of mass and volume of second eggs laid by 23 experimental females and 29 control females

Model and terms	*df*	*F*	*P*
Second-egg mass			
Treatment	1, 47	7.02	0.01
Year	1, 47	11.48	0.001
First-egg mass	1, 47	46.76	<0.0001
First-egg mass × year	1, 47	10.26	0.002
Second-egg volume			
Treatment	1, 47	28.33	<0.0001
Year	1, 47	1.73	0.19
First-egg volume	1, 44.5	184.73	<0.0001
First-egg volume × treatment	1, 47	25.91	<0.0001

In the experimental group, less than 24 h after the first egg was laid, male feet colour was manipulated to a duller blue. Data were analysed with general linear mixed models. The models included the identity of the nest as a random factor (Wald *Z* tests for random effects: egg mass, $Z=4.22$, $P<0.001$; egg volume, $Z=4.85$, $P<0.001$). The initial models included all second-degree interactions but non-significant terms were excluded to obtain the minimum adequate model

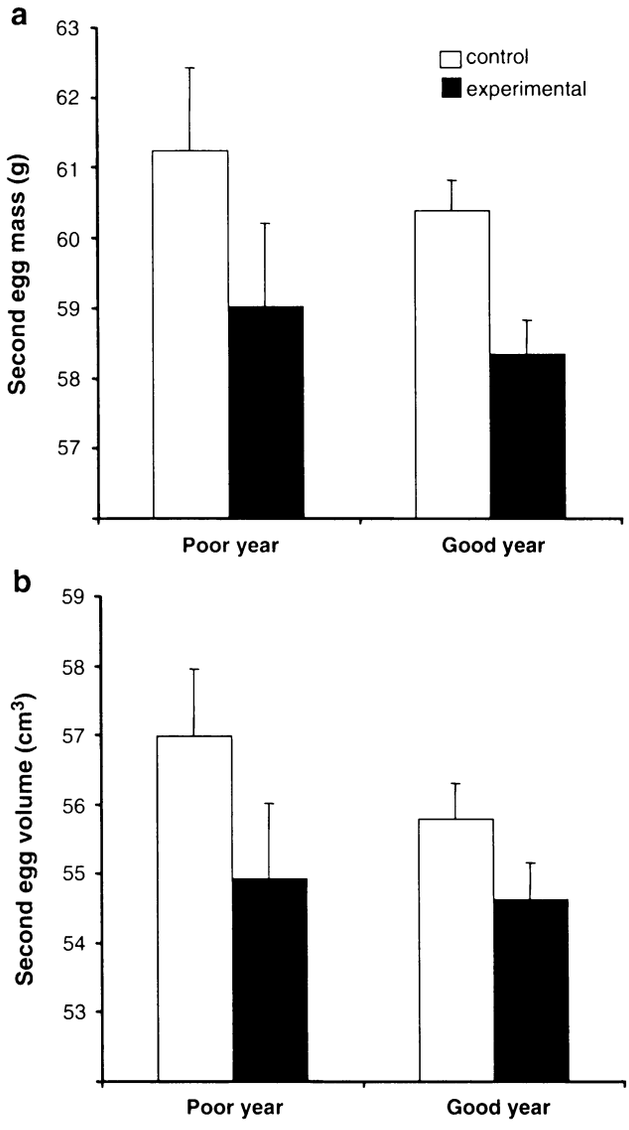

Fig. 1 Least squares means (LSM ± SE) of second-egg (**a**) mass and (**b**) volume from control and experimental clutches during a year with poor breeding conditions (control, $N=7$; experimental, $N=5$) and during a year with good breeding conditions (control, $N=22$; experimental, $N=18$). LSM were estimated from the models that included first-egg mass or volume, treatment and year of study as fixed factors and nest id as a random factor

Absolute yolk androgen concentrations

The concentration of A4 in second eggs did not differ between control and experimental groups ($F_{1, 44}=2.30$, $P=0.13$; all interactions with treatment $P>0.12$, Table 1). Concentration of A4 of first and second eggs within the clutch was positively correlated and variation among

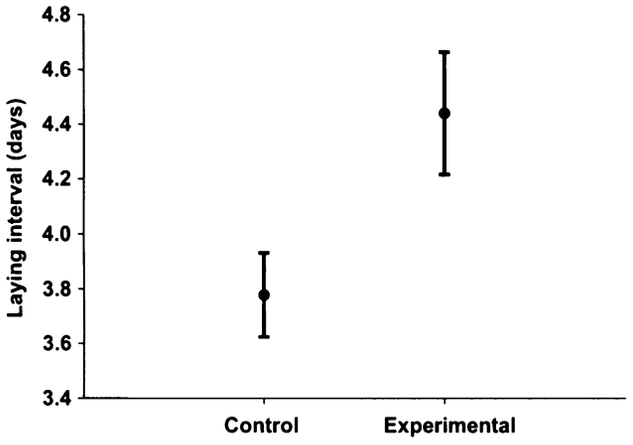

Fig. 2 Laying interval (days) between first and second eggs in the control and experimental groups from the pooled sample of both years of study

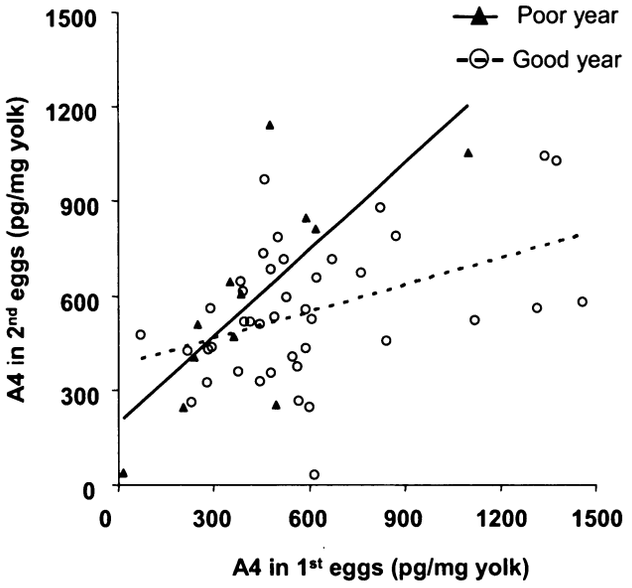

Fig. 3 Yolk A4 concentration of first and second eggs during a year with poor breeding conditions (2005) and a year with good breeding conditions (2006)

clutches was significant (first egg A4, $F_{1,\ 44}=28.84$, $P<0.0001$; random effect $Z=4.69$, $P<0.001$). Yet, A4 concentration in second eggs was on average 6.69% greater in the poor year (584.85±96.42 pg/mg of yolk) than in the good year (548.17±33.51 pg/mg of yolk), when variation in the concentration of A4 of the first egg was controlled (year $F_{1,\ 44}=3.46$, $P=0.069$, year × first egg A4, $F_{1,\ 44}=9.00$, $P=0.004$, Fig. 3).

The concentration of T in second eggs did not differ between experimental treatments ($F_{1,\ 42}=0.30$, $P=0.58$; all interactions with treatment $P>0.51$) or years ($F_{1,\ 42}=0.14$, $P=0.71$, all interactions $P>0.51$). There was significant variation among clutches in the concentration of T (random effect $Z=4.52$, $P<0.001$), but the concentration of T in the first and the second egg was not related ($F_{1,\ 42}=0.60$, $P=0.44$).

Relative allocation of androgens

The relative allocation of A4 differed between years and the interaction between treatment and year was significant (Table 3; Fig. 4). Overall, second eggs received on average relatively more A4 than first eggs during a poor year than during a good year, and post hoc comparisons showed that, compared to the control group, females paired with experimental males decreased their relative allocation of A4 in second eggs during a poor year (2005, $t_{1,\ 10}=6.81$, $P=0.01$; Fig. 4) but not in a good year (2006, $t_{1,\ 38}=2.14$, $P=0.15$, Fig. 4).

Second eggs received relatively more T than first eggs during a poor year than during a good year (Table 3). There was a significant interaction between the treatment and the year, but this was because the control groups differed between years ($t_{1,\ 27}=11.53$, $P=0.001$). During a good year the relative concentration of T in control and experimental clutches did not differ (2006, $t_{1,\ 39}=2.45$, $P=0.12$), yet, during a poor year, experimental clutches had lower relative T concentration than controls, although this difference did not reach significance (2005, $t_{1,\ 10}=3.47$, $P=0.068$).

Discussion

This study suggests that maternal investment in eggs in the blue-footed booby is influenced by expected conditions during chick development (Mousseau and Fox 1998a, b; Verboven et al. 2003; Sandell et al. 2007). Females were

Table 3 Relative concentration of androstenedione (A4) and testosterone (T) in second eggs

Terms in model	*df*	*F*	*P*
A4 in second eggs			
Treatment	1, 46	2.46	0.12
Year	1, 46	9.20	0.004
Treatment × year	1, 46	8.95	0.004
T in second eggs			
Treatment	1, 47	0.77	0.38
Year	1, 47	4.08	0.049
Treatment × year	1, 47	5.70	0.02

Data were analysed with general linear mixed models. The models included the identity of the nest as a random factor (Wald *Z* tests for random effects: A4, $Z=3.64$, $P=0.0001$; T, $Z=4.85$, $P<0.001$). Sample size were 22 experimental and 28 control clutches for the analysis of A4 and 22 experimental and 29 control clutches for the analysis of T. The initial models included egg volume and egg mass

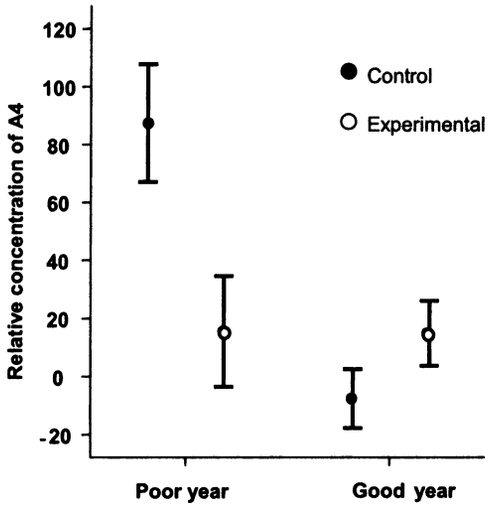

Fig. 4 Relative concentration of A4 in second eggs of control and experimental clutches during a year with poor breeding conditions (2005) and a year with good breeding conditions (2006). Relative concentration=([A4 in the second egg]×100 / [A4 in the first egg])−100. Values greater than zero indicate greater A4 concentration in second than in first eggs within a clutch

able to adjust egg size and content according to annual variation of breeding conditions and as a function of male feet colour, a trait that indicates male condition (Velando et al. 2006) and probably male feeding effort (Velando et al. 2005). After male feet colour was modified to a duller blue, females were able to rapidly adjust egg investment, not only through a decrease of second-egg volume, as found by Velando et al. (2006), but also by decreasing second-egg mass and the relative yolk concentration of A4 in second eggs during a poor year. Furthermore, females mated with experimental males deferred the laying of second eggs. The fact that females adjusted investment in eggs based on a male sexual trait supports the differential investment hypothesis (Burley 1986, 1988; Gil et al. 1999; Cunningham and Russell 2000; Groothuis et al. 2005). The results suggest that females are capable of fine-tuning various egg components depending on prevailing mate and environmental breeding conditions.

By decreasing egg mass and size, females may influence the embryo development, hatching success, size and weight at hatching, chick growth rate and survival (Williams 1994; Cunningham and Russell 2000; Christians 2002; Wagner and Williams 2007). In the blue-footed booby, egg size has a positive effect on hatching success and females seem to reduce their relative investment in second eggs compared to first eggs as the season advance and breeding conditions deteriorate (D'Alba and Torres 2007). In the present study, we found that, compared to eggs produced during a poor year, during a good year females produced heavier and larger first eggs, but relative to the first egg in the clutch, females produced heavier, but not larger, second eggs during a poor year, suggesting that females may vary their investment in eggs of different laying order according to annual breeding conditions. Moreover, when the male feet colour was modified to a duller blue, females decreased size and mass of second eggs. Thus, blue-footed booby females seem to anticipate rearing conditions and adjust investment accordingly using a combination of signals that may directly affect her own condition and access to food, such as annual variations of breeding conditions, and indirect signals, such as the male feet colour, a phenotypic trait that indicates male condition (Velando et al. 2006) and paternal investment (Velando et al. 2005).

In both years of study, females paired with experimental males delayed the laying of the second egg compared to control females. In a similar study, Velando et al. (2006) did not detect such an effect probably because in their study male feet colour manipulation was done 24 to 48 h after the first egg was laid, whereas in the present study, male feet colour was manipulated less than 24 h after the first egg was laid, giving females a longer period to vary investment in eggs and laying dates. The blue-footed booby is a species with aggressive sibling competition and facultative brood reduction (Drummond et al. 1986). The senior chick within a brood hatches on average 4 days before the junior chick, and this asynchrony at hatching is key in determining the output of sibling competition (Osorno and Drummond 1995). Experimental duplication of the hatching interval resulted in poorer growth, 50% increase of aggression and a greater mortality for junior chicks in the experimental group compared to the control group (Osorno and Drummond 1995). Thus, by postponing the laying of the second egg, females paired with males with duller blue feet are probably increasing the competitive asymmetries between brood mates and facilitating brood reduction.

Blue-footed booby females were able to transfer androgens in relation to expected breeding conditions. Females transferred more T to first eggs during a good year than during a year with poor breeding conditions, and for second-laid eggs, the absolute and relative concentration of yolk A4 and the relative concentration of yolk T were higher during a poor year compared to a good year. An increasing number of studies indicate that even minor variations of maternally derived yolk androgens can have important fitness effects on offspring (Schwabl 1996; Groothuis et al. 2005; Rubolini et al. 2006; Von Engelhardt et al. 2006; Tobler et al. 2007). For instance, in the black-legged kittiwake, *Rissa tridactyla*, concentrations in the yolk of A4 and IgGs are positively correlated (Gasparini et al. 2007), and in vitro experiments showed that, contrary to T, A4 enhances immune system (Yao and Shang 2005). Moreover, it has been suggested that higher concentrations of yolk A4 may be related to the production of highly competitive phenotypes in species with communally breed-

ing systems or colonial life (Cariello et al. 2006; Gil et al. 2007). Female condition, which partly depends on environmental breeding conditions, has been shown to influence deposition of androgens in eggs (Verboven et al. 2003; Sandell et al. 2007). Experimental manipulation of food availability previous to egg laying showed that females in good condition reduced the yolk androgen content of their eggs without altering offspring performance (Verboven et al. 2003) and modified the within-clutch pattern of yolk androgen allocation (Sandell et al. 2007). If increasing levels of yolk androgens have a positive effect on blue-footed booby offspring, by transferring more absolute and relative concentrations of A4 and more relative concentration of T into second eggs, females are probably increasing the probability of survival of second-hatched chick during a poor year.

Interestingly, when the feet colour of the mate was modified to a duller blue, females transferred relatively less A4 (but not T) to second eggs than to first eggs within the clutch during a poor year but not during a good year. In our study, first eggs in the control group had significantly more A4 than first eggs in the experimental group during the good year; thus, the interpretation of the results should be taken with caution. In principle, by analysing the relative allocation to first and second eggs within the clutch, we have taken into account this variation, yet more studies will be needed to confirm the results. At present, the results suggest that during a poor year females are probably increasing the probabilities of survival of second chicks by transferring more androgens to second eggs, but when mate feet colour deteriorates during a poor year females decrease the survival probability of second chicks by transferring relatively less A4 to second eggs. In a previous study in the blue-footed booby carried out at the end of an extended El Niño event, thus females were probably in poor condition and ecological prospects for incubating clutches and feeding chicks may also have been poor, no differential allocation of T and DHT according to laying order was detected; yet second eggs received marginally less A4 than first eggs (Drummond et al. 2008). Experimental studies have found that females modified the levels of yolk androgens according to mate attractiveness (Gil et al. 1999, 2004b; Tanvez et al. 2004; Loyau et al. 2007), although others have failed to find such an effect (Rutstein et al. 2004; Marshall et al. 2005). In our study, females were apparently able to rapidly modify the relative allocation of A4 between egg siblings according to a sudden deterioration of male feet colour during a poor year. During a year with poor breeding conditions, females are probably constrained to invest in eggs; hence, varying the yolk concentration of A4 may be an alternative to vary more expensive resources such as lipid-rich yolk component. Adjusting the relative concentration of yolk A4 between siblings may influence sibling asymmetries within a clutch (Schwabl 1997; Sockman et al. 2006; Sandell et al. 2007), which may be an adaptive strategy particularly in a siblicidal bird.

In this study, we show that blue-footed booby females are able to rapidly adjust investment in eggs according to variations in expected breeding conditions and in response to a dynamic trait such as male feet colour. In this species with a long period of parental care, reduction of paternal effort negatively affects the condition and, probably, future reproduction of females (Velando and Alonso-Alvarez 2003). Rearing a brood with a mate in poor condition during a poor breeding year is likely to be costly for females, either because females will have to compensate for a low paternal effort or because the reproductive value of the brood will decrease. Then, under these conditions, adjusting various egg components to facilitate brood reduction may be an adaptive female strategy. Moreover, this study suggests that mate evaluation and breeding decisions continue after pairing; therefore, males should maintain attractive feet colour beyond the courtship period and until laying is complete to assure paternity (Torres and Velando 2003) and to increase female investment in eggs.

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