Zur Homepage der Dissertation

Immunocompetence, testosterone and breeding success in red bishop (*Euplectes orix*)

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Gewidmet meinem Vater
ohne den ich es nie begonnen hätte
und für den ich es jetzt zu Ende bringe.

Horst Edler (1943 – 2005)

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General Introduction

Life-history, trade-offs and ecological immunology

Over the course of the last years, ecological immunology, the examination of the causes and consequences of variation in immune function in the context of evolution and of ecology, became one of the most active fields of research in both behavioural ecology and evolutionary biology (Sheldon and Verhulst 1996; Norris and Evans 2000; Schmid-Hempel 2003; Lee 2006). Against the background of the life-history theory (Stearns 1992), the investigation of trade-offs between investment in life-history components and investment in immune defence is a particularly interesting topic. Such a trade-off can be expected, if an individual's resources (e.g. food, energy) are limited and, consequently, resource-allocation becomes necessary. The costs of reproduction may then be paid in terms of energy being diverted away from body repair and maintenance and by reducing investment in immunocompetence (Sheldon and Verhulst 1996).

That immunocompetence (i.e. the ability of the immune system to cope with infection) is in fact costly to maintain has been shown in numerous studies (Lochmiller and Deerenberg 2000; Schmid-Hempel 2003; Martin et al. 2006), but the physiological mechanisms behind these costs and also the mechanisms mediating the trade-off between immune defence and reproduction are far from being completely understood. The immunocompetence handicap hypothesis (ICHH) proposed by Folstadt and Karter (1992) provides a possible explanation for these underlying physiological mechanisms via the hormone testosterone, that is thought to stimulate reproductive behaviour (e.g. development of secondary sexual ornaments, aggression toward competitors, courtship behaviour) on the one hand, and to reduce immune function on the other hand. While the positive relationship between testosterone levels and the expression of secondary sexual characteristics, as well as testosterone levels and reproductive success could be shown in numerous studies (e.g. Alatalo et al. 1996; Raouf et al. 1997; Peters et al. 2000; Gonzales et al. 2001), the support for the detrimental effect of testosterone on the immune system remains equivocal (Roberts et al. 2004). Some studies where able to link testosterone to a reduction in the immune response (Saino et al. 1995; Zuk et al. 1995; Verhulst et al. 1999; Duffy et al. 2000; Peters 2000), but others questioned an immunosuppressive effect of testosterone (Ros et al. 1997; Braude et al. 1999; Hasselquist et al. 1999),

or described testosterone as a modulator, being able to both increase and suppress the immune system (Olsen and Kovacs 1996). Thus, one of the central assumptions of the ICHH remains controversial.

The common phenomenon of extrapair mating in birds is another current topic in behavioural ecology that might benefit from the use of methods of immunological ecology. The two most widely researched hypotheses to explain the occurrence of promiscuity in birds, the 'good genes' and 'compatible genes' models (for a more detailed description of these and other hypotheses see the review by Griffith et al. 2002), both state that females gain indirect genetic benefits from extrapair copulations in terms of enhanced offspring quality of extrapair young compared with offspring from within-pair copulations. While some studies confirmed the prediction of these hypotheses by demonstrating that extrapair young fledged in better condition, survived better in cases of partial brood mortality, or had higher heterozygosity compared with within-pair young from the same brood (Kempenaers et al. 1997; Sheldon et al. 1997; Foerster et al. 2003), others failed to find significant differences between extrapair young and within-pair young (e.g. Whittingham & Dunn 2001; Schmoll et al. 2003). An individual's immunocompetence is a particularly well suited variable to determine and compare individual quality, as susceptibility to or resistance against parasites and pathogens is an important trait that determines individual quality and fitness, but studies investigating the proposed differences in immunocompetence between extrapair young and within-pair young from the same brood are scarce. Johnsen et al. (2000) were the first to compare individual quality in terms of cell-mediated immunity between maternal half-sibs from natural broods of bluethroats, *Luscinia svecica*. They found that extrapair young were more immunocompetent than their within-pair young half-sibs and their paternal half-sibs, a result meeting the predictions of the 'compatible genes' hypothesis. However, recent studies with essentially the same experimental design as in Johnsen et al. (2000) on the reed bunting, *Emberiza schoeniclus*, and the barn swallow, *Hirundo rustica*, found no significant difference in cell-mediated immune response between extrapair young and within-pair young (Kleven and Lifjeld 2004; Kleven et al. 2006), and the support for the 'good genes' and 'compatible genes' hypotheses thus remains equivocal.

Life-history theory predicts that reproducing individuals need to distribute limited resources over multiple conflicting activities. One of the trade-offs parents have to face in order to maximize their fitness (Stearns 1992), is between quantity and quality of their offspring, and increasing sibling competition in larger broods, has been shown to negatively impact immune function of individual nestlings, both in the wild (Saino et al. 1997; Hõrak et al. 1999; Chin et al. 2005) and under captive conditions (Naguib et al. 2004). In sexually dimorphic species, a negative impact of increased brood size should be more pronounced in the larger sex (Fargallo et al. 2002), due to greater energetic demands and/or food consumption of the larger individuals (Clutton-Brock et al. 1985; Wiebe and Bortolotti 1992; Anderson et al. 1993; Krijgsveld et al. 1998; Riedstra et al. 1998; Love et al. 2005) and the larger sex should consequently have a reduced immune function in large broods. Again, measurements of immune function can serve as a reliable indicator of individual quality, as it has been shown to be an important determinant of survival in nestling birds (Dawson and Bortolotti 1997; Christe et al. 2001; Merilä et al. 2001).

If different sexes in offspring yield different fitness benefits and/or entail different costs, parents should be selected to over-produce the more valuable sex, an idea first formulated by Trivers and Willard (1973) as sex-allocation theory. The correctness of this theory is still under discussion: While there are numerous studies lending support to the idea that parent birds can manipulate the sex ratio of their offspring, others were not able to find the expected bias in sex ratio, or even reported results with a skew opposite to the predicted trend (for reviews see Frank 1990; Hardy 1997; Hasselquist and Kempenaers 2002; Komdeur and Pen 2002; Krackow 2002; Ewen et al. 2004).

Objectives of the thesis

The goal of my thesis is to investigate the role of variation in immune function in relation to life-history theory, immunocompetence handicap hypothesis, 'good genes' and 'compatible genes' models, and sex-allocation theory in the red bishop (*Euplectes orix*), a polygynous, colonially breeding weaverbird species widespread in Sub-Saharan Africa. The red bishop is a sexually dimorph species, with the larger males changing into a conspicuous orange-scarlet and black breeding plumage during the breeding season, once they become sexually mature at the age of two. Females and non-breeding males have a dull brown plumage (Figure 1). The birds breed in reed beds or bullrush stands along rivers or small water bodies, where males in breeding plumage establish and defend small territories. Here, they build multiple nests that are in turn inspected and accepted by the females. The sexual ornamentation of the male birds and their strong territorial and display behaviour makes the red bishop an ideal species to test the predictions of the ICHH.

Figure 1 Male in breeding plumage (left) and female (right) red bishop (*Euplectes orix*).

Copulation is always initiated by the female, a clear indicator for female choice. Males do not guard their mates to assure paternity, but copulate frequently with them during the fertile period, that is shortly before and during the egg-laying stage. After the egg-laying phase, the female is usually ignored by the territory owner and is solely responsible for incubating and feeding nestlings. Red bishops are known to engage in frequent extrapair copulations, with an average of 30.5% nests containing extrapair young (Friedl and Klump 2002). Thus, they are an excellent model to study the predictions of the 'good genes' and 'compatible genes' hypotheses in maternal half-sibs. A more detailed description of red bishop breeding behaviour can be found in Friedl and Klump (1999; 2005) and Friedl (2004).

Chapter 1 presents an experimental study on male red bishops in an aviary in Oldenburg, Germany. Here, the testosterone mediated trade-off between reproductive effort and individual immunocondition, as proposed by the ICHH (Folstadt and Karter 1992), is tested by artificially raising testosterone levels in breeding males. The study employs multiple methods to assess general health and immune responsiveness. The following questions are addressed: 1. Does a high level of testosterone increase reproductive effort? 2. Is a high level of testosterone detrimental to general health and/or immunocondition? 3. Do innate and acquired components of the immune system react differently on raised testosterone levels?

Chapter 2 analyses differences in the T-cell-mediated immune response of within-pair young and extrapair young from mixed-paternity broods in the wild, using the phytohaemagglutinin-induced skin swelling response technique to measure immunocompetence. The study also addresses heritability of components of the immune system, by comparing offspring immunocompetence to immunological condition and health of their genetic fathers, and accounts for environmental influences on the immune system. The results are discussed in view of the different hypotheses aiming to explain the phenomenon of promiscuity in birds.

Chapter 3 deals solely with nestlings of the red bishop, especially the expected trade-off between quantity and quality of the offspring. Nestling immunological condition and viability, as assessed by multiple measurements, and their suspected relation to brood size, brood sex ratio, and environmental condition are investigated in a colony of free-ranging birds in South Africa. Results are discussed in perspective to the sex-allocation and life-history theory.

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CHAPTER 1

Effect of testosterone on reproductive behaviour and immunity in red bishops: an experimental test

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Abstract

According to the immunocompetence handicap hypothesis (ICHH), the hormone testosterone is thought to mediate a trade-off between investment into reproduction and immunological condition. In this study, we tested the ICHH in the red bishop (*Euplectes orix*), a polygynous weaverbird occurring in Sub-Saharan Africa. Males of this sexually dimorphic species show an elaborate courtship display to attract females and compete aggressively with other males for nesting sites in breeding colonies. We experimentally elevated testosterone levels in breeding male red bishops kept in an aviary in Oldenburg, Germany, under semi-natural conditions, with a subcutaneous implantation of testosterone-releasing pellets. We then compared behaviour, development of territory size, and immunological condition (as assessed through a white blood cell count) of the experimental group to a control group treated with placebos. In addition, we performed a repeated PHA-test to investigate the effect of testosterone on acquired immunity. Male individuals with elevated levels of testosterone succeeded in enlarging their territory and presented more courtship behaviour, while showing a decrease in health, expressed by an increased heterophil/lymphocyte ratio compared with the control group. Males of the control group showed an increase of the PHA-response in the second PHA-test, as expected under the assumption that repeated exposure to an antigen enhanced the immune response due to acquired immunity. However, males with experimentally increased testosterone levels did not show such an enhanced immune response in the second PHA-test, indicating that testosterone treatment directly or indirectly suppresses some component of the acquired immune response.

Introduction

The immunocompetence handicap hypothesis (ICHH) proposed by Folstad and Karter (1992) sees the hormone testosterone in the role of a double-edged sword. While testosterone is known to further the development of sexual traits and behaviour, in particular mating, territorial and mate-defence behaviour, and to amplify the probability of extrapair copulations (Wingfield et al. 1990; Collins et al. 1992; Smith 1995; Johnsen 1998; Vleck and Brown 1999; De Ridder et al. 2000; Peters et al. 2000; McDonald et al. 2001), testosterone is on the other hand also believed to be costly for the males. Proposed costs may take the form of an increase in male-male competition and therefore decreased survivorship (Dufty 1989), an increased metabolic rate with higher energy requirements (Røskaft et al. 1986), a suppression of spermatogenesis (Turek et al. 1976; Desjardins and Turek 1977), or a decrease in male parental behaviour (Wingfield et al. 1990; Ketterson et al. 1992; De Ridder et al. 2000; Peters et al. 2002; but see Lynn et al. 2002). The main drawback of testosterone in the view of Folstad and Karter (1992) is a detrimental effect on immunity, a proposition that received a lot of attention and was thoroughly studied, often with conflicting results. While some studies could link enhanced testosterone levels to a reduction in the immune response (Saino et al. 1995; Zuk et al. 1995; Verhulst et al. 1999; Duffy et al. 2000; Peters 2000; Alonso-Alvarez et al. 2007) or increased susceptibility to parasites (Duckworth et al. 2001; Deviche and Parris 2006), others questioned an immunosuppressive effect of testosterone (e.g. Ros et al. 1997; Braude et al. 1999; Hasselquist et al. 1999), or described testosterone as a modulator, being able to both increase and suppress the immune system (e.g. Olsen and Kovacs 1996; Lindström et al. 2001).

By controlled manipulation of the plasma levels of testosterone through testosterone implants, researchers can assess the individual level of immunosuppression and changes in reproductive behaviour caused by testosterone. Manipulation of plasma testosterone level was used in a number of studies to test the predictions of the ICHH. For example, by measuring antibody production initiated through an injection with sheep red blood cells, Evans et al. (2000) found that testosterone manipulations in house sparrows (*Passer domesticus*) led to immunosuppression, but suggested that the immunosuppressive effect was caused by covarying levels of corticosterone. Buchanan et al. (2003) reported a suppressive

effect of testosterone on the humoral immune response of house sparrows during the breeding season, but found no effect of testosterone on the cell-mediated immune response to a phytohaemagglutinin (PHA) injection; nor did testosterone levels appear to affect either white blood cell ratios or red blood cell counts. Experimentally elevated testosterone levels suppressed antibody production but did not significantly affect primary or secondary immune responses to PHA-injections in captive male dark-eyed juncos (*Junco hyemalis*), while cell-mediated immunity was suppressed in wild males (Casto et al. 2001). In a long term study on dark-eyed juncos (*Junco hyemalis carolinensis*), Reed et al. (2006) detected reduced survival rates in individuals with elevated testosterone levels and increased success in producing extrapair offspring. Contrary to the predictions of the ICHH, two studies on breeding blue tits (*Parus caeruleus*) reported no effect of testosterone manipulation on reproductive success and male-male competition (Foerster and Kempenaers 2004; 2005). Overall, studies that used artificial elevation of plasma testosterone levels provided mixed results in regard to the ICHH; thus, the assumption of the ICHH that testosterone acts as an immunosuppressive agent still remains equivocal (see review by Roberts et al. 2004).

We tested the proposed dualistic role of testosterone in the red bishop (*Euplectes orix*) by artificially raising testosterone levels in a group of males living under semi-natural conditions in an aviary. In our approach, we used both behavioural observations and measurements of immunological status, namely a differential white blood cell count (WBC), and the phytohaemagglutinin (PHA) skin swelling test. The latter test is routinely employed to measure T-cell-mediated immunocompetence (i.e. the ability to contain and prevent infection by a pathogen) in birds by injecting PHA subcutaneously and quantifying the resulting swelling response, usually 24h post-injection. The swelling is assumed to be a good indicator of the T-lymphocyte-mediated acquired immune system (Kennedy and Nager 2006) and fitness related traits (e.g. survival; Møller and Saino 2004), but a recent study by Martin et al. (2006a) demonstrated that the interpretation of the results of the PHA-test might have to be reconsidered. By following the immune cell infiltration after a PHA-injection over a time of 48h, Martin et al. (2006a) found at least part of the skin swelling owing to an inflammatory response, thus also involving components of the innate immune system. In consequence, they caution against the interpretation of a larger swelling as an indicator of a ‘better’ immunocompetence. To study true

acquired immunity with the PHA-test, one would have to compare subsequent exposures to the antigen to see if the immune system of an individual expresses a pathogen-specific acquired response (Kennedy and Nager 2006). We used repeated PHA-injections to assess if testosterone suppresses the acquired immune response, and if an elevated level of testosterone thus really presents a handicap for the immunocompetence.

The red bishop, our study species, is a polygynous and sexually dimorphic member of the weaverbird family occurring in Sub-Saharan Africa. Male red bishops show a striking courtship display to attract females and compete aggressively with other males for nesting sites in breeding colonies. Territorial males build their nests preferably in reed beds along rivers and small water bodies. As the number of nests a male individual can build in a given breeding season is positively related to its reproductive success (Friedl and Klump 1999), the acquisition of nesting sites is crucial (for a detailed account on red bishop behaviour see Friedl 2004; Friedl and Klump 1999; 2005).

If testosterone is furthering the reproductive success in male red bishops, we would expect individuals with an increased level of testosterone to be more aggressive, to enlarge their territory, and to spend more time building nests and trying to attract females. In addition, if testosterone has a suppressive effect on the immune system, as proposed by the ICHH, we would expect males with elevated levels of testosterone to experience a decline in immunological condition (as assessed through a white blood cell count), compared with males of the control group. Furthermore, if testosterone has a suppressive effect on acquired immunity, we would expect males with elevated levels of testosterone to show less increase of the PHA-response from the first to the second PHA-test (i.e. to show reduced acquired immunity) compared with males of the control group.

Material and Methods

Animals and experimental setup

The experiment took place between June and August 2005 in an aviary in Oldenburg, Germany, housing a breeding population of 43 female and 19 male red bishops in total. The birds were either caught in South Africa in 1994 and 2000 or hatched in the aviary. All birds in the aviary were individually marked with a numbered metal ring and 4 coloured plastic rings. The aviary consisted of two rows of six and four mesh-wire cages of 1.0 m x 2.0 m x 2.0 m (width x length x height) and a middle lane of approximately 2.0 m width, built in the middle compartment of a glassy greenhouse on the campus of the University of Oldenburg. The cages of each row were connected by holes (0.2 m x 0.2 m), roughly in the middle of each partition. Each cage was equipped with three horizontal wooden perches placed in the front, middle and back of the cage, and four vertical bamboo scaffoldings for nest building consisting of three bamboo poles each, located in the four corners of a cage. We placed coconut fibres as building material, a food dispenser with mixed bird seeds, and a water dispenser in the middle of each of the cages, to be easily accessible for all birds, and both water and bird seeds were provided *ad libitum* in all cages. The birds were kept under natural light (Oldenburg, 53°09' N, 8°13' E) all the time.

With a female-to-male-ratio of 2.0 (20 females and 10 males) in the six-cage row and 2.56 (23 females and 9 males) in the four-cage row, the two groups of red bishop were similar to each other in composition.

Male red bishops in breeding plumage defended parts of a cage or whole cages as territory to gain access to nest building opportunities in the scaffolding and on the perches. With food, water and building material readily available, building opportunities were the only limited resource. As the number of nests built by a male is an important determinant of male mating success (Friedl and Klump 1999), the acquisition of a large territory and more building opportunities is of crucial importance for individual males.

We divided the 14 males in breeding plumage randomly into two groups of seven individuals, an experimental group (Testosterone group) with artificially elevated levels of testosterone and a Control group implanted with placebos. The distribution of the animals in the aviaries was three Testosterone group and four

Control group individuals in the six-cage row aviary, and four Testosterone group and three Control group individuals in the four-cage row aviary.

We used testosterone-releasing pellets and control pellets (Innovative Research of America, Sarasota), that were inserted under the skin of the neck with a precision trochar, while the birds were anaesthetized with isoflurane (2% - 4%). The incision was closed with tissue adhesive. Testosterone pellets contained 2.5 mg of testosterone and were designed for constant release over a period of 90 days, while control pellets (placebos) consisted of pure binding material only. The pellets were designed to dissolve completely, thus making a second surgery to remove any remains unnecessary. Before surgery, we took blood samples (approximately 90µl) from the brachial vein of the right wing to determine individual levels of testosterone before the experiment, and made blood smears to assess immunological condition by counting the white blood cells (WBC). All parts of the experiment were approved by the appropriate authorities (Bezirksregierung Weser-Ems: 509.8-42502-35/10; Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit: 33.42502/27-01.02).

Behavioural observations

Focal observations of the Testosterone group and Control group took place on three consecutive days during the week before pellet injection (Week 0) and every other week for eight weeks after the injection (Week 2, Week 4, Week 6 and Week 8). As the testosterone pellets were made to last for 90 days, the duration of the whole experimental period fell well into the given time frame. Observations were done in the morning (between 07:00 and 10:00) during the time of highest activity, and lasted for 10 min per animal. The time slot in which an animal was observed changed on each of the three consecutive days according to a randomized block design. During the observations, temperatures in the aviary ranged from 21°C to 25°C.

The observer was hiding behind a screen and had no knowledge whether the focal individual was implanted with either a testosterone or a placebo pellet. Observations were recorded with a laptop computer, using a custom-made computer program (written by R.E.), that automatically calculated duration and frequencies of the different types of behaviour.

Behaviours we recorded were aggressive encounters (threat displays and fights with territory neighbours, other males in breeding plumage, or birds in non-breeding plumage), flight from opponent, courtship display, nest building, and absence from the territory. A detailed description of male behaviour in red bishops can be found in Friedl (2004).

We calculated means for the three consecutive observation days for both frequency and duration of behaviours and used them in all further analyses. During each focal observation, the size of the territory was determined as the proportion of a cage defended by the focal individual. Territory sizes for each week were calculated as mean of the three daily values per week.

On the last observation day of each week we caught all 14 males and took a blood sample (approximately 90 µl) within three minutes of catching. In addition, a blood smear was made and each individual was weighed with a Pesola spring balance to the nearest gram. Tarsus length was measured to describe skeletal growth using callipers (± 0.25 mm) in Week 0. As weight and tarsus length were significantly correlated (Week 0: $R^2 = 0.474$, $F_{1,12} = 12.73$, $P < 0.01$), we calculated a body condition index for each of the observation periods as the residuals from the linear regression of body weight on tarsus length.

Directly after weighing and measuring in Week 0, the testosterone pellets and the placebos were implanted, while in Week 2 and Week 6 the PHA-test took place after weighing.

Hormone analysis

Plasma was extracted from the blood samples after centrifugation and stored in a freezer (-20°C) until later analysis. Testosterone concentrations of each sample were determined with a radioimmunoassay following Goymann et al. (2006). Plasma samples (mean ± SD: 41.5 ± 20.5 µl) were extracted twice with dichloromethane, resulting in an average extraction recovery of 86.0 ± 3.0% (mean ± SD) for ^{3}H-testosterone added prior to extraction. The lower detection limit of the assay was 73 pg/tube, resulting in a range of lower detection limits between 39 - 169 pg/ml (depending on the amount of plasma available and the respective extraction recovery). To minimize variation, all samples were analyzed in one assay with an intraassay variation coefficient of 4.5%. Since the testosterone antibody (Esoterix

Endocrinology, Calabasas Hill, CA, USA) showed significant cross reactions with 5a-dihydrotestosterone (44%), our testosterone measurements may include a proportion of this other androgen.

PHA-test

The PHA-test to assess immunocompetence was conducted in Week 2 and Week 6 after the injection, following a test protocol based on the procedures described in Smits et al. (1999). We used a water resistant pen to mark a primary on the left wing close to the outer joint. The thickness of the patagium under the marked primary was measured to the nearest 0.01 mm using a pressure-sensitive digital micrometer (Mitutoyo Digimatic model 573-191, Mitutoyo, Tokyo). Twenty microgram (20µg) of PHA (L-9132, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 30 µl of phosphate buffered saline (PBS) were then injected subcutaneously into the patagium. The birds were recaught after 24 ± 1 h to measure the skin swelling at the injection site. We used the difference between the two measurements in all subsequent calculations.

As all measurements of the skin swelling were taken three times, repeatability was calculated using the methods described by Lessells and Boag (1987). Both repeatability before and 24 h after the PHA-injection were very high in both Week 2 and Week 6 (all $P < 0.01$), and the mean of the three measurements was used in further analysis.

White blood cell count

The basis of the vertebrate immune system is formed by leucocytes, whose main function is to protect the organism against various pathogens. Avian heterophils are non-specific phagocytosing immune cells that enter the tissue during the inflammatory response, and an increased number of heterophils signals infectious diseases and elevated stress-hormone levels (e.g. Campbell 1995; Maxwell and Robertson 1998; Samour 2000). Lymphocytes are responsible for the specific immune responses and decreased lymphocyte numbers are indicative of stress and immunosuppressive conditions (e.g. Campbell 1995; Siegel 1995; Samour 2000).

The proportion of different leucocytes is described by a white blood cell count (WBC). The WBC can be influenced by a variety of stressors, such as infectious diseases, parasite infection, malnutrition and socio-psychical disruption. In particular the heterophil/lymphocyte ratio (H/L-ratio), a measurement commonly used to assess stress levels and health state of birds (e.g. Gross and Siegel 1983; Gylstorff and Grimm 1998; Maxwell and Robertson 1998; Samour 2000), responds to those stressors with a progressive increase (Ots and Hõrak 1996; Hõrak et al. 1998; Birkhead et al. 1999; Ilmonen et al. 2003). Since stress is known to suppress immune functions (Sapolsky 1992; Apanius 1998) a high H/L-ratio is indicative of immunosuppressive conditions with a concomitant increase in susceptibility to infections (Siegel 1995; Ots et al. 1998) or parasitization (Edler et al. 2004). WBC and H/L-ratio have been previously used successfully to assess immunological status in red bishops (Edler et al. 2004; Friedl and Edler 2005)

To obtain the WBC, we made blood-smears every other week from Week 0 to Week 8. The air-dried blood smears were stained with Wright's stain and examined with a microscope under 630x magnification. In areas where blood cells formed a monolayer we counted the number of erythrocytes and leucocytes, which where further subdivided in lymphocytes, monocytes, basophilic granulocytes, eosinophilic granulocytes and heterophilic granulocytes. We examined neighbouring consecutive microscopic fields at a right angle to the direction of the blood smear. When we reached the border of the blood smear, the next field counted was always the one to the right. Thereby we avoided double counts of microscopic fields. Every smear was examined until we counted a total of 100 leucocytes. Based on these counts the H/L-ratio was calculated. The person conducting the WBC had no knowledge of the specific treatment a bird was submitted to. Repeatability of this method on red bishops has been previously tested and was found to be high (all $r > 0.6$, all $P < 0.05$). The repeatability for relative lymphocyte counts, relative heterophil counts, and the H/L-ratio was particularly high (all $r > 0.9$, all $P < 0.001$; Edler et al. 2004; Friedl and Edler 2005).

Statistical analysis

We used repeated measures ANOVAs to test for influences of treatment (Control group or Testosterone group) on testosterone level, territory size, PHA-reaction, body condition index and H/L-ratio. For all analyses, testosterone concentrations were transformed by natural logarithm to approximate normality. However, in the figures and in the text we present back-transformed data to increase readability. The two different rows of the aviary were at first included in the ANOVA as independent factor, but omitted in the final model, as they did not have a significant effect on any of the dependent variables. We used t-tests to compare the Control group and the Testosterone group in the single weeks of observations after the implantation. Again, location in the aviary was omitted from the final models.

Behavioural data for the treatment groups was analyzed with a repeated measures ANOVA. Flight from opponent and absence from the territory was excluded from the analysis, as these behaviours could only be observed in a small percentage of observations (flight < 19%, absence < 6%). Courtship behaviour with and without female presence was combined into one variable, as were all behavioural traits regarding aggression. Frequencies (mean occurrence of behaviour during 10 minutes) were used for all behavioural data for comparisons between treatment groups.

Statistics were performed using the R statistical package 2.2.0 (R Development Core Team 2005). Quoted significance values are for two-tailed tests. The level of significance was set as $P < 0.05$.

Data used in the study can be found in Appendix A.

Results

Comparisons of groups before implantation

Before the treatment, males that were assigned to the Testosterone and the Control group did not differ in testosterone level (t = 0.019, P = 0.985), territory size (t = -0.117, P = 0.909), body condition index (t = -1.937, P = 0.077), or H/L-ratio (t = -1.373, P = 0.195). In addition, there was no difference in frequencies of aggressive behaviour (t = 0.095, P = 0.926) or nest building behaviour (t = 0.731, P = 0.479), but Testosterone group males tended to display more courtship behaviour (t = -2.007, P = 0.068). We repeated these tests to compare males from the two aviaries before the implantation, but no differences were found (all P > 0.05; statistics not shown here).

Testosterone levels after implantation

Testosterone levels after implantation were – naturally – much higher in the Testosterone group than in the Control group ($F_{1,12}$ = 16.33, P = 0.002), with a significant interaction between treatment and week ($F_{3,36}$ = 20.16, P < 0.001). As illustrated in Figure 1, testosterone levels were significantly different between the two treatment groups during Week 2 (t = -9.066, P < 0.001), Week 4 (t = 3.572, P = 0.004), and Week 6 (t = -2.219, P = 0.047), but not during Week 8 (t = -1.758, P = 0.104).

The highest levels of testosterone were measured in Week 2 in the Testosterone group (mean [+SEM; -SEM]: 3.40 [0.95; 0.74], range 1.10 - 8.25 ng/ml). While testosterone levels in the aviary before implantation (mean [+SEM; -SEM]: 0.43 [0.09; 0.07] ng/ml), range 0.19 - 1.64 ng/ml) were similar to the levels found in a natural population of red bishops in the Addo Elephant National Park, South Africa (N = 18; mean [+SEM; -SEM]: 0.51 [0.07; 0.06] ng/ml, range 0.25 - 1.69 ng/ml; Alice Aidnik, Wolfgang Goymann and Thomas W. P. Friedl, unpublished data), levels of the Testosterone group males two and four weeks after implantation were significantly higher than in free-living males from the South African population (Week 2: t = 6.782, P < 0.001; Week 4: t = 3.181, P = 0.004).

Behaviour after experimental treatment

Testosterone group males did not express more nest building behaviour after experimental treatment than Control group males ($F_{1,12}$ = 2.478, P = 0.141). Testosterone group males tended to be more aggressive overall ($F_{1,12}$ = 4.720, P = 0.051), but there was no significant interaction between treatment and week ($F_{3,36}$ = 0.580, P = 0.632). In addition, Testosterone group males showed more courtship behaviour overall ($F_{1,12}$ = 6.338, P = 0.027), but again there was no significant interaction between treatment and week ($F_{3,36}$ = 1.393, P = 0.261). For details on behavioural data see Table 1.

Table 1 Frequencies of behaviours recorded during focal observations in the aviary. For each animal we calculated the means of the frequencies of behaviours shown in 10 minutes observation time on three consecutive days.

		Testosterone group		*Control group*	
	Behaviour	*Mean ± SEM*	*Range*	*Mean ± SEM*	*Range*
Week 0	*Nest building*	2.05 ± 0.50	(0.00; 4.33)	2.81 ± 0.91	(0.00; 7.67)
	Aggressive behaviour	30.29 ± 1.70	(21.67; 33.67)	21.67 ± 2.67	(14.33; 30.33)
	Courtship behaviour	12.52 ± 2.78	(0.33; 20.67)	6.38 ± 1.29	(2.00; 11.00)
Week 2	*Nest building*	3.76 ± 0.98	(0.33; 8.33)	2.14 ± 0.97	(0.33; 7.67)
	Aggressive behaviour	28.52 ± 2.41	(20.00; 37.67)	17.90 ± 3.49	(4.67; 30.00)
	Courtship behaviour	12.43 ± 1.62	(7.33; 19.00)	5.81 ± 2.10	(0.00; 17.33)
Week 4	*Nest building*	5.05 ± 0.91	(2.67; 9.00)	2.71 ± 0.69	(0.33; 5.00)
	Aggressive behaviour	23.43 ± 2.27	(15.00; 32.00)	16.43 ± 2.94	(7.00; 29.00)
	Courtship behaviour	8.14 ± 2.17	(3.33; 19.33)	4.57 ± 1.19	(0.33; 9.00)
Week 6	*Nest building*	1.48 ± 0.28	(0.33; 2.67)	0.90 ± 0.56	(0.00; 4.00)
	Aggressive behaviour	12.52 ± 2.94	(4.00; 27.33)	6.57 ± 1.46	(3.67; 15.00)
	Courtship behaviour	3.81 ± 1.39	(0.67; 10.67)	1.14 ± 0.28	(0.00; 2.33)
Week 8	*Nest building*	0.71 ± 0.37	(0.00; 2.67)	1.14 ± 0.45	(0.00; 3.00)
	Aggressive behaviour	9.67 ± 3.70	(1.00; 28.33)	7.48 ± 2.03	(1.00; 15.67)
	Courtship behaviour	3.43 ± 1.68	(0.00; 10.67)	2.10 ± 0.89	(0.00; 6.67)

Territory size, immunological condition, and body condition after experimental treatment

During treatment, territory sizes differed significantly between males of the Testosterone group and those of the Control group ($F_{1,12}$ = 9.584, P = 0.009). This effect differed among the weeks as shown by the significant interaction between treatment and week ($F_{3,36}$ = 3.984, P = 0.015). Testosterone group males started to increase their territory size at the cost of Control males in Week 2 (t = -2.242, P = 0.045), the difference peaks in Week 4 (t = -4.618, P = 0.001) and decreased again in Week 6 (t = -2.710, P = 0.019). The difference in territory size was no longer apparent in Week 8 (t = -0.515, P = 0.616; Figure 1).

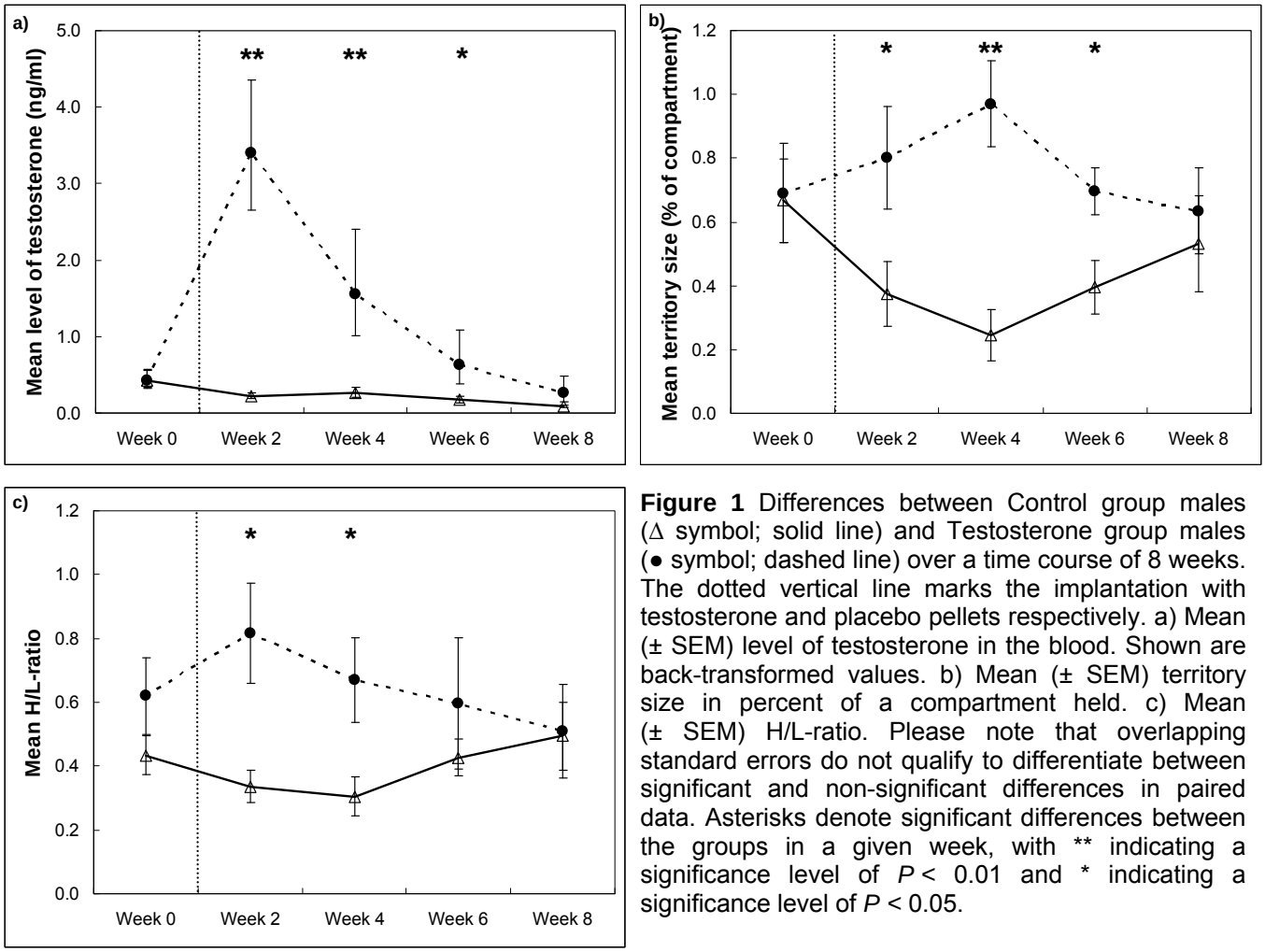

Figure 1 Differences between Control group males (Δ symbol; solid line) and Testosterone group males (● symbol; dashed line) over a time course of 8 weeks. The dotted vertical line marks the implantation with testosterone and placebo pellets respectively. a) Mean (± SEM) level of testosterone in the blood. Shown are back-transformed values. b) Mean (± SEM) territory size in percent of a compartment held. c) Mean (± SEM) H/L-ratio. Please note that overlapping standard errors do not qualify to differentiate between significant and non-significant differences in paired data. Asterisks denote significant differences between the groups in a given week, with ** indicating a significance level of P < 0.01 and * indicating a significance level of P < 0.05.

Immunological condition as assessed by the H/L-ratio takes a similar course. While the H/L-ratio of Testosterone group males was not significantly higher than that of Control group males overall after the experimental treatment ($F_{1,12}$ = 2.985, P = 0.110), the interaction between status and week was significant ($F_{3,36}$ = 3.388, P = 0.028). There was a pronounced peak in Week 2 (t = -2.898, P = 0.013) and Week 4 (t = -2.525, P = 0.027) with significant differences between the groups, indicating stress and immunosuppressive conditions (Figure 1). This was caused by an increase in heterophilic granulocytes in Testosterone group males compared with Control group males, which was significant in Week 2 (t = -2.972, P = 0.012), and nearly significant in Week 4 (t = -2.168, P = 0.051). Other parameters taken with the WBC (number of monocytes, basophilic granulocytes, and eosinophilic granulocytes) did not differ between the groups or over time (statistics not shown here).

The body condition index, another measure of health status, did not differ between experimental and control group ($F_{1,12}$ = 3.237, P = 0.097) and there was no significant interaction between treatment and week ($F_{3,36}$ = 0.241, P = 0.867).

Responses to an immune challenge

The reaction to an immune challenge with PHA did not differ between the groups in Week 2 (t = 0.644, P = 0.532) or Week 6 (t = -1.241, P = 0.238). Within the groups, there were no changes between Week 2 and Week 6 for Testosterone group males (t = -0.184, P = 0.860), but Control group males increased their response to the immune challenge from Week 2 to Week 6 (t = -2.924, P = 0.026). This result is indicative of true acquired immunity in the Control group (Figure 2).

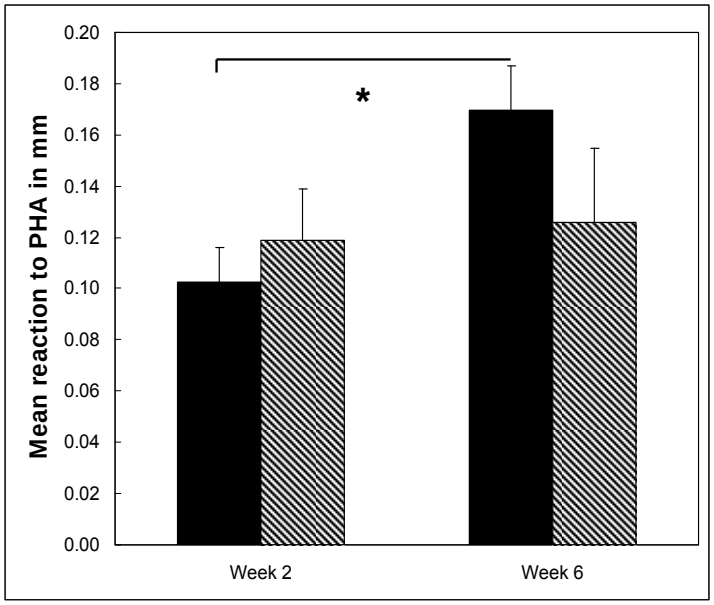

Figure 2 Reaction to an immune challenge (mean ± SEM) two and six weeks after implantation with testosterone and placebo pellets respectively. Solid bars represent Control group males, hatched bars Testosterone group males. The asterisk denotes a significance level of $P < 0.05$.

For the Testosterone group, there was a significant negative correlation between the immune response to the first PHA-injection and the immune response to the second PHA-injection (Spearman's correlation: r_S = -0.793, P = 0.033), indicating a possible trade-off between initial and secondary immune response. No such correlation was found for the Control group (Spearman's correlation: r_S = -0.043, P = 0.928).

Discussion

In the present study, we found a significant increase in territory size in testosterone implanted male red bishops. These males showed significantly more courtship behaviour and expressed a tendency to be more aggressive than males implanted with a placebo. Thus, our results add to the evidence that testosterone plays a crucial role in the expression of sexual traits and behaviour in birds (see Roberts et al. 2004 for a review). In addition, males with testosterone implants failed to show an increase in the answer to a repeated exposure to an immune challenge with PHA, while males with placebo implants showed a stronger immune response on the second exposure, indicative of true acquired immunity. This result can be interpreted as supportive evidence for the immunosuppressive character of testosterone as proposed by the ICHH.

While our data could indicate a direct immunosuppressive character of testosterone, as proposed in the original ICHH (Folstadt and Karter 1992), it remains possible that a more complex, indirect mechanism may be involved in mediating the immunosuppressive effects of testosterone. Artificially elevated levels of testosterone have been shown to covary with levels of plasma corticosterone (i.e. Klukowski et al. 1997; Duffy et al. 2000; Evans et al. 2000; Casto et al. 2001; Owen-Ashley et al. 2004), which is known to be immunosuppressive in birds and mammals (i.e. Olsen and Kovacs 1996; Buchanan 2000; Evans et al. 2000). While this idea of an indirect pathway gained some support from recent works (Buchanan et al. 2003; Owen-Ashley et al. 2004; Mougeot et al. 2005), the exact physiological mechanism of immunosuppression via corticosterone remains unclear (for a review see Martin et al. 2006b). In addition, some studies presented results conflicting with the idea of an indirect pathway of immunosuppression initiated by testosterone. In an experiment conducted on free-living house sparrows, *Passer domesticus*, testosterone implantation did not result in raised plasma corticosterone (Hegner and Wingfield 1987), a result later confirmed by Greenman et al. (2005). A study by Duckworth et al. (2001) on house finches, *Carpodacus mexicanus,* supports a direct relationship between testosterone and disease resistance. Consequently, it still remains unclear whether testosterone acts as an immunosuppressant directly or indirectly. However, as pointed out by Folstadt and Karter (1992), biologically active substances other than testosterone could mediate the ICHH; thus, the predictions of the ICHH hold true

independently of testosterone having a direct immunosuppressive effect or acting as a mediator.

Significant differences in behaviour associated with breeding success, namely the acquisition of larger territories, and an increase in courtship behaviour, could only be observed during the time when testosterone levels in the two treatment groups were also significantly different. The same was true for the H/L-ratio, which differed significantly between treatment groups in Weeks 2 and 4, while testosterone in the Testosterone group males was at its highest levels. Thus, there is a clear temporal connection between testosterone levels and behavioural and immunological changes.

When testing the supposed immunosuppressive effect of testosterone in birds, the PHA-test is arguably the most often employed test in the field of immunoecology (Martin et al. 2006a), mainly because of its simplicity to use in the field (Smits et al. 1999). However, care has to be taken when it comes to the interpretation of the results of PHA-challenges. According to a recent study on house sparrows, *Passer domesticus* (Martin et al. 2006a), a bigger swelling is not necessarily equal to a 'better' T-cell-mediated immunity, as part of the swelling can be caused by components of the non-specific components of the innate immune system. Therefore, repeated exposures to the novel antigen are required to safely interpret the results of an immune challenge with PHA in regard to an acquired immune response (Kennedy and Nagel 2006). We are aware of only two studies on birds that employed a repeated exposure of the same individual to an immune challenge with PHA. A significantly stronger immune response on the second treatment, similar to our results, was found by Casto et al. (2001) in a study on dark-eyed juncos, *Junco hyemalis*, while Alonso-Alvarez and Tella (2001) report a non-significant increase in the strength of the immune answer in the control group during a food deprivation experiment with yellow-legged gulls, *Larus cachinnans*. These data indicate the ability of the immune system to build up a learned response to the PHA-challenge. Interestingly, Alonso-Alvarez and Tella (2001) found a significant decline in the strength of the immune response in birds from both experimental groups that were either fed on one-third of the mean dietary intake of the control group or completely deprived of food. Casto et al. (2001) also used testosterone and placebo implants on captive animals, similar to our design, but in contrast to the results of our study they found an increase in both treatment groups, when comparing the strength of the

immune response to primary and secondary exposure of PHA. A reason for them failing to find a suppressive effect of testosterone on the acquired immune response might be found in a subtle difference in the experimental design: while they removed the implants three weeks before the second exposure to PHA, our experimental animals where still implanted and therefore under a constant exposure to elevated testosterone levels.

In this study, we found a negative effect of testosterone on acquired immunity using a combination of experimental elevation of testosterone levels and repeated PHA-tests on birds kept under controlled conditions in an aviary. It might be very difficult to detect such an effect in free-living birds, where the interpretation of PHA-results is complicated by environmental masking effects on individual quality. A seasonal effect on the results of the PHA-test was recently published by Garvin et al. (2006) in a study on common yellowthroats, *Geothlypis trichas*. The immune response of extrapair young was stronger than that of within-pair young, but only in the colder of two study seasons. Low temperature is likely to reduce food abundance, an environmental factor that potentially has an effect on the immune response (Lifjeld et al. 2002). In a study on free-living red bishops, we detected a similar environmental effect on nestling immune response, with within-pair young having a significantly stronger immune response than extrapair young, but only in the warmer of two seasons (Edler and Friedl, in press). Therefore, the interpretation of results on immunity obtained with the PHA-test performed outside of a controlled laboratory environment in the field needs to take possible environmental influences into account.

A high H/L-ratio, as found in the Testosterone group in Weeks 2 and 4, is indicative of immunosuppressive conditions (Siegel 1995; Ots et al. 1998). Here, the increase in the H/L-ratio is caused by an increase in heterophilic granulocytes, as is common during periods of mild to moderate physiological stress (Ewenson et al. 2001). In birds, cellular immunity is usually mediated by heterophilic granulocytes and lymphocytes (Figuerola et al. 1999), where heterophils provide a first line of defense against bacterial infections (Grasman 2002). Thus, the increase in heterophils and therefore the differences in the H/L-ratio between males of the Testosterone group and the Control group could be interpreted as an additional indicator of the direct or indirect immunosuppressive properties of testosterone.

The testosterone level in Testosterone group males increased as expected, but the subsequent decline of the testosterone level until there was no significant difference between Testosterone group and Control group in Week 8 came as a surprise, considering that the pellets used in our test were supposed to have a constant release of testosterone over a period of 90 days. Unfortunately, it seems that the release rate of the pellets was not as constant as assumed by the manufacturer, generating higher than expected testosterone levels in Weeks 2 and 4. The highest testosterone level in the Control group was 1.64 ng/ml, which is similar to the highest value of 1.70 ng/ml found in a wild population of red bishops in the Addo Elephant National Park, South Africa (Alice Aidnik, Wolfgang Goymann and Thomas W. P. Friedl, unpublished data). In the Testosterone group, the highest levels measured were 8.25 ng/ml in Week 2 (mean [+SEM; -SEM]: 3.40 [0.95; 0.74]) and 7.59 ng/ml in Week 4 (mean [+SEM; -SEM]: 1.55 [0.85; 0.55]). We lack information whether naturally occurring testosterone titres in male red bishops can reach similar high levels as observed in some males of the Testosterone group. The highest natural testosterone level found so far for red bishops was 3.52 ng/ml, obtained from a breeding male in Tanzania (Goymann et al. 2004 and Wolfgang Goymann, unpublished data). However, given that the blood samples both from the population in South Africa and from the population in Tanzania were taken from males well after territorial establishment at the beginning of the breeding season, and that testosterone levels in other species have been shown to be highest during territory establishment early in the season (i.e. Wingfield 1984; Johnsen 1998; Wingfield et al. 2001; Jawor 2007), the observed maximum level of 3.52 ng/ml might not be the maximum physiological level for red bishops. Nevertheless, unless there are no confirmed data on testosterone levels in the range of 8 ng/ml or higher for red bishop males in natural populations, we cannot completely exclude the possibility that the testosterone levels observed in some males of the Testosterone group in Week 2 and 4 are supra-physiological for this species, and that the immunosuppressive effects of testosterone found in this study are only apparent when the hormone circulates in concentrations above the physiological level.

In conclusion, our study provides support for the dualistic role of testosterone, which is the fundament of the ICHH. On the one hand, testosterone increases the ability of male red bishops to compete for territories, thus increasing their chances to attract female pairing partners. On the other hand, males with experimentally

elevated levels of testosterone experience suppression of the acquired immune system, either directly by testosterone acting as an immunosuppressant, or indirectly by testosterone mediating an immunosuppressive effect via a more complex physiological mechanism. While the results are in accordance with the propositions of the ICHH, the underlying physiological mechanism for an immunosuppressive effect of testosterone on the secondary (i.e. acquired) immune response remains to be investigated.

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CHAPTER 2

Within-pair young are more immunocompetent than extrapair young in mixed-paternity broods of the red bishop

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Abstract

Two competing hypotheses are commonly used to explain matings with multiple males in female birds. The 'genetic benefit' model sees extrapair copulations as a means to enhance offspring viability, while the 'fertility insurance' hypothesis states that females copulate with multiple males to guarantee fertilisation of eggs. According to the 'genetic benefit' hypothesis, extrapair young are expected to have a genetic advantage over their maternal half-sibs raised in the same brood. A quantifiable measure for offspring quality is the immunocompetence, the ability of an individual to cope with infections. In this study, we used the phytohaemagglutinin induced skin swelling response technique to test whether the cell-mediated immune reaction differs between extrapair and within-pair nestlings from mixed-paternity broods in the polygynous red bishop, *Euplectes orix*. The strength of the nestlings' immune response was positively linked to general health status and immunological condition of their genetic fathers, indicating a heritable component of nestling immunocompetence. We found that extrapair young were actually significantly less immunocompetent than within-pair young from the same nest, a result that does not lend support to the 'genetic benefit' hypothesis. We suggest that female red bishops mated to successful high-quality males engage in extrapair copulations to insure against temporary infertility in their social mates caused by sperm depletion because of frequent copulations with their multiple female partners. This result, however, was significant only in the hotter of two years, indicating that environmental factors can obscure variation in nestling fitness arising from female extrapair mating behaviour.

Introduction

The widespread phenomenon of promiscuity in bird species with pair-bonds is far easier to explain for the male perspective than for the female perspective. Through successful extrapair copulations, males can sire additional offspring at the cost of the female's social mate. For females, the reasons to engage in matings with multiple males are less obvious, and several possible benefits for the female have been proposed (e.g. Westneat et al. 1990; Birkhead and Møller 1992; Jennions and Petrie 2000; Griffith et al. 2002; Westneat and Stewart 2003). The 'good genes' and 'compatible genes' models (for a more detailed description of these models see the review by Griffith et al. 2002) both state that females gain indirect genetic benefits from extrapair copulations in terms of enhanced offspring viability of extrapair young compared with offspring from within-pair copulations (within-pair young). Other possible explanations for female birds engaging in extrapair copulations concentrate on non-genetic benefits such as insurance against (temporary) infertility of the social mate and material benefits provided by the extrapair male in terms of courtship feeding, nest defence, or paternal care.

In most passerine species females have the control over copulations (Birkhead and Møller 1993) and in many species they even actively seek extrapair copulations (e.g. Kempenaers et al. 1992; Sheldon 1994; Gray 1996; Otter et al. 1999). In such female-driven mating systems, the choice of a social mate might be based on material benefits (e.g. nesting sites or high-quality territory) or the lack of a better option (e.g. due to restrictions of choice imposed by social monogamy; Møller 1992), while a copulation with an extrapair male usually provides no material benefit besides sperm (Birkhead and Møller 1992). Therefore, one would expect females to engage in extrapair copulations with males whose genes enhance the genetic quality of the offspring (Andersson 1994), and there is empirical evidence, provided by observational studies, that females selectively solicit extrapair copulations with males of higher quality than their social mates (e.g. Smith 1988; Houtman 1992; Kempenaers et al. 1992; Graves et al. 1993). More support for the 'good genes' hypothesis comes from studies using molecular techniques for paternity determination, which show that extrapair males are of higher quality than the males they cuckolded (e.g. Kempenaers et al. 1992; Weatherhead and Boag 1995;

Hasselquist et al. 1995; Sundberg and Dixon 1996; Kempenaers et al. 1997; Møller and Tegelström 1997; Saino et al. 1997; Sheldon et al. 1997; Otter et al. 1998).

Unfortunately, empirical evidence for the 'good genes' hypothesis is scarce when it comes to studies investigating genetic benefits for the offspring. One of the main difficulties in the design of those studies is to single out the contribution of parental genes to offspring quality, because offspring quality may also be determined or masked by environmental and maternal factors (Kirkpatrick and Ryan 1991; Szép and Møller 1999). As pointed out by Griffith et al. (2002), a simple and effective approach to circumvent this problem, and at the same time a crucial test both for the 'good genes' and the 'compatible genes' hypothesis, is to compare maternal half-sibs from natural broods containing extrapair young and within-pair young. However, up to now surprisingly few studies have been conducted that investigated whether extrapair young differ in quality from their maternal half-sibs raised in the same brood. While some of these studies indeed showed that extrapair young fledged in better condition, survived better in cases of partial brood mortality, or had higher heterozygosity compared to within-pair young from the same brood (Kempenaers et al. 1997; Sheldon et al. 1997; Foerster et al. 2003), others failed to find quality differences between extrapair young and within-pair young (e.g. Krokene et al. 1998; Strohbach et al. 1998; Whittingham and Dunn 2001a; Schmoll et al. 2003).

Susceptibility to or resistance against parasites and pathogens is an important trait that determines individual quality and fitness; therefore, an individual's immunocompetence is a particularly well suited variable for determining and comparing individual quality. A standard technique to determine an aspect of individual immunocompetence is to quantify cell-mediated immunity by measuring the phytohaemagglutinin (PHA) induced skin swelling response (Cheng and Lamont 1988; Smits et al. 1999). The PHA-response in bird nestlings has been found to have a genetic component (e.g. Brinkhof et al. 1999; Soler et al. 2003), and also to be associated with fitness-related traits (e.g. Birkhead et al. 1999; Møller and Saino 2004; Chin et al. 2005). Johnsen et al. (2000) were the first to make use of the PHA-assay to compare individual quality in terms of cell-mediated immunity between maternal half-sibs from natural broods of bluethroats, *Luscinia svecica*, containing both extrapair young and within-pair young. They found that extrapair young were more immunocompetent (i.e. had a stronger T-cell-mediated immune response) than their within-pair young half-sibs, a result meeting the predictions of the 'good genes'

hypothesis. Extrapair young also showed a stronger immune response than their paternal half-siblings reared in the biological father's own nest, as would be expected if females seek extrapair fertilizations to obtain compatible genes, rather than 'good genes' per se. However, recent studies with essentially the same experimental design as in Johnsen et al. (2000) on two other socially monogamous passerines, the reed bunting, *Emberiza schoeniclus*, and the barn swallow, *Hirundo rustica*, found no significant difference in cell-mediated immune response between extrapair young and within-pair young (Kleven and Lifjeld 2004; Kleven et al. 2006).

A difficulty in detecting benefits of extrapair copulations to females in terms of good genes or compatible genes is that such genetic benefits can be masked by the environmental context and might only be detectable under poor environmental conditions, as demonstrated by Schmoll et al. (2005) in the coal tit, *Parus ater*. Likewise, a seasonal effect on the results of the PHA-test was recently published by Garvin et al. (2006) in a study on common yellowthroats, *Geothlypis trichas*. The immune response of extrapair young was stronger than that of within-pair young, but only in the colder of two study seasons. Low temperature is likely to lower food abundance, an environmental factor that has been shown to have an effect on the immune response (Lifjeld et al. 2002). Thus, studies that make use of PHA should include measurements of environmental conditions, and, if data were obtained in more than one year, control for seasonal effects.

An alternative for the 'good genes' or the 'compatible genes' hypothesis is known as the 'fertility insurance' hypothesis. It is easy to see why permanent infertility in males should be strongly selected against in nature. Temporary infertility, however, might occur in natural populations, especially in polygynous mating systems, where male mate guarding is absent and male paternity-insurance behaviour usually takes the form of repeated copulations (Birkhead and Møller 1992). Given that sperm numbers in ejaculates of birds have been shown to decline after frequent copulations (Birkhead 1991; Birkhead et al. 1994; 1995; Westneat et al. 1998), successful males mated to many females simultaneously may suffer from temporary sperm depletion, thus becoming temporarily infertile. Mechanisms of sperm limitation or sperm depletion in males have been reported for such diverse organisms as birds, sheep, moths, sandflies, crabs, and fish (Preston et al. 2001; review in Wedell et al. 2002).

We investigated extrapair paternity in the red bishop, *Euplectes orix*, a passerine species with a polygynous mating system. Males provide no paternal care

and foraging takes place away from the territories; the only material benefit females obtain from their mates is a nest. Since the supply of nests built by males always outnumbers female demand for nests (Metz et al. 2007), there is no competition among females for nesting sites. Thus, in the mating system of the red bishop there are no resources or other material benefits provided by territorial males that have to be shared among the females on the territory. Red bishops engage frequently in extrapair copulations, with about 30% of the broods containing extrapair young (Friedl and Klump 2002). Copulations are always initiated by the female, and extrapair copulations are usually performed in the territory of the extrapair male, indicating that females actively visited males for the purpose of seeking extrapair copulations (Friedl and Klump 2005). However, an investigation of pattern and frequency of extrapair paternity in the red bishop yielded inconsistent results with regard to the 'good genes' hypothesis (Friedl and Klump 2002). While extrapair fathers had longer tarsi and were older than the within-pair fathers they cuckolded (in accordance with expectations derived from the 'good genes' hypothesis), no differences between within-pair and extrapair fathers were found with regard to territory tenure and number of nests built, which have previously been found to be good indicators for male quality in red bishops (Friedl and Klump 2002). Furthermore, females mated to high-quality males were as likely to engage in extrapair copulations as females mated to low-quality males (Friedl and Klump 2002).

Recently, Friedl and Klump (2005) provided some evidence that female red bishops mated to successful high-quality males might follow a fertility insurance strategy. Successful male red bishops can have ≤3 female partners at the same time (i.e. simultaneous polygyny) and up to 18 social mates in one breeding season (Friedl and Klump 1999). In addition, male mate guarding is absent and males engage in frequent copulations with their social mates to ensure paternity (Friedl 1998). Thus, male red bishops are in theory prone to sperm depletion, a prerequisite for the 'fertility insurance' hypothesis. As can be expected in a polygynous species where successful high-quality males suffer from sperm depletion because of frequent copulations, unhatched eggs were more common in seasons with high overall breeding activity, and the proportion of unhatched eggs was higher in territories of high-quality males than in those of low-quality males. In addition, the proportion of unhatched eggs within the territory was positively correlated to the number of simultaneously fertile females within the territory for high-quality males only.

Furthermore, evidence that females mated to high-quality males follow a mating strategy to insure fertility is given by the fact that for these females the frequency of cuckoldry increased in seasons with high overall breeding activity, that is in seasons with a high risk of temporary sperm depletion because of frequent copulations (while the frequency of cuckoldry was unrelated to overall breeding activity for females mated to low-quality males). Finally, broods of high-quality males without unhatched eggs had a significantly higher proportion of extrapair young than broods of high-quality males with unhatched eggs. In conclusion, the available data suggest that the 'fertility insurance' hypothesis can explain female extrapair mating strategies in red bishop females which are mated to high-quality males (Friedl and Klump 2005).

Our goal in this study was to investigate in more detail whether female red bishops gain indirect benefits through extrapair copulations in terms of increased offspring viability, namely a better immunocompetence. We compared the cell-mediated immune response between extrapair young and their maternal half-sibs raised in the same brood, using a very similar study design as in Johnsen et al. (2000), Kleven and Lifjeld (2004), and Kleven et al. (2006). If female red bishops engage in extrapair copulations to gain a genetic benefit for their offspring, we would expect the extrapair young to be more immunocompetent than their maternal half-sibs from within-pair copulations. Alternatively, if females seek extrapair copulations as insurance against temporary infertility of their social mates, we would expect within-pair young to be as or even more immunocompetent than their maternal half-sibs. A central assumption of any study testing for genetic benefits of extrapair copulations in terms of increased offspring immunocompetence is a heritable component of offspring immunocondition. Therefore, we also test if offspring cell-mediated immune response is related to immunological condition and health of their genetic fathers. Finally, we investigate if possible differences in the cell-mediated immune response between extrapair young and within-pair young from the same brood vary among seasons, and if so, whether such variation can be explained by environmental conditions.

Material and Methods

Study species and study site

Red bishops are sexually dimorphic weaverbirds widely distributed in Sub-Saharan Africa. They usually breed in colonies in reed beds or bulrush stands in marshes, dams and rivers. At the beginning of the breeding season, males moult into a conspicuous orange-scarlet and black breeding plumage. They establish and defend small territories and construct several nests within their territories to which they try to attract females that are solely responsible for incubation and nestling provisioning. Most of the males are polygynous, with male reproductive success mainly being determined by the number of nests built within the territory, which is also a good indicator of male quality (Friedl and Klump 1999). The study was conducted in a breeding colony (approx. 70 territorial males) of red bishops in the Addo Elephant National Park in the Eastern Cape Region, South Africa (S 33° 26', E 25° 45') during two consecutive breeding seasons (approx. October to March) between 2002 and 2004. The breeding site was a circular small dam (approx. 250 m²) completely surrounded by an inner belt of bulrushes, *Typha capensis*, and an outer belt of common reeds, *Phragmites australis*. More details on the breeding colony at our study site and on red bishop breeding behaviour are given in Friedl (2004) and Friedl and Klump (1999; 2005).

Field methods

Throughout the breeding season, information on locations of territories and nests, identities of territory holders, and social parents were obtained through detailed daily observations. Adult red bishops were caught in mist nets or live traps. We took a blood sample (approximately 90 µl) from the brachial vein using sterile needles to determine genetic parentage in the laboratory and made a blood smear to be able to assess male health through determination of white blood cell counts (see below). As the colony has been extensively studied since 1993, a large proportion of breeding birds is already ringed with an individual combination of four coloured plastic rings, thus allowing for easy recognition. Nests were checked every other day,

and we recorded all eggs laid and nestlings hatched. A more detailed description of general field methods is provided in Friedl and Klump (1999).

To obtain data on nestling immunocompetence, we caught every nestling on day 10 ± 1 after hatching, approximately two days before leaving the nest, and ringed them individually with a numbered metal ring (provided by the South African Bird Ringing Unit, Cape Town) and four coloured plastic rings. The nestlings were weighed (using a 50 g Pesola spring balance, ± 0.5 g) and their tarsus length (using callipers, ± 0.25mm) was measured to describe skeletal growth. As weight and tarsus length are significantly correlated, we calculated a body condition index as the residuals from the linear regression of body weight on tarsus length (R^2 = 0.347, $F_{1,273}$ = 146.8, P < 0.001).

The protocol of the PHA-test followed the procedures described in Smits et al. (1999). We marked a primary on the left wing close to the outer joint with a water resistant pen. The thickness of the patagium was measured under the marked primary to the nearest 0.01 mm using a pressure-sensitive digital micrometer (Mitutoyo Digimatic model 573-191, Mitutoyo, Tokyo). Twenty microgram (20 µg) of PHA (L-9132, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 30 µl of phosphate buffered saline (PBS) was then injected subcutaneously in the patagium. Blood samples (approximately 30 µl) were taken from the brachial vein of the right wing to determine genetic parentage in the laboratory. The nestlings were recaught after 24 ± 1 h to measure the skin swelling of the injection site. The swelling (thickness 24 h after injection minus thickness before injection in millimetre) is thought to be a good indicator of the immune status of individuals (e.g. Smits et al. 1999; Johnsen et al. 2000). All measurements of the skin swelling were taken three times. Repeatability was calculated using the methods described by Lessells and Boag (1987). Both repeatability before (r = 0.918, $F_{274,550}$ = 32.6, P < 0.001) and 24 h after (r = 0.983, $F_{235,472}$ = 142.5, P < 0.001) the PHA-injection were high, and the mean of the three measurements was used in further analysis.

Our sample contained all the nestlings that reached the age of 10 ± 1 days in the colony in the two study seasons (N = 275 nestlings from 110 broods). We were able to take the second measurement of the PHA-test on 85,8 % of those nestlings; thus complete data for the cell-mediated immune response were available for 236 nestlings from 103 broods.

Determination of paternity

Genetic paternity was determined by non-radioactive multilocus DNA fingerprinting using the digoxigenin-labelled oligonucleotide probe $(GGAT)_4$. Genetic analysis and paternity assignment were conducted following the procedures described in detail by Friedl and Klump (1999; 2002). We determined genetic paternity for 162 nestlings from 66 broods. Data on both genetic paternity and immunocompetence were available for 22 broods with mixed-paternity (27 extrapair young and 30 within-pair young), 17 broods with within-pair young only (40 nestlings) and 8 broods with extrapair young only (18 nestlings).

Male health status and immunological condition

A central assumption of this and any other study testing for genetic benefits of extrapair copulations in terms of increased offspring immunocompetence is a heritable component of offspring immunocompetence that is linked to male quality. One possible indicator of male quality in red bishops is the number of nests a male built in a season, since the number of nests built is positively correlated to male mating success (Friedl and Klump 1999; Lawes et al. 2002). However, since the number of nests built and male mating success are often, but not always, positively correlated to male health (Friedl and Edler 2005), and since we want to test for the heritability of a health-related trait in nestlings, that is their immunocompetence, we chose to use a direct measurement for male health and immunological condition in form of the heterophil/lymphocyte ratio.

The heterophil/lymphocyte ratio responds to stressors such as infectious diseases, parasite infection, malnutrition and social-psychical disruption with a progressive increase (Ots and Hõrak 1996; Birkhead et al. 1998; Hõrak et al. 1998; Ilmonen et al. 2003), and is thus a measurement commonly used to assess stress levels of birds (e.g. Gross and Siegel 1983; Maxwell and Robertson 1998; Samour 2000). Moreover, given that the heterophil/lymphocyte ratio shows long-term individual consistency (Hõrak et al. 2002), it is also a useful measure for the general health status and immunological condition of an individual (Friedl and Edler 2005).

The air-dried blood smears were stained with Wright's stain and examined with a microscope under 630 x magnification. We counted the number of erythrocytes and

leucocytes (subdivided in lymphocytes, monocytes, basophilic granulocytes, eosinophilic granulocytes and heterophilic granulocytes) in each blood smear until we reached a total of 100 leucocytes (for more details see Edler et al. 2004). Based on these counts the heterophil/lymphocyte ratio was calculated. The blood smears were marked only with a number; thus the person conducting the examination had no knowledge of the identity of the male it belonged to. We have previously tested the repeatability of this method using 12 blood smears that were randomly chosen and recounted without knowledge of the results of the first count, and found a high and significant repeatability ($N = 12$, $r > 0.9$, $P < 0.001$; Edler et al. 2004).

We were able to obtain blood smears from 20 territorial males from our study colony that had within-pair offspring for which PHA-data were available. As three of those males were breeding in both seasons, we randomly chose one of the two blood smears to insure statistical independence.

Molecular sexing

Sex of nestlings sampled in the breeding season 2002/2003 ($N = 112$) was determined by amplification of parts of both the W-linked CHD gene (unique to females) and the non-W-linked CHD gene (occurs in both sexes), treatment with the restriction endonuclease *Bsu*RI, and subsequent separation of the PCR products by electrophoresis on 3% agarose gels (after Griffiths et al. 1996). The untreated PCR products show a 110 bp band both for males and females. After the PCR products have been cut with *Bsu*RI, the 110 bp band disappears in males since the non-W-linked CHD gene of both males and females is cut into two fragments (65 bp and 45 bp), while the W-linked CHD gene of females remains uncut, resulting in a female-specific PCR product in the 110 bp range. Nestlings sampled in the season 2003/2004 ($N = 156$) were sexed by amplification of a larger fragment of the CHD gene including introns that are longer in the W-linked CHD gene compared to the Z-linked CHD gene, thus not requiring separation of the PCR products with a restriction enzyme (Griffith et al. 1998). This method results in one PCR product of around 350 bp corresponding to the amplified fragment of the Z-linked CHD gene in both males and females, and an additional female-specific PCR product of around 400 bp which corresponds to the longer fragment of the W-linked CHD gene. To test the validity and reliability of the two methods, especially whether the restriction

enzyme *Bsu*RI used in the first method reliably cut the 110 bp fragment of the non-W-linked CHD gene of both males and females, we additionally sexed 47 of the nestlings sampled in the season 2002/2003 using the method of Griffith et al. (1998); in all cases the results obtained with both sexing methods were the same.

We determined the sex of 268 nestlings from 105 nests, of which 144 were male. The proportion of males was 53.7 % and did not differ significantly from unity (binomial-test, $P = 0.246$).

Statistics

Statistics were performed using the R statistical package 2.2.0 (R Development Core Team 2005). Quoted significance values are for two tailed tests. The level of significance was set as $P < 0.05$.

As the body condition index and the skin swelling are weakly, but significantly correlated ($R^2 = 0.025$, $F_{1,234} = 7.174$, $P < 0.01$) we calculated the residuals of a linear regression using the skin swelling as dependent variable and the body condition index as independent variable. Thus, we obtained a measure of the immune reaction independent from nestling body condition, which is used in further analysis.

Given that the data structure for tests of differences between sexes and differences between extrapair young and within-pair young is highly unbalanced, we used a restricted maximum-likelihood (REML) design within the GLMM (General Linear Mixed Model) framework, with nest as random factor.

As male red bishops are highly polygynous and return to the same breeding colonies year after year, there is a high probability of males entering our data repeatedly, either by fathering more than one nest in a season and/or by breeding in both study seasons. In total, our 236 nestlings were from clutches of 46 individual social fathers. Five males were breeding in both seasons, 13 out of 25 males entered our data as the social fathers of more than one nest in season 2002/2003, 17 out of 26 males were the social fathers of more than one nest in season 2003/2004. Thus, we used the social fathers as a random factor in all our REML analyses.

Temperature data recorded by an automated weather station close to the Addo Elephant National Park were provided by the South African Weather Service. We determined the average daily temperature as the mean of daily maximum and

minimum temperature, and calculated the average temperature of the 30 day period for each nest before the PHA-test was administered to a clutch. This 30 day period covers the estimated time from copulation of the parents to the near-fledgling state of the nestlings, thus providing a measure of a possible environmental influence for the whole breeding period of a clutch.

Data used in the study can be found in Appendices B and C.

Ethical note

Phytohaemagglutinin (PHA) has been criticized to be potentially harmful, but a detailed study by Merino et al. (1999) found no adverse effects on nestling northern house martins in terms of increased heat shock protein levels or altered haematological parameters, other than the ones imposed by handling. To reduce any possible negative impact of PHA on the nestlings, we injected the same small amount of PHA (0.02 mg) as used in the study by Merino et al. (1999), which is considerably less than in most other studies using the PHA-assay to assess cell-mediated immunity in nestling birds of approximately the same size (range 0.05 mg to 0.1 mg; Johnsen et al. 2000; Kleven and Lifjeld 2004; Garvin et al. 2006; Kleven et al. 2006). In addition, to reduce handling-induced stress in nestlings we used the simplified techniques for the PHA assay as described by Smits et al. (1999), allowing us to inject only once per nestling. We observed no negative effects of the PHA-injection in our study. The research was approved by the National Parks Board of South Africa and the Province of the Eastern Cape, Department of Economic Affairs, Environment and Tourism, Chief Directorate Environmental Affairs (License nos. WRO 10/02WR and WRO 10/03WR).

Results

Phytohaemagglutinin (PHA) induced wing swelling ranged from -0.03 to 0.68 mm (mean = 0.130 mm, SD ± 0.1 mm, *N* = 236). A negative result for the wing swelling was obtained in 6 of 236 cases. All negative values are very small (-0.01 to -0.03 mm) and are most likely caused by measurement errors because of the elasticity of the patagium.

Male nestlings close to fledging had longer tarsi (REML: $F_{1,160}$ = 124.6, *N* = 268 nestlings, *P* < 0.0001) and were heavier ($F_{1,160}$ = 158.0, *N* = 268 nestlings, *P* < 0.0001) than female nestlings, and they had a higher body condition index ($F_{1,160}$ = 35.8, *N* = 268 nestlings, *P* < 0.0001). Using the residual immune response (i.e. immune response corrected for body condition index), we found no differences between sexes with respect to immune response in broods with mixed sexes ($F_{1,84}$ = 1.032, *N* = 51 nests, *P* = 0. 313) or in mixed-paternity broods ($F_{1,34}$ = 1.317, *N* = 22 nests, *P* = 0.259). The sex ratio did not differ between extrapair young and within-pair young (Fisher's Exact Test: *N* = 22 nests, *P* = 0.568).

Of a total of 27 broods containing maternal half-siblings (extrapair young and within-pair young), we were able to use 22 broods (with 27 extrapair young and 30 within-pair young) for comparisons of the immune response of extrapair young and

Table 1 Mixed-model analysis of nestling weight, tarsus length and body condition index. The analysis compares extrapair young (*N* = 27) and within-pair young (*N* = 30) from 22 mixed-paternity broods. Brood and identity of the social father were included as random factors. A positive estimate indicates larger values of within-pair young compared to extrapair young.

Response	*Fixed effect*	*Estimate*	*F*	*P*
Weight	*Paternity*	-0.646	0.177	0.677
	Season	-2.082	0.217	0.661
	Sex	1.472	17.789	0.0002
	*Paternity*Season*	1.321	2.743	0.108
Tarsus	*Paternity*	-0.394	0.887	0.353
	Season	-0.980	1.437	0.284
	Sex	1.338	25.996	<0.0001
	*Paternity*Season*	0.982	2.529	0.122
Body condition index	*Paternity*	-0.253	0.014	0.907
	Season	-1.250	0.791	0.414
	Sex	0.148	0.525	0.474
	*Paternity*Season*	0.551	0.626	0.435

within-pair young. There were no significant differences between extrapair young and within-pair young from mixed broods with regard to body condition index (with sex controlled for), tarsus length, and weight (see Table 1). However, we found a significant difference in the reaction to PHA between extrapair young and within-pair young in broods with mixed-paternity (REML: $F_{1,33} = 5.482$, $N = 22$ nests, $P = 0.025$), with within-pair young showing a stronger residual immune response in 16 of 22 broods (Figure 1). Expressed in standard deviations of extrapair young, the mean difference between within-pair young and extrapair young (within-pair young – extrapair young) was 0.765.

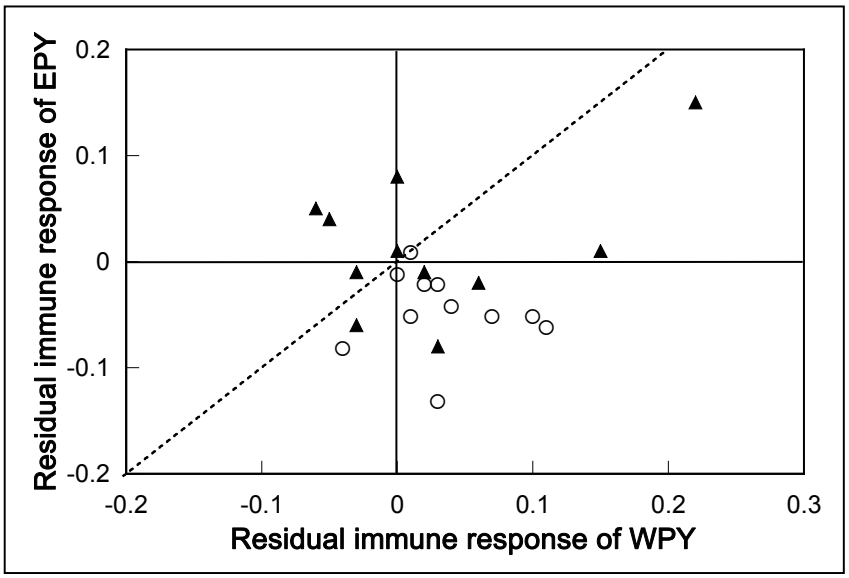

Figure 1 Relationship between PHA-induced immune response of extrapair (EPY) and within-pair young (WPY) of the red bishop, *Euplectes orix*, after controlling for nestling body condition ($N = 22$ broods with mixed-paternity). Triangles represent brood means for the season 2002/2003, circles represent brood means for the season 2003/2004. The dashed line indicates an identical response of extrapair and within-pair young.

We found no difference in the immune reaction between the two seasons when we compared the reaction to PHA of all nestlings (REML: $F_{1,34} = 0.826$, $N = 142$ nestlings from 63 nests, $P = 0.370$). We repeated the test with only the mixed-paternity nests, but there was also no difference between the two seasons (REML: $F_{1,5} = 0.512$, $N = 57$ nestlings from 22 nests, $P = 0.495$); however, the interaction between season and paternity proved to be significant (REML: $F_{1,33} = 4.867$, $P = 0.034$). There were no significant effects of the interaction between season and paternity on tarsus length, weight, or body condition index of nestlings in mixed-paternity broods (see Table 1). Thus, in neither season were there any differences between extrapair young and within-pair young with regard to tarsus length, weight, or body condition

index which could explain the significant effect of the interaction between season and paternity on the PHA-response of nestlings in mixed-paternity broods. To further investigate this result, we calculated brood means of the cell-mediated immune response of pure extrapair young broods, pure within-pair young broods, and extrapair young and within-pair young from mixed-paternity broods for each season separately. If a male was the social father of more than one nest, we used means for all broods of this male combined. There was no difference in PHA-response between broods of different paternity types in 2002/2003 (ANOVA: $F_{1,21} = 1.229$, $P = 0.328$; see Figure 2a), while there were significant differences in PHA-response between broods of different paternity types in 2003/2004 (ANOVA: $F_{1,29} = 9.415$, $P < 0.001$; see Figure 2b). In 2003/2004, within-pair young from mixed-paternity broods were more immunocompetent than extrapair young from mixed broods (post-hoc-test after Scheffe: $P < 0.001$), extrapair young from pure broods (post-hoc-test after Scheffe: $P = 0.015$) and within-pair young from pure broods (post-hoc-test after Scheffe: $P = 0.02$), while extrapair young from mixed broods, extrapair young from pure broods and within-pair young from pure broods did not differ significantly from each other with respect to their PHA-response (post-hoc-tests after Scheffe: all $P > 0.6$).

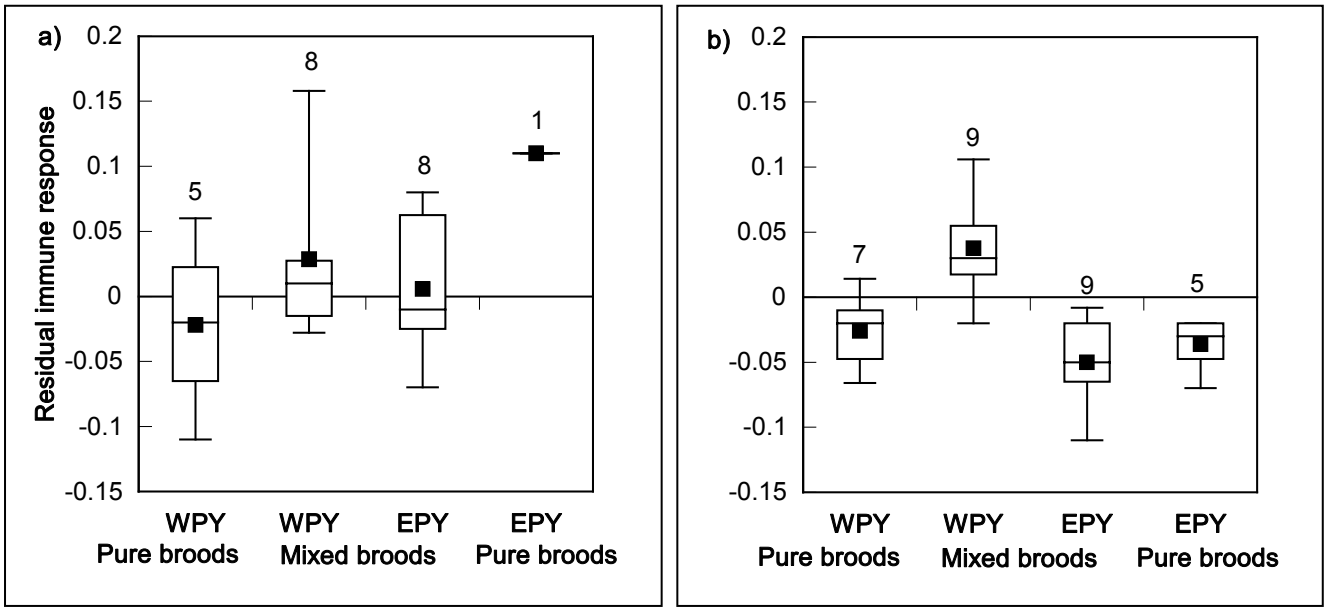

Figure 2 Standardized residual PHA-induced immune reaction of nestling red bishops (brood means) in relation to brood composition of extrapair (EPY) and within-pair young (WPY), after controlling for nestling body condition for (a) season 2002/2003 and (b) season 2003/2004. Black rectangles indicate brood means, horizontal lines indicate medians, boxes indicate second and third quartiles, and whiskers indicate 10th and 90th percentiles. Sample sizes are indicated above the lines.

A correlation between the health status of male red bishops, as assessed by the heterophil/lymphocyte ratio, and the immunocondition of their genetic offspring, expressed by the mean residual PHA-reaction of all their within-pair young for which these data were available, failed to reach significance, but has a strong tendency towards the expected result: males with a low heterophil/lymphocyte ratio, indicative for good health and immunological condition, sired offspring with better immunocompetence (Pearson correlation: $r = -0.469$, $P = 0.057$, $N = 17$ males), suggesting a heritable component of the nestlings' cell-mediated immunity.

Average temperature on the day of PHA-injection was known for 72 broods. Daily temperature had no influence on the mean immune reaction of all nestlings within broods (REML: $F_{1,43} = 0.227$, $N = 72$ broods, $P = 0.636$). To investigate whether environmental conditions during development differed between mixed-paternity broods in the two study seasons, we analysed and compared the average temperature in the 30 day period before the immune challenge (i.e. approximately the period from copulation to fledging; see Methods). The mean temperature in the 30 day period before the immune challenge was significantly higher for mixed-paternity broods in 2003/2004 compared to mixed-paternity broods in 2002/2003 (t-test: $t_{20} = -4.37$, $P < 0.001$), with the mean temperature in 2002/2003 being on average 3.1°C lower than in 2003/2004.

Discussion

The results from our study on nestling immunocompetence do not support the 'good genes' or 'compatible genes' hypotheses, as we found no genetic advantage, expressed by a higher cell-mediated immune response, in extrapair young compared to their maternal half-siblings from natural broods of the red bishop. On the contrary, within-pair young were significantly more immunocompetent than their maternal half-siblings when data for both seasons were combined. When data were analysed separately for the two study seasons, within-pair young were significantly more immunocompetent than their maternal half-siblings in the season 2003/2004, while in the season 2002/2003 within-pair young were on average only marginally but not significantly more immunocompetent than their maternal half-siblings.

How can such differences within mixed-paternity broods be explained? Some studies suggest a correlation between nestling biometry and immunocompetence, with differences in nestling biometry being caused by environmental factors (e.g. Christe et al. 2001; Naguib et al. 2004). Sex is also a possible source for differences in biometry, with male nestlings of the red bishop being bigger, heavier and of better body condition than female nestlings (see results). However, extrapair young and within-pair young in mixed-paternity broods did not differ in weight, size, body condition, or sex ratio (see results). Furthermore, we corrected the PHA-response for possible sex-related differences in body condition. Thus, our results indicate a 'true' genetic advantage of the within-pair young immune system, which is not caused by differences in nestling biometry or sex ratio between within-pair young and extrapair young.

An alternative reason for the observed differences in PHA-response between within-pair young and extrapair young could be differences in parental care for within-pair young and extrapair young. However, it remains to be shown that parents are able to distinguish between within-pair young and extrapair young in a clutch (Kempenaers and Sheldon 1996; Whittingham and Dunn 2001b). Even if parents should possess this ability, only cuckolded males would have an interest to discriminate between extrapair young and within-pair young and to provide resources mainly to their own genetic offspring, thus giving an advantage to within-pair young. As males in red bishops provide no parental care (Friedl and Klump 1999) and, as stated above, there is no difference in nestling biometry between extrapair young and

within-pair young raised in the same brood, it is very unlikely that differential parental care has influenced our results.

In view of the previous work on red bishops, suggesting that successful high-quality males might suffer from temporary sperm depletion and that females seem to adjust their extrapair mating behaviour to the risk of temporary infertility of their social mates (Friedl and Klump 2005; see also Introduction), the results of this study are, in our opinion, best explained with the 'fertility insurance' hypothesis. If females mated to high-quality males indeed seek extrapair copulations for fertility insurance, then within-pair young are expected to be of better immunocompetence than their maternal half-sibs, in accordance with our findings in this study. This is because these females should avoid high-quality males as extrapair mates, given that other high-quality males are as likely to suffer from temporary sperm depletion due to frequent copulations as the female's own social mate. A critical assumption to explain differences between extrapair young and within-pair young as resulting from differences in the quality of their respective fathers is a heritable component of nestling immunocompetence. Such heritability has been previously shown in blue tits, *Cyanistes caeruleus*, formerly *Parus caeruleus,* (Råberg et al. 2003), great tits, *Parus major*, (Brinkhof et al. 1999), and pied flycatchers, *Ficedula hypoleuca*, (Soler et al. 2003). The positive and almost significant correlation (despite a rather small sample size) between the nestlings' immune response and general health status of their genetic fathers found in this study likewise points to a heritable component of nestling immunocompetence. Thus, a pattern with higher immunocompetence of within-pair young compared to extrapair young in mixed-paternity broods as found in this study is in accordance with expectations derived from the hypothesis that red bishop females mated to healthy and successful high-quality males perform extrapair copulations with less healthy low-quality males to insure against temporary infertility of their social mate (see also Friedl and Klump 2005).

In a mating system where 'fertility insurance' against sperm depletion of successful males is the main reason for females seeking extrapair copulations, one would also expect nestlings from pure within-pair young broods being of lower quality than the within-pair young from mixed-paternity broods. This is because females that are socially paired with high-quality males engage in extrapair copulations more often than females mated to low-quality males to guard themselves against sperm depletion. Consequently, pure broods would be expected to occur more often in

females mated to low-quality partners. Our results are in accordance with this expectation, since within-pair young in mixed-paternity broods showed a stronger cell-mediated immune response than within-pair young in broods without extrapair young in 2003/2004.

Our results show a clear difference between the two study seasons in the reaction of extrapair young and within-pair young of mixed broods to an immune challenge, with within-pair young showing a significantly higher PHA-response than extrapair young raised in the same brood in one season, and almost no difference in PHA-response between within-pair young and extrapair young in the other season. As in the study of Garvin et al. (2006), there was a significant difference in temperature between the two study seasons, but in our study it was in the hotter, not the colder of the two seasons, that the significant difference in PHA-response between maternal half-siblings could be observed. These contrasting findings might be explained by the location of the two study sites: while Garvin et al. (2006) conducted their field research in Wisconsin, USA, we worked in the Eastern Cape Province, South Africa. In the arid climate of the Eastern Cape with very hot summers, heat rather than cold might have a negative effect on the brood or, more likely as there was no significant influence of the temperature on the nestling immune reaction, on breeding performance. Female red bishops feed seeds and insects to their young, but during very hot periods both food sources might become scarce and nestling body condition index can decline. While there is no difference in the body condition index between the study seasons for the nestlings from mixed-paternity broods only (see Results), a comparison of all nestlings showed that the nestling body condition index was significantly lower in the hotter season (t-test: $t_{273} = 4.26$, $P < 0.001$). Thus, the higher temperature in season 2003/2004 might well be the 'poor' environmental condition, that makes genetic benefits detectable (see Schmoll et al. 2005). In accordance with this idea, a significantly stronger cell-mediated immune response of within-pair young in mixed-paternity broods compared to pure within-pair young broods was also only found in the hotter of the two study seasons.

While the differences in the two study seasons point to the necessity to take environmental factors into account, they also show the limitation of a study presenting data from two seasons only. A conclusive statement on the effects of environmental factors on female extrapair mating behaviour and the benefits thereof would require a data set obtained in more than just two breeding seasons.

In conclusion, our results do not lend support to the 'good genes' or 'compatible genes' models, rather they indicate that fertility insurance is the driving force for female red bishops to engage in extrapair copulations, at least for females mated to high-quality males. We still can not exclude the possibility that female red bishops mated to low-quality males seek extrapair copulations for genetic benefits (e.g. in terms of increased offspring immunocompetence), as proposed by 'good genes' or 'compatible genes' models. Unfortunately, we lack sufficient data to test if extrapair young in nests of females mated to low-quality males are of better immunocompetence than their maternal half-sibs as predicted by the genetic benefits hypotheses due to low sample sizes of offspring from females mated to low-quality males. Nevertheless, our results show that the 'good genes' and 'compatible genes' hypotheses alone are not sufficient to explain female extrapair copulations in all species of passerine birds. To our knowledge, this is the first study to demonstrate a higher immunocompetence in within-pair young of mixed-paternity nests compared to their extrapair young half-sibs. Our findings thus add a new perspective to the ongoing discussion about the reasons for the occurrence of matings with multiple males in female birds. In addition, our results add to the growing number of studies indicating that environmental conditions can obscure variation in nestling genetic constitution due to female extrapair mating behaviour.

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CHAPTER 3

No trade-off between offspring quantity and quality in a subtropical bird species, the red bishop (*Euplectes orix*)

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Abstract

As predicted by life-history theory, parents are expected to face a trade-off between quantity and quality of the offspring they produce. Nestling birds from larger broods should thus have a decreased viability and/or immunological condition compared with nestlings of smaller broods. Nestlings from the larger, more expensive sex are expected be less common in large broods and to suffer more from increased competition in larger broods or from adverse environmental conditions than nestlings from the smaller sex. Furthermore, nestling health might vary in relation to nestling sex, brood sex ratio, season, or environmental conditions. Here, we investigate how clutch and brood size, nestling sex, season and weather affect nestling condition and immunocompetence (as assessed by a PHA-test) in a free-living colony of the red bishop (*Euplectes orix*), a polygynous weaverbird common in Sub-Saharan Africa. Nestling condition and immunocompetence differed significantly among the three study seasons. Male nestlings had a better body condition and were heavier than female nestlings of the same age, but there were no significant differences between the sexes in nestling immunocompetence. We found no decrease in nestling condition or immunocompetence with increasing clutch or brood size. Likewise, there was no evidence that male nestlings as the larger sex were more susceptible to increased competition in large broods or adverse weather conditions than female nestlings. Finally, we found no biased brood sex ratios in broods of different sizes. We argue that the lack of such trade-offs, which are proposed by life-history and sex-allocation theory, in the red bishop might be explained by a slower 'pace of life' of tropical and subtropical birds as compared to temperate zone bird species.

Introduction

According to life-history theory, reproducing birds need to distribute their effort over multiple conflicting activities in the face of generally limited resources. One of the possible trade-offs parents have to deal with in order to produce the number and type of offspring that maximizes their fitness (Stearns 1992), is between quantity and quality of their offspring. Support for this expected trade-off comes from studies indicating that increasing sibling competition, via increases in brood size, negatively impacts immune function of individual nestlings under both wild (Saino et al. 1997; Hõrak et al. 1999; Chin et al. 2005) and captive conditions (Naguib et al. 2004). Variation in immune function is an important determinant of survival in nestling birds, and thus a reliable measure of offspring quality (Dawson and Bortolotti 1997; Christe et al. 2001; Merilä et al. 2001). Nestlings that elicit a stronger immune response have been shown to survive better both during pre-fledging and post-fledging period (Lochmiller et al. 1993; Gonzalez et al. 1999; Moreno et al. 2005). Increases in brood size are also known to reduce nestling body condition (Fargallo et al. 2002; Török et al. 2004), another important determinant of an individual's fitness (Green 2001). In a sexually dimorphic species, the negative impact of increased brood size should be more strongly felt by the larger sex, due to greater energetic demands and/or food consumption of the larger individuals (Clutton-Brock et al. 1985; Wiebe and Bortolotti 1992; Anderson et al. 1993; Krijgsveld et al. 1998; Riedstra et al. 1998; Fargallo et al. 2002; Love et al. 2005). Consequently, within a certain brood size, nestlings of the larger sex should therefore have a reduced immune response or body condition compared with nestlings of the smaller sex of the same age.

The development of the immune system appears to be strongly resource-dependent (Lochmiller and Deerenberg 2000; Norris and Evans 2000; Alonso-Alvarez and Tella 2001; Soler et al. 2003). Thus, the proposed relationship between the number of offspring and offspring quality might be detectable only in poor environmental conditions (Chin et al. 2005). A test on the proposed relationship of individual nestling quality, as assessed by the strength of the immune system and nestling body condition, and clutch or brood size respectively, should therefore aim to cover more than one breeding season, to check for possible seasonal effects. Among the environmental conditions that are suspected to account for differences in breeding seasons, both rainfall and temperature have been shown to influence breeding

success and offspring condition (Christe et al. 2001; Friedl 2002; Lifjeld et al. 2002; Acquarone et al. 2003; Weatherhead 2005), and could thus mediate individual nestling quality and clutch or brood size.

Another possibility for breeding birds to maximize their fitness is described in the sex-allocation theory (Trivers and Willard 1973), which predicts that parents should be selected to over-produce the more valuable sex whenever sons and daughters yield different fitness benefits and/or entail different costs. Sex-allocation in birds has been widely studied in recent years, and while the underlying mechanisms are still far from being completely understood, there is considerable theoretical and empirical evidence to support the idea that parent birds can, and do, manipulate the sex ratio of their offspring (for reviews see Frank 1990; Hardy 1997; Hasselquist and Kempenaers 2002; Komdeur and Pen 2002; Krackow 2002) although there is no evidence of an adaptive bias across all studies conducted so far (Ewen et al. 2004). Environmental variables (such as differences in temperature and rainfall) might alter the relative cost of raising males and females, or their condition (Post et al. 1999). A skewed bias towards one sex has been related to female body condition (e.g. Nager et al. 1999; Alonso-Alvarez and Velando 2003), male quality (e.g. Dreiss et al. 2006), habitat quality (e.g. Stauss et al. 2005), weather, namely temperature prior to breeding (Dyrcz et al. 2004), or the availability of food resources (e.g. Wiebe and Bortolotti 1992; Kilner 1998; Arroyo 2002). As the larger sex is expected to have higher energy demands and to be more susceptible to food stress in terms of growth and survival (Røskaft and Slagsvold 1985; Teather and Weatherhead 1989), larger broods should consequently be biased towards the smaller sex, thus minimizing intra-clutch competition for the limited resources, and maximizing survival in the broods (Dijkstra et al. 1998; Dyrcz et al. 2004). On the other hand, if the costlier sex is also the sex with the higher potential rate of reproduction as well as the greater variance in fertilisation success, as it is the case with males in polygynous species (e.g. Hartley et al. 1999), one would expect this sex to be abundant in smaller broods where sibling competition is reduced and survival probabilities are better compared to larger broods.

Incubation in small terrestrial birds can require as much energy as nestling provisioning (Tatner and Bryant 1993; Monaghan and Nager 1997; Reid et al. 2002; Dobbs et al. 2006). Experimental clutch enlargements have been found to increase maternal energy expenditure during incubation (Moreno and Sanz 1994; Thomson et al. 1998). In species, in which only the female incubates, foraging trips away from the

nest and the necessary rewarming of the eggs on return, create additional metabolic demands during incubation (Williams 1991). As the occurrence of hatching failure is not uncommon in small passerines (Cichoń et al. 2005), the trade-off between quality and quantity, and the adjustment of the offspring sex ratio can be masked by a high proportion of broods smaller than the initial clutches. Provided there is a parental trade-off between quality and quantity of their offspring, these broods containing one or more unhatched eggs could appear to be of lower quality than their size, in terms of number of nestlings, would lead to expect. Consequently, we used both the initial clutch size (number of eggs) and the brood size (number of nestlings) as size determinants.

This study aims to test predictions derived from life-history and sex-allocation theory in a subtropical bird species, the red bishop (*Euplectes orix*). Most studies that found evidence for the proposed trade-off between offspring quality and quantity in birds focused on populations in the temperate zone (e.g. Saino et al. 1997; Hõrak et al. 1999; Fargallo et al. 2002; Török et al. 2004; Chin et al. 2005), while studies in the subtropics or tropics with comparable results are rare. To our knowledge, no study to date reported a decrease of offspring immune response in large clutches in naturally occurring broods in subtropical or tropical bird populations, a trade-off well documented in temperate zone birds.

The three predictions from life-history and sex-allocation theory tested here are: (1) Nestlings from larger clutches experience a reduction in immune function, as assessed by a phytohaemagglutinin (PHA) skin swelling test, and body condition, as assessed by nestling weight and a body condition index relating body weight to body size. (2) Male nestlings, as the larger sex in red bishops, should suffer more than female nestlings from this decline in immune response and body condition with increasing brood/clutch size. (3) Larger clutches/broods should be female biased, while smaller broods should be male biased. In addition, we tested for possible effects of weather conditions on nestling condition, brood sex ratios, and clutch or brood size.

Material and Methods

Study species and study site

Red bishops are sexually dimorphic weaverbirds widely distributed in Sub-Saharan Africa. They usually breed in colonies, building nests in the reed beds or bulrush stands of marshes, dams and rivers. While females and males are coloured in an inconspicuous brown outside the breeding season, males moult into an orange-scarlet and black breeding plumage at the beginning of the breeding cycle. Males establish and defend small territories, where they construct several nests to which they try to attract females that are solely responsible for incubation and nestling provisioning. Most of the males are polygynous, with male reproductive success mainly being determined by the number of nests built within the territory, which also serves as a good indicator of male quality (Friedl and Klump 1999). In our study species, the larger sex is the male, and even at nestling stage males are both heavier and larger (as assessed by tarsus length) than female nestlings of the same age (Edler and Friedl, in press).

The study was conducted in a breeding colony (approx. 70 territorial males) of red bishops in the Addo Elephant National Park in the Eastern Cape Region, South Africa (S 33° 26', E 25° 45') during three consecutive breeding seasons (approx. October to March) between 2002 and 2005. The breeding site was a circular small dam (approx. 250 m²) completely surrounded by an inner belt of bulrushes, *Typha capensis*, and an outer belt of common reeds, *Phragmites australis*. More details on the breeding colony at our study site and on red bishop breeding behaviour are given in Friedl (2004), and Friedl and Klump (1999; 2005).

Field methods

As the colony has been extensively studied since 1993, a large proportion of breeding birds was already ringed with an individual combination of four coloured plastic rings (in addition to a numbered metal ring provided by the South African Avian Demographic Unit, Cape Town) at the beginning of this study, thus allowing for easy recognition. During the course of this study, additional adult red bishops were caught in live traps or mist nets during the breeding seasons for individual ringing.

Throughout the breeding season, information on locations of territories and nests, as well as identities of territory holders and social parents were obtained through detailed daily observations. Nests were checked every other day, and we recorded all eggs laid and nestlings hatched. A more detailed description of general field methods is provided in Friedl and Klump (1999).

To obtain data on nestling size and health status, we caught every nestling on day 10 ± 1 after hatching, approximately two days before leaving the nest, and ringed them individually with a numbered metal ring (provided by the South African Bird Ringing Unit, Cape Town) and four coloured plastic rings. The nestlings were weighed to the nearest 0.5 g using a 50 g Pesola spring balance, and their tarsus length was measured with callipers to the nearest 0.25 mm to describe skeletal growth. Given that weight and tarsus length were significantly correlated, we calculated a body condition index as the residuals from the linear regression of body weight on tarsus length ($F_{1,315}$ = 26.55, P < 0.001).

The PHA-test to measure nestling immune status was conducted in the seasons 2002/2003 and 2003/2004, but not in the season 2004/2005. This test is routinely employed to measure T-cell-mediated immunocompetence (i.e. the ability to contain and prevent infection by a pathogen) in birds by injecting PHA subcutaneously and quantifying the resulting swelling response. The swelling (thickness 24 h after injection minus thickness before injection in millimetre) is thought to be a good indicator of the immune status of individuals (e.g. Smits et al. 1999; Johnsen et al. 2000; Kennedy and Nager 2006; but see Martin et al. 2006a) and fitness related traits (e.g. survival; Møller and Saino 2004). We used a protocol of the PHA-test that followed the procedures described in Smits et al. (1999). First, we marked a primary on the left wing close to the outer joint with a water resistant pen and measured the thickness of the patagium under the marked primary to the nearest 0.01 mm using a pressure-sensitive digital micrometer (Mitutoyo Digimatic model 573-191, Mitutoyo, Tokyo). We then injected 20 µg of PHA (L-9132, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 30 µl of phosphate buffered saline (PBS) subcutaneously in the patagium of the left wing. A blood sample (approximately 30 µl) was taken immediately afterwards from the brachial vein of the right wing for sexing in the laboratory. The nestlings were recaught after 24 ± 1h to measure the skin swelling at the injection site. All measurements of the skin swelling were taken three times and repeatability was calculated using the methods described by Lessells and Boag

(1987). Both repeatability before and 24h after the PHA-injection were very high (all $P < 0.01$), and the mean of the three measurements was used in further analysis (Edler and Friedl, in press).

Phytohaemagglutinin (PHA)-induced wing swelling ranged from -0.03 to 0.68 mm (mean = 0.130 mm, SD ± 0.1 mm, $N = 236$). A negative result for the wing swelling was obtained in 6 of 236 cases. All negative values are very small (-0.01 to -0.03 mm) and are most likely caused by measurement errors due to the elasticity of the patagium.

Our sample contained all the nestlings that reached the age of 10 ± 1 days in the colony in the three study seasons ($N = 316$ nestlings from 126 broods), but excluded all nestlings from nests in which partial brood loss occurred (3.1%; 4 of 130 nests). The PHA-test was conducted on 275 nestlings from 103 broods, and we were able to take the second measurement on 85.8 % of those nestlings; thus complete data for the cell-mediated immune response were available for 236 nestlings from 103 broods.

Molecular sexing

We used two methods to determine nestling sex. In the breeding season 2002/2003 ($N = 112$) nestling sex was determined by amplification of parts of both the W-linked CHD gene (unique to females) and the non-W-linked CHD gene (occurs in both sexes), treatment with the restriction endonuclease *Bsu*RI, and subsequent separation of the PCR products by electrophoresis on 3% agarose gels (after Griffiths et al. 1996). The untreated PCR products show a 110 bp band both for males and females. After treating the PCR products with *Bsu*RI, the non-W-linked CHD gene of both males and females are cut into two fragments (65 bp and 45 bp), while the W-linked CHD gene of females remains uncut. Thus, the 110 bp band disappears in males resulting in a female-specific PCR product in the 110 bp range. Nestlings sampled in the season 2003/2004 *(*$N = 156$) and 2004/2005 *(*$N = 39$) were sexed by amplification of a larger fragment of the CHD gene including introns that are longer in the W-linked CHD gene compared to the Z-linked CHD gene, thus not requiring separation of the PCR products with a restriction enzyme (Griffiths et al. 1998). This method results in one PCR product of around 350 bp corresponding to the amplified fragment of the Z-linked CHD gene in both males and females, and an additional

female-specific PCR product of around 400 bp which corresponds to the longer fragment of the W-linked CHD gene. To test the validity and reliability of the two methods, especially whether the restriction enzyme *Bsu*RI used in the first method reliably cut the 110 bp fragment of the non-W-linked CHD gene of both males and females, we additionally sexed 47 of the nestlings sampled in the season 2002/2003 using the method of Griffiths et al. (1998) used in the two latter seasons. The results obtained with both sexing methods were the same in all cases.

We determined the sex of 307 nestlings from 127 nests, of which 164 were male. The proportion of males was 53.4% and did not differ significantly from unity (binomial-test, $P = 0.254$).

Temperature and rainfall

Temperature and rainfall data recorded by an automated weather station close to the Addo Elephant National Park were provided by the South African Weather Service. We determined the average daily temperature as the mean of daily maximum and minimum temperature, and calculated the average temperature of the 30 day period for each nest before the PHA-injection was administered. Total rainfall for the 30 days was calculated as the amount of rain in millimetre registered in this period. This 30 day period covers the estimated time from copulation of the parents to the fledgling state of the nestlings, thus providing a measure of a possible environmental influence for the whole breeding period of a clutch.

Statistics

Statistics were performed using SPSS 13.0 (SPSS Inc.) with the level of significance set as $P < 0.05$. As the body condition index and the skin swelling are not significantly correlated in our sample (Spearman's correlation: $r_S = 0.177$, $N = 228$, $P = 0.078$) we did not calculate a correction factor but used the skin swelling directly as a measure of the immune reaction in further analysis. We used stepwise general linear mixed models (GLMM) to test for influences of 'brood size', 'nestling sex', 'season', and 'presence of unhatched eggs in the brood' on health indices. The dependent variables in the models were 'PHA', 'BCI' and, additionally, 'nestling weight', as a study by Green (2001) has shown that indices of body condition, calculated as residuals of mass on a measure of body size, might in some cases

produce misleading results given that the measure of size is not always independent from body mass. We repeated all GLMMs, replacing the independent variable 'brood size' (number of nestlings hatched) with the variable 'clutch size' (number of eggs in the clutch), as it is known that selection on the number of offspring produced by parents does not only act during the nestling phase, but also during earlier phases in the reproductive cycle, such as the incubation phase (Monaghan and Nager 1997; Visser and Lessells 2001; de Heij et al. 2006). We incorporated 'nest identity' as a random effect in all models to account for the non-independence of chicks from the same brood. Models initially included all explanatory variables and interactions. The final models were selected by sequentially dropping all non-significant interactions until only significant interactions and the main effects remained in the model.

As the weather conditions during the breeding period act on all nestlings of a clutch in the same way, we calculated nest means for the BCI, nestling weight for all complete (all eggs hatched) nests *(N* = 104 nests), and the mean reaction to PHA for complete nests of the first two study seasons *(N* = 74 nests). We determined the sex ratio as the proportion of males in the brood (Wilson and Hardy 2002), using only nests where all eggs hatched and the sex of all nestlings could be determined *(N* = 99). To test for influences of rainfall and temperature during the 30 day prior to hatching on nestling condition, clutch or brood size, and sex ratio, we used Spearman's correlation. We examined differences in the sex ratio of offspring in clutches of different sizes using the Kruskall-Wallis-Test. Descriptive statistics are given as mean ± SD, and the significance level was set at $P < 0.05$.

Data used in the study can be found in Appendices B and C.

Results

Clutch size and brood size

The 126 clutches in the three study seasons contained at least 2 eggs and a maximum of 5 eggs. On average, a clutch consisted of 2.88 ± 0.615 eggs. Broods contained between 1 and 5 young, with an average brood size at hatching of 2.67 ± 0.780. Divided by season, clutches and broods were largest in season 2002/2003 (clutches: 3.21 ± 0.68, $N = 43$, range 2 – 5; broods: 3.12 ± 0.76, $N = 43$, Range 2 – 5), and somewhat smaller in season 2003/2004 (clutches: 2.68 ± 0.50, $N = 65$, range 2 – 4; broods: 2.45 ± 0.66, $N = 65$, range 1 – 3) and season 2004/2005 (clutches: 2.83 ± 0.51, $N = 18$, range 2 – 4; broods: 2.39 ± 0.78, $N = 18$, range 1 – 3). A test for differences in the distribution of clutch sizes between the three study seasons proved to be significant (Kruskall-Wallis-Test: $\chi^2 = 18.071$, $N = 126$ nests, $P < 0.001$) with clutches being larger in 2002/2003 than in season 2003/2004 (Mann-Whitney-U-Test: $Z = -4.194$, $N = 108$, $P < 0.001$), and in season 2004/2005 ($Z = -2.078$, $N = 61$, $P = 0.038$). There is no significant difference between seasons 2003/2004 and 2004/2005 ($Z = -1.107$, $N = 83$, $P = 0.268$). Similarly, the distribution of brood sizes also differed between seasons (Kruskall-Wallis-Test: $\chi^2 = 19.398$, $N = 126$ nests, $P < 0.001$). Season 2002/2003 differs significantly from both following seasons 2003/2004 (Mann-Whitney-U-Test: $Z = -4.217$, $N = 108$, $P < 0.001$) and 2004/2005 ($Z = -2.968$, $N = 61$, $P = 0.003$). There is again no significant difference between seasons 2003/2004 and 2004/2005 ($Z = -0.136$, $N = 83$, $P = 0.892$) (Figure 1).

Frequency of unhatched eggs

Unhatched eggs occurred in 22 of 126 broods (17.5%) in all seasons combined. While the frequency of unhatched eggs was lowest in the season 2002/2003 (4 of 43 nests; 9.3%), larger in 2003/2004 (12 of 65 nests; 18.5%), and largest in 2004/2005 (6 of 18 nests; 33.3%), the difference fails to reach the level of significance (Fisher's Exact Test: $N = 126$ nests, $P = 0.086$).

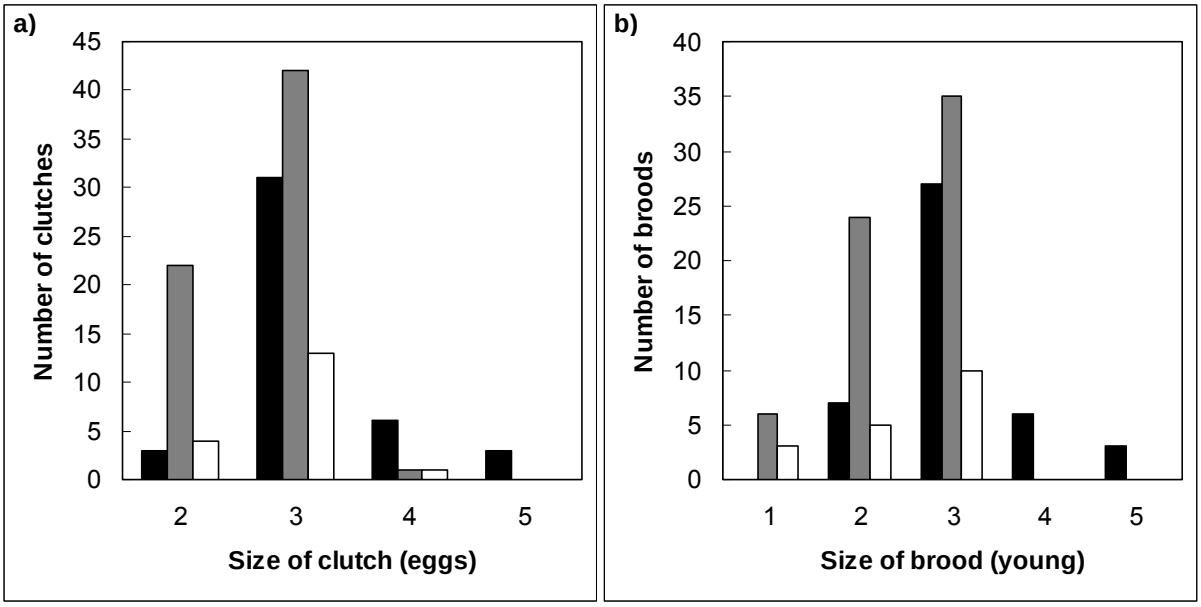

Figure 1 Distribution of nests of different clutch sizes (a), and brood sizes (b) in the study seasons 2002/2003 (black bars), 2003/2004 (grey bars), and 2004/2005 (white bars).

Sex ratio in broods and seasons

The sex ratio did not differ between different clutch sizes in clutches where all eggs hatched and the sex of all nestlings could be determined (Kruskall-Wallis-Test: $\chi^2 = 3.239$, $N = 99$ nests, $P = 0.356$) when data from all seasons were pooled. Broken down for single seasons, there were also no significant differences in the sex ratio in clutches of different sizes (2002/2003: $\chi^2 = 3.422$, $N = 35$ nests, $P = 0.331$; 2003/2004: $\chi^2 = 1.081$, $N = 52$ nests, $P = 0.298$; 2004/2005: $\chi^2 = 0.455$, $N = 12$ nests, $P = 0.500$). Thus, no adjustment of brood sex ratios in relation to clutch size could be observed. There was no significant difference in brood sex ratios between the three study seasons ($\chi^2 = 0.456$, $N = 99$ nests, $P = 0.796$).

Effects of season, sex and clutch/brood size on nestling BCI and weight

A GLMM with the BCI of nestlings as the response variable (Table 1) showed significant effects of season and sex, independently of the model being calculated with the number of young or the number of eggs as size variable (GLMM; all $P < 0.001$). Male nestlings had a higher BCI than female nestlings (Mann-Whitney-U: $Z = -5.505$, $N = 307$, $P < 0.001$), and mean BCI was highest in season 2002/2003 (Figure 2a). In

addition, when the number of young was used, the effect of brood size was also significant ($F_{4,135}$ = 2.533, N = 307, P = 0.043), with nestlings in broods of five having the highest average BCI (Figure 3a).

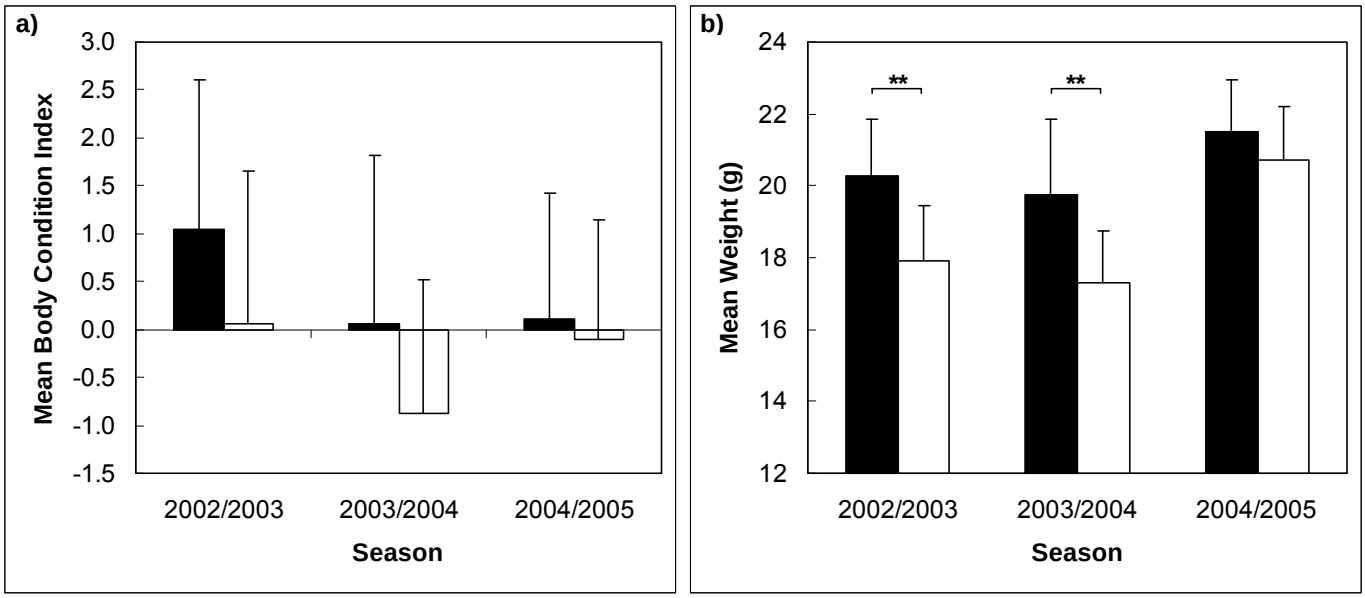

Figure 2 Nestling BCI (a) and nestling weight (b), shown as means ± SD, in the three study seasons. Black bars represent male nestlings, white bars represent female nestlings. Asterisks denote significant differences between the sexes with ** indicating a significance level of $P < 0.01$.

If one sex would suffer more from increased sibling competition in larger broods, one would expect the interaction between brood size and sex to be significant, but it was not ($F_{4,151}$ = 1.035, N = 307, P = 0.390). Likewise, if such an effect would only show in seasons with adverse environmental conditions, one would expect the three-way interaction between brood size, sex, and season to be significant, but it was not either ($F_{6,171}$ = 0. 544, N = 307, P = 0.774).

When we used nestling weight as dependent variable in the GLMM (Table 1), the influence of season and sex was again highly significant, both in the model with the number of eggs and the number of young as independent variable (all $P < 0.001$). Male nestlings were heavier than female nestlings (Mann-Whitney-U-Test: Z = -9.325, N = 307, $P < 0.001$), and both sexes show seasonal differences in their mean weight (Kruskall-Wallis-Test: male nestlings: χ^2 = 13.987, N = 164, P = 0.001, female nestlings: χ^2 = 44.912, N = 143, $P < 0.001$) with individuals being heaviest in 2004/2005 (Figure 2b). Brood size again had a significant influence when the number of young was used in the model (GLMM: $F_{4,135}$ = 3.355, N = 307, P = 0.012), with

nestlings in broods of five being the heaviest (Figure 3b). In addition, the interaction between seasonal effects and sex was significant, both when the model was calculated with the number of eggs ($F_{2,136}$ = 6.195, N = 307, P = 0.002) and the number of young ($F_{2,141}$ = 5.891, N = 307, P = 0.003). The significance of this interaction is because of the fact that male and female nestlings differ significantly in their mean weight in 2002/2003 (Mann-Whitney-U-Test: Z = -6.777, N = 110, P < 0.001) and 2003/2004 (Z = -7.467, N = 156, P < 0.001), but not in 2004/2005 (Z = 1.636, N = 41, P = 0.102). As in the analysis with BCI as dependent variable, there was no significant two-way interaction between brood size and sex (GLMM: $F_{4,151}$ = 1.889, N = 307, P = 0.113) and no significant three-way interaction between brood size, sex and season ($F_{6,171}$ = 0.922, N = 307, P = 0.480).

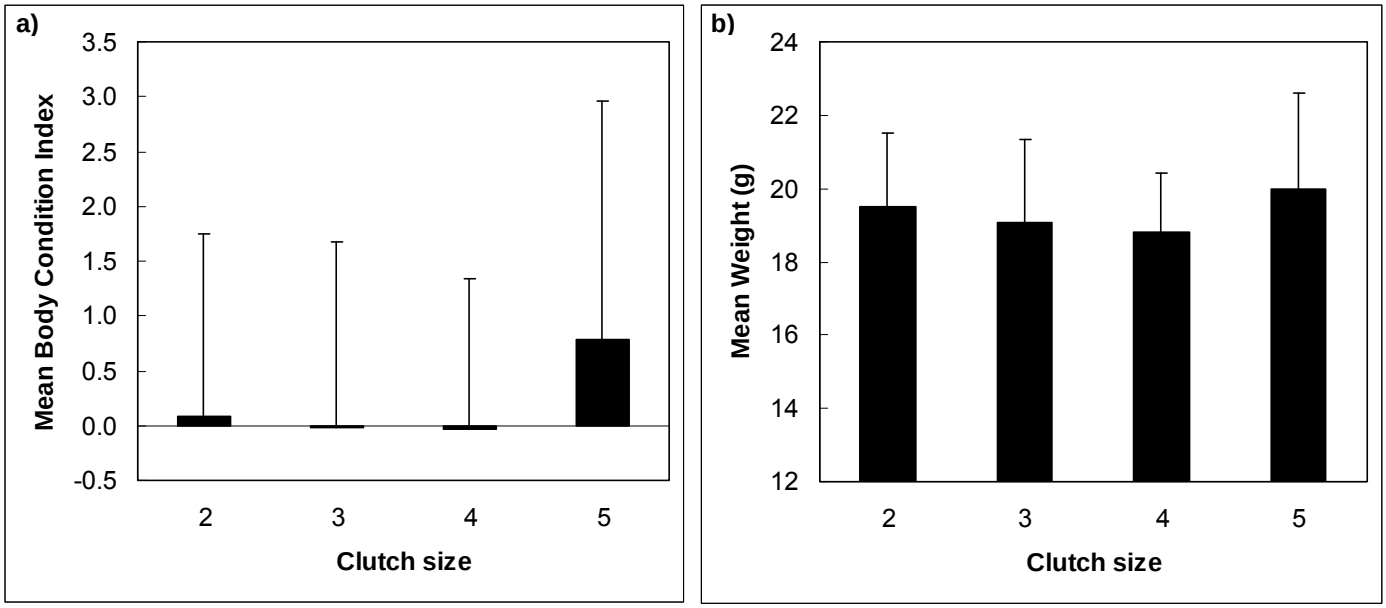

Figure 3 Nestling BCI (a) and nestling weight (b), shown as means ± SD, in relation to clutch size.

To test if nestling BCI and nestling weight declined linearly with an increase in brood size, we used the mean BCI and mean weight for complete nest in a linear regression with the mean BCI ($F_{1,103}$ = 0.035, N = 104 nests, P = 0.852) and the mean weight ($F_{1,103}$ = 0.312, N = 104 nests, P = 0.578) respectively as dependent variables, and the brood size as independent variable.

Effects of season, sex and clutch/brood size on nestling immune response

A GLMM to test for possible influences of season, nestling sex, and clutch or brood size on nestling immune response showed a significant seasonal effect, both for the model using number of eggs ($F_{4,109}$ = 9.038, N = 228, P = 0.004), and for the model using number of nestlings ($F_{4,109}$ = 8.463, N = 228, P = 0.005), with the average PHA-response being higher in season 2002/2003 compared to season 2003/2004 (Figure 4). There was no significant effect of nestling sex, clutch or brood size (Table 1).

Table 1 Analysis of a deviance table resulting from two General Linear Mixed Models (GLMM) to test for effects of season, nestling sex, and either clutch size or brood size on nestling health indices. a) GLMM with nestling body condition index (BCI) as response variable *(N* = 307 nestlings from 3 seasons); b) GLMM with nestling weight as response variable *(N* = 307 nestlings from 3 seasons) c) GLMM with the skin swelling resulting from a PHA-test as response variable *(N* = 228 nestlings from 2 seasons). Nest identity was used as a random factor in all models. All non-significant (p > 0.05) interaction terms have been removed from the final model.

		GLMM with clutch size			*GLMM with brood size*		
Model		*Df*	*F*	*P*	*Df*	*F*	*P*
a) BCI	*Intercept*	1	0.053	0.818	1	0.168	0.683
	Season	2	8.758	<0.001	2	11.424	<0.001
	Sex	1	42.126	<0.001	1	43.381	<0.001
	Size	3	0.996	0.397	4	2.533	0.043
b) Weight	*Intercept*	1	5313.267	<0.001	1	6514.935	<0.001
	Season	2	25.902	<0.001	2	27.392	<0.001
	Sex	1	108.961	<0.001	1	112.288	<0.001
	Size	3	1.676	0.176	4	3.020	0.020
	*Season*Sex*	2	6.195	0.002	2	5.891	0.003
c) PHA	*Intercept*	1	74.583	<0.001	1	74.879	<0.001
	Season	1	9.038	0.004	1	8.463	0.005
	Sex	1	2.093	0.149	1	2.056	0.153
	Size	3	0.189	0.904	4	0.476	0.753

Influence of weather conditions

Since the distribution of data for total rainfall and average temperature in the 30 days prior to fledging differed strongly and significantly from a normal distribution (Kolmogorov-Smirnov test, both $P < 0.001$), non-parametric statistics were used in the following. Total amount of rainfall differed significantly between the three study seasons (Kruskall-Wallis-Test: $\chi^2 = 23.757$, $N = 126$ nests, $P < 0.001$), as did average temperature ($\chi^2 = 55.529$, $N = 126$ nests, $P < 0.001$). Temperature was lowest in 2002/2003 (19.58 ± 1.58°C) and significantly different compared to both the season 2003/2004 (23.15 ± 0.96°C; Mann-Whitney-U-Test: $Z = -7.107$, $N = 108$, $P < 0.001$) and the season 2004/2005 (22.94 ± 0.51°C; $Z = -5.005$, $N = 61$, $P < 0.001$). Similarly, total rainfall was lowest in 2002/2003 (11.05 ± 6.90 mm) and significantly different compared to both the season 2003/2004 (22.93 ± 18.51 mm; Mann-Whitney-U-Test: $Z = -4.575$, $N = 108$, $P < 0.001$) and the season 2004/2005 (30.67 ± 25.21 mm; $Z = -3.418$, $N = 61$, $P = 0.001$). Thus, the season with the nestlings in the best condition (highest BCI) and the highest parental investment (largest clutches), was the season with the lowest average temperature and lowest rainfall during the 30 days prior to fledging.

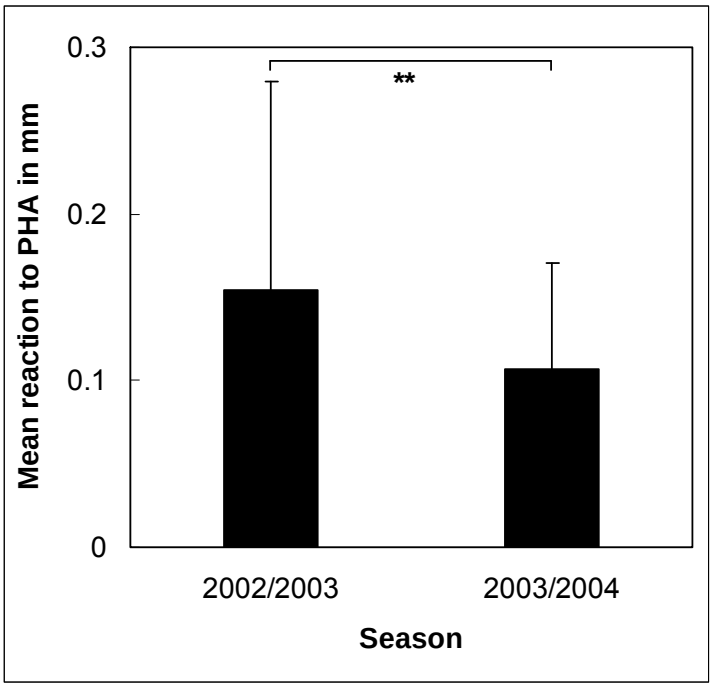

Figure 4 Immune response (mean ± SD) to a PHA skin-swelling test in the two seasons 2002/2003 and 2003/2004. Asterisks denote significant differences between the seasons with ** indicating a significance level of $P < 0.01$.

There was no significant correlation between brood sex ratios and average temperature in the 30 days prior to fledging (Spearman's correlation: $r_S = -0.071$, $N = 99$ nests, $P = 0.486$) or total rainfall in the 30 days prior to fledging ($r_S = -0.098$, $N = 99$ nests, $P = 0.333$), when we pooled data for all seasons. Likewise, in the single seasons there were no significant correlations of brood sex ratios with average

temperature (2002/2003: r_S = 0.006, N = 35 nests, P = 0.974; 2003/2004: r_S = -0.121, N = 53 nests, P = 0.391; 2004/2005: r_S = 0.083, N = 12 nests, P = 0.798) or total rainfall (2002/2003: r_S = -0.017, N = 35 nests, P = 0.921; 2003/2004: r_S = 0.156, N = 52 nests, P = 0.268; 2004/2005: r_S = 0.195, N = 12 nests, P = 0.544).

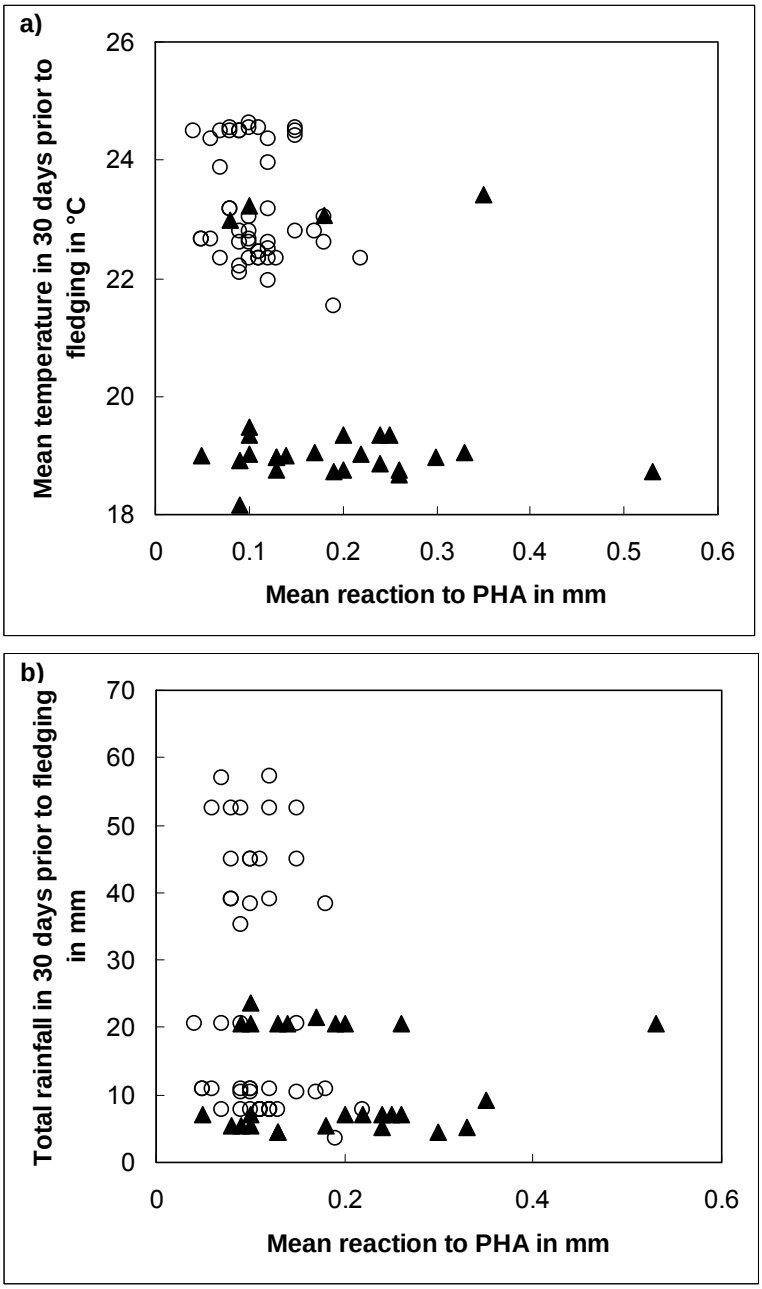

Figure 5 Relationship between the reaction to a challenge of the immune system with PHA (brood means) and a) average temperature and b) total rainfall in the 30 day period prior to fledging. Filled triangles represent broods from 2002/2003, open circles broods from 2003/2004.

Immune response, as assessed by the swelling after injection of PHA, correlated significantly negative with both average temperature (r_S = -0.424, N = 74 nests, P < 0.001) and total rainfall (r_S = -0.292, N = 74 nests, P = 0.012) 30 days prior to fledging, when we combined data for the seasons 2002/2003 and 2003/2004 (Figure 5). Analysed for each season separately, these relationships were still negative for both seasons, but no longer significant (all P > 0.10).

When we pooled data for all three seasons, BCI was higher when average temperatures (r_S = -0.400, N = 104 nests, P < 0.001) and total rainfall (r_S = -0.218, N = 104 nests, P = 0.026) were low, but both average temperature (r_S = -0.174, N = 104 nests, P = 0.077) and total rainfall had no effect on nestling weight (r_S = -0.091, N = 104 nests, P = 0.356). The correlation between average temperature and BCI was not apparent in the single

seasons (2002/2003: r_S = -0.222, N = 39 nests, P = 0.174; 2003/2004: r_S = -0.106, N = 53 nests, P = 0.452; 2004/2005: r_S = -0.571, N = 12 nests, P = 0.052). Total rainfall and BCI were positively correlated in 2002/2003 (r_S = 0.359, N = 39 nests, P = 0.025), not correlated in 2003/2004 (r_S = -0.199, N = 53 nests, P = 0.153) and negatively correlated in 2004/2005 (r_S = -0.864, N = 12 nests, P < 0.001).

Discussion

Our results do not confirm the predictions derived from sex-allocation theory and life-history theory. While there is a weak effect of brood size on the parameters indicating nestling status (BCI, weight), a decline of these health indicators with increasing brood size cannot be shown. Male and female nestlings did not respond differently to increasing clutch and brood size, and there was no difference in the sex ratio of broods of different sizes. The main determinants for nestling condition seem to be environmental factors and nestling sex, with males as the larger sex being, contrary to the prediction, in better condition than females.

The range in brood size (clutch size) from 1 to 5 young (2 to 5 eggs) found in red bishops is comparable to other studies aiming to test a possible relationship between brood size (clutch size) and brood sex ratio. Dijkstra et al. (1998), for example, presented a study on seven bird species (six raptor and one passerine species, all from the temperate zone) with brood sizes ranging from 1 to 4 at a minimum, and 1 to 7 at a maximum, where the sex ratio at fledging varied systematically with brood size. A study by Dyrcz et al. (2004) on the aquatic warbler, *Acrocephalus paludicola*, demonstrated sex ratio variation dependent on brood size, with broods containing between 2 and 6 young. Larger broods had a significant bias towards the smaller sex, which is females, while smaller broods were male biased. In addition, low temperature before the breeding period increased the proportion of females in complete broods. But, contrary to those studies, the higher brood and clutch size classes (4 or 5 eggs or young) in red bishops are relatively uncommon, a phenomenon common to subtropical and tropical bird species, where mean brood sizes are known to be smaller than in temperate zones (Martin et al. 2006b). Thus, while our sample in itself is not small, the variation in clutch and brood size, and the very small number of large clutches and broods might be too low to make conclusive statements about sex ratio variation or nestling condition in relation to clutch and brood size. Interestingly, we found a significantly better condition of nestlings in large broods, which is contrary to the prediction that nestling health should decline with brood size. This could point to an increased breeding investment by females able to support large broods, but given that the data on clutches with 5 nestlings stems from only 7 individuals from 3 different broods, the sample size is too small for conclusive statements.

We could neither detect a male-biased sex ratio in larger broods, nor sex-specific differences in nestling condition and immune response dependent on brood size. Such a bias does not need to be adaptive, but can also be caused by the differential susceptibility of the sexes to adverse environmental conditions, for example food limitation (Arroyo 2002; Styrsky et al. 2005). Due to their higher levels of androgens which may depress immune function (Folstadt and Karter 1992), male nestlings could be expected to be more sensitive to this time of adverse condition (Fletcher and Hamer 2004; Gonzales-Solis et al. 2005). However, a higher female-biased mortality has been reported for zebra finches, *Poephila guttata*, in times of restricted food resources (Kilner 1998; Martins 2004), and in great tits, *Parus major*, male nestlings as the larger sex compete better in poor rearing conditions than their female siblings, consequently creating a male bias in broods (Oddie 2000). Thus, partial brood loss does not necessarily result in female-biased broods. Partial brood mortality, the loss of some nestlings of a brood commonly associated with competition among nestlings and starvation (Weatherhead and Dufour 2005), is generally low in red bishops. In previous studies on the same red bishop colony, partial mortality occurred in about 8% of the broods (Friedl 2004; Friedl and Klump 2005) while in our study period partial mortality occurred in only 3.1% of the broods. With a sample size that small, we could not determine if the sex ratio in partial broods differs from the sex ratio in complete broods.

Previous studies have shown that immunological parameters both in adult and nestling red bishops can change drastically between seasons (Friedl and Edler 2005; Edler and Friedl in press), and especially T-cell-mediated immunity can show short-term (Lifjeld et al. 2002) or seasonal (Garvin et al. 2006) variations as a result of environmental conditions. The fact that both the strength of the immune response and the BCI of nestlings were highest in 2002/2003 is most likely the result of favourable weather conditions in the first study season. Interestingly enough, it is in the season with the lower rainfall, that larger clutches occur and nestlings are of better condition, while Brooke (1966) and Friedl (2002) both describe a reduction of average clutch size in seasons with lower rainfall, while no influence of rainfall on clutch size was detected by Craig (1982). The highest average clutch size found by Friedl (2002) in previous studies on the same colony was 3.14 in 1996/1997, and thus smaller than the average clutch size found in 2002/2003 (3.21 ± 0.68), while total rainfall (from 1st of September to 31st of March) in the season 1996/1997 with 475.4 mm was considerably higher

than the total rainfall in season 2002/2003 for the same period (189.6 mm). Low rainfall is generally assumed to reduce the amount of insects and grass seeds, the main food source of adult and nestling red bishops (Friedl 2002), and the resulting low food abundance has been shown to have a negative effect on the PHA-reaction (Lifjeld et al. 2002). However, such a reduction in the immune response with low rainfall could not be found in our study, where reduced average temperature rather than rainfall seems a more likely proximate factor in mediating nestling condition. In a bird living in semi-arid subtropics, such as the red bishop, 'bad weather' is more likely linked to high temperatures, which might dry out open water that is a breeding ground for insects (Acquarone et al. 2003). High temperatures during the breeding season could also constrain parental activity, as has been demonstrated for the neotropical green-backed firecrown (*Sephanoides sephanoides*), whose activity levels dropped with ambient temperature to reduce body mass loss (López-Calleja and Bozinovic 2003). Reduced parental activity and feeding rates have in turn been shown to reduce immunological condition, namely the PHA-response, in nestlings (Saino et al. 1997; Chin et al. 2005). Lower temperatures on the other hand would not hamper parents in their provisioning effort, thus increasing nestling condition and allowing for larger clutches, such as occurring in season 2002/2003.

The significant differences in nestling condition and clutch sizes between seasons found in our study, which were most likely caused by different weather conditions, highlight the influence of environmental factors on nestling condition and breeding success that has been also shown in many other studies (e.g. Birkhead et al. 1999; Christe et al. 2001; Friedl 2002; Lifjeld et al. 2002; Acquarone et al. 2003; Chin et al. 2005; Weatherhead 2005). However, we could not find a reduction in nestling immune function and nestling body condition with increasing brood or clutch size, or a female bias in larger broods, in any of the three study seasons. While the environmental conditions between the three study seasons clearly differ, they might not differ enough. A reduction in nestling condition with increasing brood size or female-biased brood sex ratios in larger broods, as predicted by sex-allocation and life-history theory, might only be detectable in seasons with far more adverse conditions than were present in the study seasons.

While our study adds to the large number of studies that were not able to find the expected bias in sex ratio, or even reported results with a skew opposite to the predicted trend (for reviews see Krackow 2002; Ewen et al. 2004), the evidence for an

adjustment of offspring sex ratio in relation to the environmental and social circumstances experienced by parents during reproduction is continuously growing (for reviews see Frank 1990; Hardy 1997; Hasselquist and Kempenaers 2002; Komdeur and Pen 2002). However, the mechanisms underlying biases in sex ratio are still poorly understood (Pike and Petrie 2003).

Overall, the expected trade-off between quality and quantity of offspring, which is well documented in species breeding in the temperate zone (e.g. Saino et al. 1997; Hõrak et al. 1999; Fargallo et al. 2002; Török et al. 2004; Chin et al. 2005), could not be confirmed in our study on a subtropical bird species, the red bishop. Results from subtropical or tropical species similar to those reported from the temperate zone are scarce. Strysky et al. (2005) report decreased survival in nestlings of enlarged broods in the spotted antbird (*Hylophylax naevioides*) in central Panama. Young (1996), in an experimental study on house wrens (*Troglodytes aedon*) in a population in Costa Rica, found decreased survivorship and growth in nestlings of large broods, but only in one of three study seasons. To our knowledge, a decrease in offspring immune response with increasing clutch size, as was found for several temperate zone species (e.g. Hõrak et al. 1999; Fargallo et al. 2002; Chin et al. 2005), was never reported in a subtropical or tropical species. The 'pace-of-life-syndrome' connecting demographic parameters related to survival and reproduction, and various physiological attributes, including metabolism and immune function (Ricklefs and Wikelski 2002; Tieleman et al. 2005) might explain this geographical variation. Bird species from tropical zones are known to have a slow 'pace-of-life' (Wiersma et al. 2007), a phenomenon commonly associated with a more robust immune response (Tieleman et al. 2005). In combination with the smaller brood sizes in tropical birds (see Martin 1996), this might make the detection of possible trade-offs between offspring number and immune response extremely difficult. There is the urgent need for more detailed studies comparing immunological trade-offs in temperate and tropical zone birds, to decide if the lack of results demonstrating a trade-off between offspring quantity and quality in tropical birds is indicative of different life-history strategies in birds of different geographical regions.

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APPENDIX

APPENDIX A

Male red bishops from the aviary in Oldenburg (Germany)

Data presented here are blood testosterone level (ng/ml), territory size (proportion of compartments), heterophil/lymphocyte ratio, reaction to PHA (mm), frequency of nest building behaviour in 10 min, frequency of aggressive encounters in 10 min, and frequency of courtship behaviour in 10 min for 14 breeding male red bishop in the aviary, Oldenburg, Germany. Measurements were taken five times in two week intervals.

MALE	*IMPLANT*	*WEEK*	*TESTOSTERONE (ng/ml)*	*TERRITORY SIZE*	*H/L-RATIO*	*BODY CONDITION INDEX*	*PHA (mm)*	*NEST-BUILDING*	*AGGRESSION*	*COURTSHIP*
16 TUM		0	0.55	0.50	0.19	1.63		2.67	11.67	11.00
17 TUM		0	0.29	0.78	0.50	-3.87		2.33	3.67	9.33
86 TUM		0	0.27	0.33	0.30	0.53		1.00	3.33	6.67
88 TUM		0	0.24	1.00	0.36	2.27		3.00	11.00	2.00
94 TUM		0	0.39	0.50	0.54	-2.27		7.67	16.33	2.33
99 TUM		0	0.28	1.22	0.46	-6.03		3.00	18.67	8.00
113 TUM		0	0.21	0.33	0.69	-5.83		0.00	5.00	5.33
4 TUM		0	0.27	0.33	0.66	5.84		1.67	10.67	19.00
5 TUM		0	0.94	1.00	0.69	2.30		1.33	14.33	13.00
22 TUM		0	0.27	0.50	0.90	2.63		2.33	9.67	9.33
69 TUM		0	1.64	1.00	0.24	-5.90		2.67	15.67	7.33
95 TUM		0	0.36	1.00	0.44	6.10		2.00	9.33	20.67
109 TUM		0	0.14	0.00	0.30	1.73		0.00	0.00	0.33
114 TUM		0	0.19	1.00	1.11	0.85		4.33	8.00	18.00
16 TUM	Placebo	2	0.33	0.50	0.29	0.99	0.11	1.00	8.67	17.33
17 TUM	Placebo	2	0.39	0.67	0.19	-4.56	0.08	2.67	5.00	3.00

86 TUM	Placebo	2	0.15	0.56	0.58	0.39	0.12	0.67	7.67	5.00
88 TUM	Placebo	2	0.16	0.00	0.22	4.92	0.08	0.67	5.33	0.00
94 TUM	Placebo	2	8.25	0.50	0.30	-3.46	0.06	7.67	12.67	6.33
99 TUM	Placebo	2	1.95	0.00	0.43	-4.88	0.09	0.33	1.00	2.67
113 TUM	Placebo	2	1.10	0.39	0.34	-7.08	0.17	2.00	8.00	6.33
4 TUM	Testosterone	2	4.48	0.44	1.44	7.94	0.05	2.33	3.33	13.33
5 TUM	Testosterone	2	3.73	0.50	0.89	1.15	0.14	5.33	12.33	7.33
22 TUM	Testosterone	2	4.08	0.50	1.00	1.99	0.14	4.33	9.33	9.67
69 TUM	Testosterone	2	4.39	1.00	0.34	-3.50	0.11	2.00	23.33	8.00
95 TUM	Testosterone	2	0.42	0.50	0.23	6.96	0.05	0.33	9.67	15.67
109 TUM	Testosterone	2	0.43	1.56	1.03	-0.86	0.13	3.67	14.00	14.00
114 TUM	Testosterone	2	0.15	1.11	0.78	0.01	0.20	8.33	9.33	19.00
16 TUM	Placebo	4	0.52	0.50	0.65	-1.18		5.00	13.00	9.00
17 TUM	Placebo	4	0.22	0.33	0.21	-0.98		3.00	2.00	2.00
86 TUM	Placebo	4	0.30	0.00	0.29	-1.38		1.00	2.00	0.33
88 TUM	Placebo	4	0.08	0.13	0.23	5.05		1.33	8.67	7.33
94 TUM	Placebo	4	4.84	0.50	0.34	-5.28		4.33	13.00	6.00
99 TUM	Placebo	4	0.27	0.00	0.17	-2.35		0.33	3.00	5.00
113 TUM	Placebo	4	1.12	0.25	0.24	-5.43		4.00	6.67	2.33
4 TUM	Testosterone	4	2.43	0.75	1.24	5.93		2.67	9.67	19.33
5 TUM	Testosterone	4	0.67	1.00	0.78	3.20		3.00	10.67	3.33
22 TUM	Testosterone	4	7.59	0.50	0.60	1.62		6.33	5.33	10.67
69 TUM	Testosterone	4	1.24	0.88	0.32	-4.01		6.33	4.67	3.33
95 TUM	Testosterone	4	0.12	1.00	0.40	3.48		5.33	8.67	6.33
109 TUM	Testosterone	4	0.07	1.67	0.98	2.42		2.67	14.00	9.67
114 TUM	Testosterone	4	0.64	1.00	0.37	-1.08		9.00	9.67	4.33
16 TUM	Placebo	6	0.12	0.50	0.22	3.73	0.15	0.00	2.33	1.00
17 TUM	Placebo	6	0.28	0.50	0.54	-2.00	0.15	0.00	1.33	1.00
86 TUM	Placebo	6	0.14	0.04	0.60	2.53	0.10	0.33	0.67	1.33
88 TUM	Placebo	6	0.17	0.33	0.22	2.41	0.25	1.67	3.00	1.67
94 TUM	Placebo	6	3.77	0.67	0.47	-4.90	0.17	4.00	9.00	0.67
99 TUM	Placebo	6	0.34	0.56	0.52	-6.59	0.16	0.00	3.00	2.33

113 TUM	Placebo	6	0.15	0.17	0.42	-6.44	0.20	0.33	2.33	0.00
4 TUM	Testosterone	6	0.99	0.83	1.09	5.15	0.17	2.00	12.33	10.67
5 TUM	Testosterone	6	0.13	0.60	0.72	2.04	0.04	1.67	3.00	1.33
22 TUM	Testosterone	6	4.00	0.50	0.35	2.33	0.03	1.33	6.00	2.33
69 TUM	Testosterone	6	0.42	0.67	0.05	-7.78	0.19	0.33	5.67	5.00
95 TUM	Testosterone	6	0.06	0.67	0.16	5.19	0.24	2.67	6.67	0.67
109 TUM	Testosterone	6	0.08	1.06	1.53	2.60	0.12	1.33	7.33	6.00
114 TUM	Testosterone	6	0.04	0.56	0.28	1.74	0.09	1.00	1.67	0.67
16 TUM	Placebo	8	0.16	0.50	0.54	2.90		1.00	0.67	0.33
17 TUM	Placebo	8	0.15	1.17	0.23	-4.62		0.33	0.00	0.33
86 TUM	Placebo	8	0.13	0.08	0.89	-2.70		0.33	6.00	2.00
88 TUM	Placebo	8	0.06	0.47	0.33	2.55		3.00	6.33	2.00
94 TUM	Placebo	8	1.92	0.92	0.63	-1.42		2.67	5.00	6.67
99 TUM	Placebo	8	0.05	0.50	0.11	-4.85		0.00	3.00	3.33
113 TUM	Placebo	8	0.07	0.08	0.73	-6.32		0.67	2.67	0.00
4 TUM	Testosterone	8	0.92	1.00	1.29	8.01		2.67	14.33	10.67
5 TUM	Testosterone	8	0.21	0.00	0.60	1.54		0.00	0.00	0.00
22 TUM	Testosterone	8	1.24	0.50	0.60	2.50		0.00	3.00	0.33
69 TUM	Testosterone	8	0.06	0.61	0.38	-6.87		0.33	4.33	7.67
95 TUM	Testosterone	8	5.36	1.00	0.12	6.01		1.33	7.67	5.33
109 TUM	Testosterone	8	7.12	0.83	0.30	1.88		0.00	0.33	0.00
114 TUM	Testosterone	8	4.09	0.50	0.28	1.39		0.67	0.00	0.00

APPENDIX B

Nestlings from Addo Elephant Park (South Africa) in seasons 2002/2003 to 2004/2005

Data presented here are number of eggs in nest, number of young in nest, nestling sex, paternity (extrapair or within-pair young), body condition index, and reaction to immune challenge with PHA (mm) for individual nestlings of the red bishop in three conclusive seasons in the Addo Elephant National Park, South Africa. All measurements were taken on day 10 ± 1 after hatching.

SEASON	BIRD	NEST	EGGS in NEST	YOUNG in NEST	SEX	PATERNITY	WEIGHT (g)	BODY CONDITION INDEX	PHA (mm)
2002/2003	BH09002	BE4	3	3	male	WPY	18	0.26	0.07
2002/2003	BH09003	BE4	3	3	male	WPY	17	-1.68	0.11
2002/2003	BH09004	BE4	3	3	female	EPY	18	0.26	0.08
2002/2003	BH09005	F1	5	5	male	EPY	22	3.32	0.13
2002/2003	BH09006	F1	5	5	male	EPY	19	-0.62	0.00
2002/2003	BH09007	F1	5	5	unknown	unknown	21	1.38	0.04
2002/2003	BH09009	F2	5	5	male	unknown	21	1.38	0.42
2002/2003	BH09010	F2	5	5	male	WPY	23	3.38	0.09
2002/2003	BH09011	AR1	3	3	male	unknown	22	1.44	0.11
2002/2003	BH09012	AR1	3	3	male	unknown	23	2.44	
2002/2003	BH09013	C4	4	4	female	EPY	19	0.32	
2002/2003	BH09014	C4	4	4	male	EPY	21	1.38	
2002/2003	BH09015	C4	4	4	male	WPY	21	3.26	0.04
2002/2003	BH09016	C4	4	4	female	WPY	19	1.26	
2002/2003	BH09017	BB1	5	5	female	unknown	15	-2.74	
2002/2003	BH09018	BB1	5	5	male	unknown	20	0.38	0.04
2002/2003	BH09019	BB1	5	5	male	unknown	20	0.38	

2002/2003	BH09020	BA5	4	4	male	WPY	19	-1.56	
2002/2003	BH09021	BA5	4	4	female	EPY	17	0.19	
2002/2003	BH09023	W3	3	3	male	unknown	22	2.38	0.14
2002/2003	BH09024	W3	3	3	female	unknown	18	0.26	-0.02
2002/2003	BH09025	W3	3	3	female	unknown	17	0.19	0.03
2002/2003	BH09026	BB2	3	3	male	unknown	19	-0.62	0.11
2002/2003	BH09027	BB2	3	3	male	unknown	21	1.38	0.10
2002/2003	BH09028	BB2	3	3	female	unknown	15	-2.74	
2002/2003	BH09030	V1	3	2	female	WPY	19	1.26	0.03
2002/2003	BH09031	V1	3	2	female	WPY	18	-0.68	
2002/2003	BH09032	AI1	3	3	male	WPY	22	2.38	
2002/2003	BH09033	AI1	3	3	female	WPY	18	0.26	
2002/2003	BH09034	AI1	3	3	male	WPY	21	1.38	0.10
2002/2003	BH09035	AU2	4	4	male	WPY	20	0.38	0.29
2002/2003	BH09036	AU2	4	4	male	unknown	16	-3.62	
2002/2003	BH09037	AU2	4	4	male	EPY	19	0.32	0.14
2002/2003	BH09051	P2	4	4	male	unknown	19	0.32	0.27
2002/2003	BH09052	P2	4	4	male	EPY	19	0.32	0.19
2002/2003	BH09053	P2	4	4	female	EPY	18	-0.68	0.25
2002/2003	BH09054	P2	4	4	male	unknown	20	1.32	0.23
2002/2003	BH09055	P4	3	3	male	unknown	21	2.32	0.33
2002/2003	BH09056	P4	3	3	female	WPY	19	1.26	0.08
2002/2003	BH09057	P4	3	3	male	EPY	20	2.26	0.20
2002/2003	BH09058	AG1	3	3	female	EPY	17	-1.68	0.03
2002/2003	BH09059	AG1	3	3	female	WPY	18	0.26	
2002/2003	BH09060	AG1	3	3	female	WPY	17	-0.74	0.16
2002/2003	BH09061	AI3	2	2	female	EPY	20	1.32	0.34
2002/2003	BH09062	AI3	2	2	female	EPY	18	0.26	0.16
2002/2003	BH09063	AT1	3	3	male	WPY	20	1.32	0.03
2002/2003	BH09064	AT1	3	3	male	WPY	21	1.38	0.09
2002/2003	BH09065	AT1	3	3	female	EPY	18	0.26	
2002/2003	BH09066	BA6	3	3	female	EPY	18	-0.68	

2002/2003	BH09067	BA6	3	3	male	WPY	22	2.38	
2002/2003	BH09068	BA6	3	3	male	WPY	22	2.38	
2002/2003	BH09069	Y2	3	3	male	unknown	21	2.32	0.11
2002/2003	BH09070	Y2	3	3	unknown	unknown	21	1.38	0.40
2002/2003	BH09071	Y2	3	3	male	unknown	19	0.32	0.49
2002/2003	BH09073	S2	3	3	female	WPY	19	1.26	0.07
2002/2003	BH09074	S2	3	3	unknown	unknown	21	1.38	0.54
2002/2003	BH09075	S2	3	3	male	WPY	20	1.32	0.11
2002/2003	BH09076	AN2	3	3	female	WPY	19	1.26	0.15
2002/2003	BH09077	AN2	3	3	female	EPY	18	2.13	
2002/2003	BH09078	AU1	3	3	female	EPY	17	-1.68	0.26
2002/2003	BH09079	AU1	3	3	male	WPY	18	-1.62	0.33
2002/2003	BH09080	AY2	3	3	female	unknown	17	-0.74	0.17
2002/2003	BH09081	AY2	3	3	female	unknown	16	-2.68	0.18
2002/2003	BH09082	AY2	3	3	male	unknown	20	-0.56	0.03
2002/2003	BH09084	BE3	4	4	female	EPY	17	-0.74	0.10
2002/2003	BH09085	BE3	4	4	female	EPY	16	-1.74	0.04
2002/2003	BH09086	BE3	4	4	male	EPY	20	0.38	0.26
2002/2003	BH09087	BE3	4	4	male	WPY	18	-0.68	0.13
2002/2003	BH09088	U5	4	4	female	EPY	17	-0.74	0.17
2002/2003	BH09089	U5	4	4	male	unknown	18	-1.62	0.06
2002/2003	BH09090	U5	4	4	male	WPY	19	0.32	0.09
2002/2003	BH09091	U5	4	4	female	WPY	18	-1.62	0.05
2002/2003	BH09092	AQ3	3	2	female	unknown	18	0.26	-0.02
2002/2003	BH09093	AQ3	3	2	male	unknown	22	3.32	0.03
2002/2003	BH09094	BB5	3	3	female	unknown	24	6.26	0.45
2002/2003	BH09095	BB5	3	3	female	unknown	18	-0.68	
2002/2003	BH09096	BF2	3	3	female	unknown	19	0.32	0.15
2002/2003	BH09097	BF2	3	3	female	unknown	20	1.32	0.45
2002/2003	BH09098	BF2	3	3	male	unknown	18	-1.62	0.18
2002/2003	BH09099	BE5	3	3	female	WPY	18	0.26	0.08
2002/2003	BH09100	BE5	3	3	male	WPY	21	1.38	0.13

2002/2003	BH09101	BE5	3	3	female	unknown	18	0.26	
2002/2003	BH09102	AI5	3	3	female	WPY	19	0.32	0.38
2002/2003	BH09103	AI5	3	3	male	WPY	21	1.38	0.68
2002/2003	BH09104	U6	3	3	male	WPY	22	2.38	0.26
2002/2003	BH09105	U6	3	3	male	WPY	21	2.32	0.18
2002/2003	BH09106	U6	3	3	female	EPY	19	1.26	0.12
2002/2003	BH09107	AG3	3	3	male	WPY	21	1.38	0.28
2002/2003	BH09108	AG3	3	3	female	WPY	18	0.26	0.04
2002/2003	BH09109	AG3	3	3	male	WPY	21	1.38	0.27
2002/2003	BH09110	AO2	3	3	male	unknown	20	1.32	0.18
2002/2003	BH09111	AO2	3	3	female	unknown	20	1.32	0.04
2002/2003	BH09112	AO2	3	3	male	unknown	21	2.32	0.17
2002/2003	BH09116	BF4	2	2	female	unknown	18	1.19	
2002/2003	BH09117	BF4	2	2	female	unknown	19	1.26	0.10
2002/2003	BH09118	U7	3	3	male	WPY	20	1.32	0.08
2002/2003	BH09119	U7	3	3	female	WPY	17	-0.74	0.10
2002/2003	BH09120	U7	3	3	male	WPY	19	0.32	0.23
2002/2003	BH09122	AY4	3	3	female	unknown	20	3.19	0.10
2002/2003	BH09123	AY4	3	3	male	unknown	21	3.26	0.23
2002/2003	BH09135	S8	3	3	male	WPY	23	3.38	
2002/2003	BH09136	S8	3	3	male	EPY	22	3.32	
2002/2003	BH09137	S8	3	3	female	WPY	18	1.19	0.10
2002/2003	BH09140	BH9	2	2	male	WPY	21	0.44	0.13
2002/2003	BH09141	BH9	2	2	male	EPY	22	2.38	0.23
2002/2003	BH09150	AK12	3	3	female	WPY	16	-2.68	0.09
2002/2003	BH09151	AK12	3	3	male	WPY	20	0.38	0.05
2002/2003	BH09152	AK12	3	3	male	WPY	18	-1.62	0.16
2002/2003	BH09154	E13	3	2	female	unknown	18	1.19	
2002/2003	BH09155	E13	3	2	female	unknown	18	-0.68	0.04
2002/2003	BH09156	BA19	3	3	female	WPY	16	-2.68	0.04
2002/2003	BH09157	BA19	3	3	female	EPY	15	-1.81	0.11
2002/2003	BH09158	BA19	3	3	female	WPY	16	0.13	0.08

2002/2003	BH09166	T13	3	3	unknown	unknown	18	-0.68	0.55
2002/2003	BH09167	T13	3	3	unknown	unknown	17	0.19	0.16
2002/2003	BH09168	T13	3	3	unknown	unknown	15	-2.74	0.34
2002/2003	BH09169	E14	3	2	unknown	WPY	19	-0.62	0.15
2002/2003	BH09170	E14	3	2	unknown	EPY	12	-4.81	0.07
2003/2004	BH09173	A1	2	2	female	unknown	15	-0.87	0.24
2003/2004	BH09175	A1	2	2	male	unknown	18	-0.68	0.13
2003/2004	BH09176	G1	2	1	male	EPY	17	-3.56	0.10
2003/2004	BH09194	A5	3	3	female	unknown	18	0.26	0.04
2003/2004	BH09195	A5	3	3	female	unknown	18	0.26	0.14
2003/2004	BH09196	A5	3	3	female	unknown	19	1.26	0.10
2003/2004	BH09216	A8	2	2	female	unknown	18	-0.68	0.12
2003/2004	BH09217	A8	2	2	male	unknown	20	0.38	0.08
2003/2004	BH09218	E11	2	2	male	unknown	18	-3.49	0.06
2003/2004	BH09219	E11	2	2	female	unknown	17	-2.62	0.09
2003/2004	BH09220	D5	2	2	female	WPY	19	1.26	0.22
2003/2004	BH09221	D5	2	2	male	EPY	21	-0.49	0.07
2003/2004	BH09222	AB5	3	3	male	EPY	18	-2.56	0.16
2003/2004	BH09223	AB5	3	3	female	WPY	18	0.26	0.16
2003/2004	BH09224	AB5	3	3	female	EPY	16	-2.68	0.01
2003/2004	BH09225	U6	2	2	female	unknown	18	-1.62	0.06
2003/2004	BH09226	U6	2	2	male	unknown	19	0.32	0.13
2003/2004	BH09227	I5	2	2	male	WPY	20	-0.56	0.10
2003/2004	BH09228	I5	2	2	male	WPY	20	-0.56	
2003/2004	BH09229	D6	3	2	female	EPY	19	0.32	0.08
2003/2004	BH09230	D6	3	2	female	WPY	18	-1.62	0.12
2003/2004	BH09231	A9	2	2	female	unknown	20	0.38	0.08
2003/2004	BH09232	A9	2	2	male	unknown	22	3.32	0.10
2003/2004	BH09233	D7	2	2	female	WPY	18	0.26	0.22
2003/2004	BH09234	D7	2	2	female	WPY	17	-2.62	0.07
2003/2004	BH09235	U4	2	2	male	unknown	22	2.38	0.10
2003/2004	BH09236	U4	2	2	male	unknown	21	2.32	-0.02

2003/2004	BH09237	X6	2	1	male	unknown	20	-0.56	-0.02
2003/2004	BH09238	B9	2	2	female	unknown	18	-0.68	0.05
2003/2004	BH09239	B9	2	2	male	unknown	21	0.44	0.08
2003/2004	BH09240	H12	3	2	female	WPY	20	2.26	0.05
2003/2004	BH09241	I6	3	2	male	unknown	19	0.32	0.11
2003/2004	BH09242	I6	3	2	male	unknown	21	0.44	0.08
2003/2004	BH09243	W6	3	3	male	unknown	17	-2.62	0.09
2003/2004	BH09244	W6	3	3	male	unknown	20	1.32	0.07
2003/2004	BH09245	W6	3	3	male	unknown	14	-1.87	0.02
2003/2004	BH09246	U7	3	3	male	unknown	21	2.32	0.12
2003/2004	BH09247	U7	3	3	female	unknown	18	-2.56	0.08
2003/2004	BH09248	U7	3	3	female	unknown	15	-1.81	0.17
2003/2004	BH09249	X8	3	3	female	unknown	17	-0.74	0.17
2003/2004	BH09250	X8	3	3	male	unknown	20	-1.49	0.14
2003/2004	BH09251	X8	3	3	male	unknown	19	-1.56	-0.03
2003/2004	BH09252	AB6	2	2	female	WPY	16	-2.68	0.08
2003/2004	BH09253	AB6	2	2	male	WPY	20	1.32	0.07
2003/2004	BH09254	N2	3	3	male	WPY	21	0.44	0.22
2003/2004	BH09255	N2	3	3	male	WPY	21	0.44	0.08
2003/2004	BH09256	N2	3	3	unknown	unknown	17	-1.68	
2003/2004	BH09257	G9	3	3	female	WPY	17	-0.74	
2003/2004	BH09258	I9	2	2	female	unknown	18	-0.68	0.06
2003/2004	BH09259	I9	2	2	female	unknown	17	-1.68	0.08
2003/2004	BH09260	F6	2	2	male	WPY	18	-1.62	0.12
2003/2004	BH09261	F6	2	2	male	EPY	18	-2.56	0.11
2003/2004	BH09262	J3	4	1	female	unknown	17	0.19	0.21
2003/2004	BH09263	M9	3	3	female	unknown	17	-2.62	0.05
2003/2004	BH09264	M9	3	3	male	unknown	20	-0.56	0.07
2003/2004	BH09265	M9	3	3	male	unknown	19	-0.62	0.13
2003/2004	BH09266	AS1	3	3	female	WPY	15	-0.87	0.04
2003/2004	BH09267	AS1	3	3	male	EPY	20	0.38	0.06
2003/2004	BH09268	AS1	3	3	male	WPY	18	-0.68	0.13

2003/2004	BH09269	AG3	2	2	male	EPY	22	3.32	0.15
2003/2004	BH09270	AG3	2	2	female	EPY	17	-0.74	0.09
2003/2004	BH09271	A12	3	3	female	unknown	17	-0.74	0.10
2003/2004	BH09272	A12	3	3	male	unknown	16	-0.81	0.38
2003/2004	BH09273	A12	3	3	male	unknown	21	0.44	0.05
2003/2004	BH09274	AB7	3	3	female	WPY	16	-1.74	
2003/2004	BH09275	AB7	3	3	male	EPY	21	2.32	0.05
2003/2004	BH09276	AB7	3	3	female	EPY	18	-0.68	0.14
2003/2004	BH09277	AO1	2	2	male	EPY	20	0.38	0.09
2003/2004	BH09278	AO1	2	2	female	WPY	18	1.19	0.24
2003/2004	BH09279	U8	3	3	female	unknown	19	1.26	0.06
2003/2004	BH09280	U8	3	3	female	unknown	16	-0.81	0.10
2003/2004	BH09281	U8	3	3	male	unknown	20	1.32	0.11
2003/2004	BH09282	I5	2	1	male	unknown	21	1.38	
2003/2004	BH09283	X10	3	3	female	unknown	18	-0.68	0.15
2003/2004	BH09284	X10	3	3	male	unknown	23	2.44	0.11
2003/2004	BH09285	X10	3	3	female	unknown	17	0.19	0.18
2003/2004	BH09286	AT2	2	1	male	unknown	17	0.19	
2003/2004	BH09287	C13	3	3	female	EPY	17	-0.74	0.11
2003/2004	BH09288	C13	3	3	female	EPY	16	-0.81	0.06
2003/2004	BH09289	C13	3	3	male	EPY	21	1.38	0.12
2003/2004	BH09290	AI2	3	3	male	WPY	20	-0.56	0.22
2003/2004	BH09291	AI2	3	3	female	WPY	19	1.26	0.12
2003/2004	BH09292	AI2	3	3	female	WPY	16	-2.68	0.10
2003/2004	BH09293	S7	3	3	female	EPY	18	-1.62	0.06
2003/2004	BH09294	S7	3	3	female	WPY	16	-1.74	0.15
2003/2004	BH09295	S7	3	3	male	EPY	19	-0.62	0.10
2003/2004	BH09296	S4	3	1	male	EPY	19	0.32	0.18
2003/2004	BH09297	F8	3	3	male	WPY	21	0.44	0.04
2003/2004	BH09298	F8	3	3	female	WPY	16	-3.62	0.05
2003/2004	BH09299	F8	3	3	female	WPY	16	-2.68	0.05
2003/2004	BH09300	BA2	3	3	female	unknown	17	-1.68	

2003/2004	BH09301	BA2	3	3	female	unknown	16	-1.74	0.05
2003/2004	BH09302	BA2	3	3	female	unknown	17	-1.68	0.04
2003/2004	BH09303	D10	2	2	male	WPY	21	2.32	0.04
2003/2004	BH09304	D10	2	2	female	WPY	17	-1.68	0.08
2003/2004	BH09305	AI2	3	3	female	EPY	15	0.07	0.11
2003/2004	BH09306	AI2	3	3	male	EPY	22	1.44	0.04
2003/2004	BH09307	E15	3	3	male	unknown	11	-3.93	0.08
2003/2004	BH09308	E15	3	3	male	unknown	16	-2.68	0.13
2003/2004	BH09309	E15	3	3	female	unknown	14	-3.74	0.07
2003/2004	BH09310	I8	2	1	male	unknown	23	3.38	0.12
2003/2004	BH09311	C12	2	2	male	EPY	20	-0.56	0.10
2003/2004	BH09312	C12	2	2	male	EPY	20	0.38	0.10
2003/2004	BH09313	O6	3	3	female	EPY	19	1.26	0.08
2003/2004	BH09314	O6	3	3	female	WPY	18	-0.68	0.13
2003/2004	BH09315	O6	3	3	female	WPY	18	-1.62	0.32
2003/2004	BH09316	F9	3	3	male	WPY	17	-3.56	0.08
2003/2004	BH09317	F9	3	3	male	WPY	19	0.32	0.02
2003/2004	BH09318	F9	3	3	male	WPY	20	-0.56	0.24
2003/2004	BH09319	H14	3	3	female	WPY	18	-0.68	0.17
2003/2004	BH09320	H14	3	3	female	EPY	17	1.13	0.01
2003/2004	BH09321	H14	3	3	female	WPY	16	-1.74	0.14
2003/2004	BH09324	X9	3	3	male	EPY	19	-0.62	0.07
2003/2004	BH09325	X9	3	3	male	EPY	19	-0.62	0.13
2003/2004	BH09326	X9	3	3	male	WPY	20	-0.56	0.15
2003/2004	BH09327	S2	3	2	male	WPY	18	0.26	0.18
2003/2004	BH09328	S2	3	2	male	WPY	19	-1.56	0.05
2003/2004	BH09329	AH2	3	3	female	EPY	17	-1.68	
2003/2004	BH09330	AH2	3	3	male	EPY	21	-0.49	0.08
2003/2004	BH09331	AH2	3	3	male	EPY	18	0.26	0.08
2003/2004	BH09332	F5	3	3	female	WPY	17	-2.62	0.09
2003/2004	BH09333	F5	3	3	female	WPY	17	-0.74	0.11
2003/2004	BH09334	F5	3	3	female	WPY	16	-2.68	0.09

2003/2004	BH09335	F10	3	3	male	EPY	19	-0.62	0.12
2003/2004	BH09336	F10	3	3	female	WPY	15	-1.81	0.17
2003/2004	BH09337	F10	3	3	female	WPY	15	-2.74	0.05
2003/2004	BH09338	B8	3	3	male	unknown	24	3.44	0.28
2003/2004	BH09339	B8	3	3	female	unknown	19	0.32	0.20
2003/2004	BH09340	B8	3	3	female	unknown	16	-1.74	0.18
2003/2004	BH09341	AX1	3	3	male	unknown	17	-0.74	0.13
2003/2004	BH09342	AX1	3	3	female	unknown	15	-1.81	0.10
2003/2004	BH09343	AX1	3	3	male	unknown	18	-1.62	0.17
2003/2004	BH09344	X7	3	3	male	WPY	22	2.38	0.08
2003/2004	BH09345	X7	3	3	female	WPY	19	0.32	0.04
2003/2004	BH09346	X7	3	3	female	WPY	19	0.32	0.10
2003/2004	BH09347	G10	3	3	male	EPY	22	0.51	0.05
2003/2004	BH09348	G10	3	3	male	EPY	21	-0.49	0.18
2003/2004	BH09349	W7	3	3	female	unknown	18	-0.68	
2003/2004	BH09350	W7	3	3	male	unknown	23	1.51	0.10
2003/2004	BH09351	W7	3	3	male	unknown	22	1.44	0.18
2003/2004	BH09352	AH1	3	3	male	EPY	20	0.38	0.10
2003/2004	BH09353	AH1	3	3	male	EPY	19	1.26	0.12
2003/2004	BH09354	AH1	3	3	female	EPY	18	-0.68	0.11
2003/2004	BH09355	AQ1	2	2	male	unknown	21	0.44	
2003/2004	BH09356	AQ1	2	2	male	unknown	21	0.44	
2003/2004	BH09357	AT1	3	3	female	unknown	19	1.26	
2003/2004	BH09358	AT1	3	3	male	unknown	21	2.32	
2003/2004	BH09359	AT1	3	3	male	unknown	20	-1.49	
2003/2004	BH09360	BC1	3	3	male	WPY	20	-0.56	0.14
2003/2004	BH09361	BC1	3	3	male	WPY	22	0.51	0.04
2003/2004	BH09362	AB8	3	2	male	EPY	23	3.38	0.08
2003/2004	BH09363	AB8	3	2	female	EPY	22	2.38	0.09
2003/2004	BH09376	D11	3	2	male	WPY	22	3.32	0.14
2003/2004	BH09377	D11	3	2	male	WPY	23	1.51	0.00
2003/2004	BH09378	I12	3	3	male	WPY	18	-1.62	0.16

2003/2004	BH09379	I12	3	3	male	WPY	16	-2.68	0.14
2003/2004	BH09380	I12	3	3	male	WPY	17	-3.56	0.05
2004/2005	BH09414	Q2	2	2	female	WPY	22	0.74	
2004/2005	BH09415	Q2	2	2	male	WPY	23	1.06	
2004/2005	BH09416	J2	3	3	female	unknown	20	-0.57	
2004/2005	BH09417	J2	3	3	female	unknown	21	0.43	
2004/2005	BH09418	J2	3	3	male	unknown	23	2.09	
2004/2005	BH09419	H3	3	3	male	EPY	22	1.77	
2004/2005	BH09420	H3	3	3	female	EPY	20	0.11	
2004/2005	BH09421	H3	3	3	male	EPY	23	2.43	
2004/2005	BH09431	N1	3	1	male	WPY	24	1.04	
2004/2005	BH09432	AF1	2	2	female	WPY	23	1.74	
2004/2005	BH09437	T3	3	3	male	EPY	24	2.06	
2004/2005	BH09438	T3	3	3	female	WPY	22	1.09	
2004/2005	BH09439	T3	3	3	female	WPY	20	0.11	
2004/2005	BH09466	AL4	3	3	female	WPY	23	1.74	
2004/2005	BH09467	AL4	3	3	female	WPY	19	-1.57	
2004/2005	BH09468	AL4	3	3	male	WPY	20	-0.23	
2004/2005	BH09469	C3	3	2	female	unknown	22	0.40	
2004/2005	BH09470	C3	3	2	male	unknown	21	-0.60	
2004/2005	BH09471	AF4	3	2	female	unknown	22	0.40	
2004/2005	BH09472	AF4	3	2	female	WPY	22	1.09	
2004/2005	BH09473	P8	4	3	male	WPY	21	0.77	
2004/2005	BH09474	P8	4	3	female	WPY	22	1.43	
2004/2005	BH09475	P8	4	3	male	WPY	20	0.11	
2004/2005	BH09485	AT7	3	1	female	unknown	18	-2.57	
2004/2005	BH09486	N6	2	1	male	WPY	23	0.38	
2004/2005	BH09487	Z11	3	3	female	WPY	19	-1.23	
2004/2005	BH09488	Z11	3	3	female	WPY	19	-1.57	
2004/2005	BH09489	Z11	3	3	male	unknown	19	-1.57	
2004/2005	BH09492	AL11	3	3	male	unknown	21	-1.62	
2004/2005	BH09493	AL11	3	3	male	EPY	20	-1.26	

2004/2005	BH09494	AL11	3	3	male	WPY	21	-1.28	
2004/2005	BH09495	BC1	3	3	male	WPY	20	-1.60	
2004/2005	BH09496	BC1	3	3	male	WPY	21	-0.94	
2004/2005	BH09497	AB7	2	2	female	unknown	19	-1.57	
2004/2005	BH09498	AB7	2	2	male	unknown	22	0.74	
2004/2005	BH09499	BB1	3	3	female	unknown	21	-0.26	
2004/2005	BH09500	BB1	3	3	male	unknown	20	-1.26	
2004/2005	BH36301	BB1	3	3	female	unknown	20	-1.60	
2004/2005	BH36302	BA2	3	3	male	unknown	22	-0.28	
2004/2005	BH36303	BA2	3	3	female	unknown	20	-0.57	
2004/2005	BH36304	BA2	3	3	male	unknown	22	0.40	

APPENDIX C

Nests from Addo Elephant Park (South Africa) in seasons 2002/2003 to 2004/2005

Data presented here are number of eggs in nest, number of young in nest, fledging success (all eggs hatched, all young fledged), sex ratio (proportion of males), average weight of nestlings, average body condition index of nestlings, average reaction to immune challenge with PHA (mm) , average temperature 30 days before fledging, total rainfall before fledging for nests of the red bishop in three conclusive seasons in the Addo Elephant National Park, South Africa. All measurements were taken on day 10 ± 1 after hatching.

SEASON	NEST	EGGS in NEST	YOUNG in NEST	FLEDGED COMPLETE	SEX RATIO	AVERAGE WEIGHT (g)	AVERAGE BODY CONDITION INDEX	AVERAGE PHA (mm)	AVERAGE TEMPERATUR 30 DAYS (°C)	TOTAL RAIN 30 DAYS (mm)
2002/2003	AG1	3	3	YES	0.00	17.33	-0.72	0.10	19.36	7.00
2002/2003	AG3	3	3	YES	0.67	20.00	1.01	0.20	18.75	20.60
2002/2003	AI1	3	3	YES	0.67	20.33	1.34	.	19.02	7.00
2002/2003	AI3	2	2	YES	0.00	19.00	0.79	0.25	19.36	7.00
2002/2003	AI5	3	3	YES	0.50	20.00	0.85	0.53	18.74	20.60
2002/2003	AK12	3	3	YES	0.67	18.00	-1.31	0.10	23.22	5.40
2002/2003	AN2	3	3	YES	0.00	18.50	1.69	.	18.87	5.10
2002/2003	AO2	3	3	YES	0.67	20.33	1.65	0.13	18.75	20.60
2002/2003	AQ3	3	2	NO	0.50	20.00	1.79	0.01	18.75	20.60
2002/2003	AR1	3	3	YES	1.00	22.50	1.94	.	18.66	7.00
2002/2003	AT1	3	3	YES	0.67	19.67	0.98	.	19.36	7.00
2002/2003	AU1	3	3	YES	0.50	17.50	-1.65	0.30	18.97	4.60
2002/2003	AU2	4	4	YES	1.00	18.33	-0.97	0.22	19.02	7.00

2002/2003	AY2	3	3	YES	0.33	17.67	-1.33	0.13	18.97	4.60
2002/2003	AY4	3	3	YES	0.50	20.50	3.22	0.17	19.06	21.60
2002/2003	BA19	3	3	YES	0.00	15.67	-1.45	0.08	22.99	5.40
2002/2003	BA5	4	4	YES	0.50	18.00	-0.68	.	18.97	7.00
2002/2003	BA6	3	3	YES	0.67	20.67	1.36	.	19.36	7.00
2002/2003	BB1	5	5	YES	0.67	18.33	-0.66	.	18.97	7.00
2002/2003	BB2	3	3	YES	0.67	18.33	-0.66	.	19.01	7.00
2002/2003	BB5	3	3	YES	0.00	21.00	2.79	.	18.75	20.60
2002/2003	BE3	4	4	YES	0.50	17.75	-0.70	0.13	18.97	4.60
2002/2003	BE4	3	3	YES	0.67	17.67	-0.39	0.09	18.17	5.50
2002/2003	BE5	3	3	YES	0.33	19.00	0.63	.	18.74	20.60
2002/2003	BF2	3	3	YES	0.33	19.00	0.01	0.26	18.75	20.60
2002/2003	BF4	2	2	YES	0.00	18.50	1.22	0.10	19.03	20.60
2002/2003	BH9	2	2	YES	1.00	21.50	1.41	0.18	23.07	5.40
2002/2003	C4	4	4	YES	0.50	20.00	1.55	.	18.97	7.00
2002/2003	E13	3	2	NO	0.00	18.00	0.26	0.04	22.96	5.40
2002/2003	E14	3	2	NO	0.00	15.50	-2.71	0.11	24.61	18.70
2002/2003	F1	5	5	YES	0.67	20.67	1.36	.	18.66	7.00
2002/2003	F2	5	5	YES	1.00	22.00	2.38	0.26	18.66	7.00
2002/2003	P2	4	4	YES	0.75	19.00	0.32	0.24	19.36	7.00
2002/2003	P4	3	3	YES	0.67	20.00	1.94	0.20	19.36	7.00
2002/2003	S2	3	3	YES	0.33	20.00	1.32	0.24	18.87	5.10
2002/2003	S8	3	3	YES	0.67	21.00	2.63	0.10	19.49	23.60
2002/2003	T13	3	3	YES	0.00	16.67	-1.08	0.35	23.41	9.30
2002/2003	U5	4	4	YES	0.50	18.00	-0.92	0.09	18.92	20.60
2002/2003	U6	3	3	YES	0.67	20.67	1.98	0.19	18.74	20.60
2002/2003	U7	3	3	YES	0.67	18.67	0.30	0.14	19.00	20.60
2002/2003	V1	3	2	NO	0.00	18.50	0.29	.	19.02	7.00
2002/2003	W3	3	3	YES	0.33	19.00	0.94	0.05	19.01	7.00
2002/2003	Y2	3	3	YES	0.67	20.33	1.34	0.33	19.05	5.10
2003/2004	A1	2	2	YES	0.50	16.50	-0.78	0.19	21.53	3.50
2003/2004	A12	3	3	YES	0.67	18.00	-0.37	0.18	23.05	38.30

2003/2004	A5	3	3	YES	0.00	18.33	0.59	0.09	22.10	35.20
2003/2004	A8	2	2	YES	0.50	19.00	-0.15	0.10	24.54	44.90
2003/2004	A9	2	2	YES	0.50	21.00	1.85	0.09	24.48	20.50
2003/2004	AB5	3	3	YES	0.33	17.33	-1.66	0.11	24.54	44.90
2003/2004	AB6	2	2	YES	0.50	18.00	-0.68	0.08	24.49	52.40
2003/2004	AB7	3	3	YES	0.33	18.33	-0.04	0.10	23.05	38.30
2003/2004	AB8	3	2	NO	0.50	22.50	2.88	0.09	22.21	7.70
2003/2004	AG3	2	2	YES	0.50	19.50	1.29	0.12	23.17	39.10
2003/2004	AH1	3	3	YES	0.67	19.00	0.32	0.11	22.33	7.70
2003/2004	AH2	3	3	YES	0.67	18.67	-0.64	.	22.49	7.70
2003/2004	AI2	3	3	YES	0.40	18.40	-0.09	0.12	22.61	10.80
2003/2004	AO1	2	2	YES	0.50	19.00	0.79	0.17	22.79	10.30
2003/2004	AQ1	2	2	YES	1.00	21.00	0.44	.	22.21	7.70
2003/2004	AS1	3	3	YES	0.67	17.67	-0.39	0.08	23.17	39.10
2003/2004	AT1	3	3	YES	0.67	20.00	0.69	.	22.21	7.70
2003/2004	AT2	2	1	NO	1.00	17.00	0.19	.	22.79	10.30
2003/2004	AX1	3	3	YES	0.67	16.67	-1.39	0.13	22.33	7.70
2003/2004	B8	3	3	YES	0.33	19.67	0.67	0.22	22.33	7.70
2003/2004	B9	2	2	YES	0.50	19.50	-0.12	0.07	24.48	20.50
2003/2004	BA2	3	3	YES	0.00	16.67	-1.70	0.05	22.65	10.80
2003/2004	BC1	3	3	YES	1.00	21.00	-0.02	0.09	22.21	7.70
2003/2004	C12	2	2	YES	1.00	20.00	-0.09	0.10	22.61	10.80
2003/2004	C13	3	3	YES	0.33	18.00	-0.06	0.10	22.79	10.30
2003/2004	D10	2	2	YES	0.50	19.00	0.32	0.06	22.65	10.80
2003/2004	D11	3	2	NO	1.00	22.50	2.41	.	21.95	7.70
2003/2004	D5	2	2	YES	0.50	20.00	0.38	0.15	24.54	44.90
2003/2004	D6	3	2	NO	0.00	18.50	-0.65	0.10	24.48	20.50
2003/2004	D7	2	2	YES	0.00	17.50	-1.18	0.15	24.48	20.50
2003/2004	E11	2	2	YES	0.50	17.50	-3.06	0.08	24.54	44.90
2003/2004	E15	3	3	YES	0.67	13.67	-3.45	0.09	22.61	10.80
2003/2004	F10	3	3	YES	0.33	16.33	-1.72	0.11	22.33	7.70
2003/2004	F5	3	3	YES	0.00	16.67	-2.02	0.10	22.33	7.70

2003/2004	F6	2	2	YES	1.00	18.00	-2.09	0.12	23.94	57.30
2003/2004	F8	3	3	YES	0.33	17.67	-1.95	0.05	22.65	10.80
2003/2004	F9	3	3	YES	1.00	18.67	-1.26	0.11	22.45	7.70
2003/2004	G1	2	1	NO	1.00	17.00	-3.56	0.10	21.53	3.50
2003/2004	G10	3	3	YES	1.00	21.50	0.01	0.12	22.33	7.70
2003/2004	G9	3	3	YES	0.00	17.00	-0.74	.	24.42	52.40
2003/2004	H12	3	2	NO	0.00	20.00	2.26	0.05	24.39	52.40
2003/2004	H14	3	3	YES	0.00	17.00	-0.43	0.11	22.45	7.70
2003/2004	I12	3	3	YES	1.00	17.00	-2.62	0.12	21.95	7.70
2003/2004	I5	2	2	YES	1.00	20.33	0.09	.	22.79	10.30
2003/2004	I6	3	2	NO	1.00	20.00	0.38	0.10	24.36	52.40
2003/2004	I8	2	1	NO	1.00	23.00	3.38	0.12	22.61	10.80
2003/2004	I9	2	2	YES	0.00	17.50	-1.18	0.07	23.88	57.00
2003/2004	J3	4	1	NO	0.00	17.00	0.19	0.21	23.94	57.30
2003/2004	M9	3	3	YES	0.67	18.67	-1.26	0.08	23.17	39.10
2003/2004	N2	3	3	YES	0.67	19.67	-0.26	0.15	24.42	52.40
2003/2004	O6	3	3	YES	0.00	18.33	-0.35	0.18	22.61	10.80
2003/2004	S2	3	2	NO	1.00	18.50	-0.65	0.12	22.49	7.70
2003/2004	S4	3	1	NO	1.00	19.00	0.32	0.18	22.65	10.80
2003/2004	S7	3	3	YES	0.33	17.67	-1.33	0.10	22.65	10.80
2003/2004	U4	2	2	YES	1.00	21.50	2.35	0.04	24.48	20.50
2003/2004	U6	2	2	YES	0.50	18.50	-0.65	0.10	24.63	44.90
2003/2004	U7	3	3	YES	0.33	18.00	-0.68	0.12	24.36	52.40
2003/2004	U8	3	3	YES	0.33	18.33	0.59	0.09	22.79	10.30
2003/2004	W6	3	3	YES	1.00	17.00	-1.06	0.06	24.36	52.40
2003/2004	W7	3	3	YES	0.67	21.00	0.76	.	22.33	7.70
2003/2004	X10	3	3	YES	0.33	19.33	0.65	0.15	22.79	10.30
2003/2004	X6	2	1	NO	1.00	20.00	-0.56	-0.02	24.48	20.50
2003/2004	X7	3	3	YES	0.33	20.00	1.01	0.07	22.33	7.70
2003/2004	X8	3	3	YES	0.67	18.67	-1.26	0.09	24.49	52.40
2003/2004	X9	3	3	YES	1.00	19.33	-0.60	0.12	22.49	7.70
2004/2005	AB7	2	2	YES	0.50	20.50	-0.41	1.00	23.04	32.40

2004/2005	AF1	2	2	YES	0.00	23.00	1.74	1.00	22.62	8.40
2004/2005	AF4	3	2	NO	0.00	22.00	0.74	1.00	23.35	29.00
2004/2005	AL11	3	3	YES	1.00	20.67	-1.38	1.00	23.04	32.40
2004/2005	AL4	3	3	YES	0.33	20.67	-0.02	1.00	23.44	31.00
2004/2005	AT7	3	1	NO	0.00	18.00	-2.57	1.00	23.03	79.30
2004/2005	BA2	3	3	YES	0.67	21.33	-0.15	1.00	23.14	32.40
2004/2005	BB1	3	3	YES	0.33	20.33	-1.04	1.00	23.14	32.40
2004/2005	BC1	3	3	YES	1.00	20.50	-1.27	1.00	23.04	32.40
2004/2005	C3	3	2	NO	0.50	21.50	-0.10	1.00	23.44	31.00
2004/2005	H3	3	3	YES	0.67	21.67	1.44	1.00	22.01	3.80
2004/2005	J2	3	3	YES	0.33	21.33	0.65	1.00	22.07	3.80
2004/2005	N1	3	1	NO	1.00	24.00	1.04	1.00	22.62	8.40
2004/2005	N6	2	1	NO	1.00	23.00	0.38	1.00	23.03	79.30
2004/2005	P8	4	3	NO	0.67	21.00	0.77	1.00	23.92	24.60
2004/2005	Q2	2	2	YES	0.50	22.50	0.90	1.00	22.06	3.80
2004/2005	T3	3	3	YES	0.33	22.00	1.09	1.00	22.84	8.40
2004/2005	Z11	3	3	YES	0.33	19.00	-1.46	1.00	23.03	79.30

SUMMARY

Summary in English

The life-history theory postulates that individuals need to allocate limited resources (i.e. energy expenditure, time, effort) to multiple conflicting life functions (i.e. growth, health maintenance, reproduction). Resources available to any given individual are always finite and any investment in a life function thus diminishes the resources available for another. For example, the costs of reproduction may be paid in terms of energy being diverted away from body repair and maintenance and by reducing investment in immunological competence, thus involving a trade-off between reproductive success and health. In my thesis, I present three studies centred around trade-offs between reproductive success and health in adult and nestling red bishops (*Euplectes orix*), a colonial breeding weaverbird common in Sub-Saharan Africa. Red bishops are sexually dimorph, they have a polygynous breeding system, and a high degree of extrapair matings.

In the first part of my thesis, I present an experimental study with artificially elevated testosterone levels in breeding male red bishops kept in an aviary in Oldenburg, Germany. According to the immunocompetence handicap hypothesis (ICHH), the hormone testosterone is thought to take the role of a double-edged sword, mediating a trade-off between investment into reproduction and immunological condition. Breeding males were divided into two groups. One, the experimental group, received a subcutaneous implantation of testosterone-releasing pellets, while a control group was treated with placebos. I then compared behaviour, development of territory size, and immunological condition (as assessed through a white blood cell count) of the two groups. To assess the effect of testosterone on acquired immunity, I performed a repeated phytohaemagglutinin skin swelling test (PHA-test), a common measure of the T-cell-mediated immune response in birds. Males with elevated levels of testosterone succeeded in enlarging their territory and presented more courtship behaviour, while at the same time showing a decrease in their immunological condition, expressed by an increased heterophil/lymphocyte ratio compared with the control group. The response to a repeated immune challenge with PHA showed an increase in the control group between the first and second PHA-test. This result can be expected under the assumption that repeated exposure to an antigen (PHA) enhances the immune response, because the acquired immune

system ‘learns’ how to cope with the antigen. Males from the experimental group did not show an enhanced immune response in the second PHA-test, thus indicating that testosterone treatment directly or indirectly suppresses some component of the acquired immune response.

Female promiscuity in birds is often explained by the ‘genetic benefit’ model, which sees extrapair copulations as a means to enhance offspring viability, and thus female life-time reproductive success. If the ‘genetic benefit’ hypothesis holds true, extrapair young should have a genetic advantage over their maternal half-sibs raised in the same brood. In the second part of my thesis, I used the nestlings’ immunocompetence, the ability to cope with infections, as a quantifiable measure for offspring quality. I tested whether the cell-mediated immune reaction, as assessed by the PHA-test, differs between extrapair and within-pair nestlings from naturally occurring mixed-paternity broods. The strength of the nestlings’ immune response was positively linked to general health status and immunological condition of their genetic fathers, therefore nestling immunocompetence seems to be determined by a heritable component. Extrapair young in this study were actually significantly less immunocompetent than their within-pair half-sibs, a result that does not lend support to the ‘genetic benefit’ hypothesis. A competing theory, the ‘fertility insurance’ hypothesis, states that females copulate with multiple males simply to guarantee fertilisation of eggs, as their mates might suffer from sperm depletion because of frequent copulations with their multiple female partners. I conclude that female red bishops paired with a successful high-quality male engage in extrapair copulations to insure against temporary infertility in their social mates. As this result was significant only in the hotter of two study seasons, environmental factors might obscure variation in nestling fitness arising from female extrapair mating behaviour.

Another trade-off parents are expected to face is the topic of the final part of my thesis. According to the life-history theory, quantity and quality of the offspring is subjected to a trade-off: nestling birds from larger broods should thus have a decreased viability and/or immunological condition compared with nestlings of smaller broods. In addition, the sex ratio in larger broods should be skewed in favour of the smaller sex, as proposed by the sex-allocation theory. Nestlings from the smaller sex, the females in red bishops, are ‘cheaper’ in terms of provisioning, and

they suffer less from increased competition in larger broods or from adverse environmental conditions. Furthermore, nestling health might vary in relation to nestling sex, brood sex ratio, season, or environmental conditions. I investigated how nestling sex, clutch and brood size, season and weather affect nestling condition and immunocompetence (as assessed by a PHA-test) in a free-living colony of the subtropical red bishop. Nestling condition and immunocompetence differed significantly among the three study seasons, indicating a strong environmental component in nestling health. In general, male nestlings had a better body condition and they were heavier than female nestlings of the same age, but the sexes did not differ significantly in their immunocompetence. I found no decrease in nestling condition or immunocompetence with increasing clutch or brood size. Likewise, there was no evidence that the larger sex was more susceptible to increased competition in large broods or adverse weather conditions, nor did I find biased brood sex ratios in broods of different sizes. In conclusion, I argue that the lack of the trade-offs which are proposed by life-history and sex-allocation theory, might be explained by a slower 'pace of life' of tropical and subtropical birds as compared to temperate zone bird species.

Zusammenfassung auf Deutsch

Die so genannte „Life-history“-Theorie besagt, dass Individuen ihre limitierten Ressourcen (z. B. Energie, Nahrung, Zeit) auf mehrere miteinander konkurrierende lebensnotwendige Aufgaben (z. B. Wachstum, Gesundheit, Fortpflanzung) aufteilen müssen. Da die einem Individuum zur Verfügung stehenden Ressourcen immer endlich sind, mindert jede Investition in eine Aufgabe folglich die für andere Aufgaben verfügbaren Ressourcen. So können zum Beispiel die Kosten für einen erhöhten Fortpflanzungsaufwand durch Energie gedeckt werden, die andernfalls in die Gesundheit investiert worden wäre. Eine solche Verminderung der Investition in das Immunsystem zu Gunsten des Fortpflanzungserfolges wird als „Trade-off“ bezeichnet. In meiner Doktorarbeit stelle ich drei Studien vor, die sich mit „Trade-offs“ zwischen Gesundheit und Fortpflanzungserfolg bei erwachsenen Oryxwebern (*Euplectes orix*) und ihren Nestlingen beschäftigen. Die polygynen Oryxweber sind eine im südlichen Afrika weit verbreitete, sexuell dimorphe Webervogelart mit einem hohen Grad an so genannten Außer-Paar-Kopulationen (Kopulationen, die nicht zwischen verpaarten Individuen stattfinden).

Im ersten Teil meiner Doktorarbeit stelle ich eine experimentelle Studie vor, in der die Testosteronlevel von brütenden männlichen Oryxwebern, die in einer Voliere in Oldenburg, Deutschland, gehalten werden, künstlich erhöht wurden. Nach der so genannten “Immunocompetence handicap”-Hypothese (ICHH) fällt dem Hormon Testosteron die Rolle eines zweischneidigen Schwertes zu, das die Regulation des “Trade-offs” zwischen Fortpflanzungsaufwand und Gesundheitszustand steuert, indem es den Fortpflanzungserfolg steigert, das Immunsystem aber unterdrückt. Brütende Männchen wurden in zwei Gruppen aufgeteilt. Der ersten Gruppe, der Experimentalgruppe, wurde ein Testosteron freisetzendes Pellet unter die Haut injiziert, während der Kontrollgruppe ein Placebopellet eingesetzt wurde. Anschließend habe ich das Verhalten, die Entwicklung der Reviergröße (ein Maßstab für den Fortpflanzungserfolg) und den Gesundheitszustand (bestimmt durch ein Differenzialblutbild) der zwei Gruppen miteinander verglichen. Um den vermuteten negativen Einfluss des Testosterons auf die erworbene Immunantwort zu bestimmen, habe ich einen wiederholten Phytohaemagglutinin-Hautschwellungstest (PHA-Test), ein Standardtest für die Stärke der T-Zell-Aktivität in Vögeln, durchgeführt. Männchen

mit erhöhtem Testosteronlevel waren in der Lage ihr Territorium zu vergrößern und zeigten erhöhtes Balzverhalten, gleichzeitig verschlechterte sich ihr Gesundheitszustand, gemessen am Anstieg der Heterophilen/Lymphozyten-Ratio, im Vergleich zu Männchen der Kontrollgruppe. Die Stärke der Immunantwort, ausgelöst durch die Konfrontation des Immunsystems mit einem neuen Antigen (PHA), steigerte sich in der Kontrollgruppe von der ersten zur zweiten Exposition mit PHA. Dieses Ergebnis würde man unter der Vorraussetzung erwarten, dass die wiederholte Exposition zu einer Verstärkung der Immunantwort durch Komponenten der erlernten Immunantwort führt. Männchen der Experimentalgruppe zeigten keine erhöhte Immunreaktion beim zweiten PHA-Test, ein Hinweis auf die direkte oder indirekte Unterdrückung der erlernten Antwort des Immunsystems durch Testosteron.

Promiskuität in weiblichen Vögeln wird oftmals durch das „Genetic benefit"-Modell erklärt, das Verpaarungen außerhalb der Paarbindung als Versuch der Weibchen ansieht, die Qualität ihrer Nachkommen zu erhöhen und somit den eigenen Lebenszeit-Reproduktionserfolg zu steigern. Wenn dieses Modell zum tragen kommt, sollten Nachkommen aus Außer-Paar-Kopulationen einen genetischen Vorteil gegenüber ihren Halbgeschwistern mütterlicherseits aus dem selben Nest besitzen. Im zweiten Teil meiner Doktorarbeit habe ich die Immunokompetenz von Nestlingen, ihre Fähigkeit Infektionen effektiv zu bekämpfen, als Maßstab für die Qualität der Nestlinge verwendet. Ich habe untersucht, ob sich die Stärke der T-Zell-Aktivität, gemessen durch einem PHA-Test, zwischen Außer-Paar-Nachkommen und Inner-Paar-Nachkommen in natürlich vorkommenden gemischten Bruten unterscheidet. Die Stärke des Immunsystems der Nestlinge hängt signifikant positiv mit dem allgemeinen Gesundheitszustand und der Stärke des Immunsystems ihrer genetischen Väter zusammen, woraus sich schließen lässt, dass die Immunokompetenz bei Oryxwebern zum Teil vererbt wird. Außer-Paar-Nachkommen in dieser Studie hatten eine deutlich geringere Immunokompetenz als ihre Inner-Paar-Halbgeschwister, ein Ergebnis, das den Vorhersagen des „Genetic benefit"-Modells widerspricht. Eine konkurrierende Theorie, die „Fertility insurance"-Hypothese, besagt, dass Weibchen mit mehreren Männchen kopulieren, um die Befruchtung ihrer Eier sicher zu stellen, da ihre Partner auf Grund von häufigen Kopulation mit ihren zahlreichen Partnerinnen unter Erschöpfung des Spermienvorrates leiden können. Ich folgere aus den Ergebnissen, dass Weibchen

des Oryxwebers die mit erfolgreichen, qualitativ hochwertigen Männchen verpaart sind, Außer-Paar-Kopulationen durchführen, um sich gegen zeitweilige Unfruchtbarkeit ihres Partners abzusichern. Da dieses Ergebnis ausschließlich in der heißeren von zwei Saisons signifikant ist, schließe ich, dass Umwelteinflüsse Unterschiede in der Fitness der Nestlinge, die durch das weibliche Paarungsverhalten bedingt wurden, überlagern können.

Ein weiterer „Trade-off“, dem sich Eltern ausgesetzt sehen, ist das Thema des abschließenden Teils meiner Doktorarbeit. Nach der „Life-History“-Theorie sollten Qualität und Quantität des Nachwuchses einem „Trade-off“ unterliegen: demzufolge sollten Nachkommen aus größeren Bruten im Vergleich zu Nestlingen aus kleineren Gelegen eine herabgesetzte Lebensfähigkeit und/oder verminderte Gesundheit aufweisen. Zusätzlich sollte nach einer Voraussage der so genannten „Sex-allocation“-Theorie in großen Gelegen der Anteil an Nachkommen des kleineren Geschlechtes maximiert werden. Die Nestlinge des kleineren Geschlechts, im Falle der Oryxweber sind dies die Weibchen, sind weniger kostenintensiv in der Versorgung durch die Eltern und weniger empfindlich für negative Umwelteinflüsse. Die Gesundheit der Nestlinge kann außerdem mit dem Geschlecht, dem Geschlechterverhältnis in der Brut, der Saison und durch Umwelteinflüsse variieren. Ich habe untersucht, wie das Geschlecht, Gelege- und Brutgröße, Saison und Wetter auf die Immunokompetenz (bestimmt durch einen PHA-Test) und den Entwicklungszustand von Nestlingen einer Brutkolonie des subtropischen Oryxwebers Einfluss nehmen. Die Gesundheit und Immunokompetenz der Nestlinge unterschied sich signifikant zwischen den drei Untersuchungssaisons, ein Hinweis auf einen starken Einfluss von Umweltfaktoren. Männliche Nestlinge hatten einen besseren Entwicklungszustand und waren schwerer als Weibchen des selben Alters, aber die Geschlechter unterschieden sich nicht im Bezug auf die Immunokompetenz. Ebenso gab es keine Hinweise, dass das größere Geschlecht stärker unter erhöhter Konkurrenz in großen Bruten oder negativen Umwelteinflüssen leidet, noch konnte ich eine Anpassung des Geschlechterverhältnisses an die Brutgröße feststellen. Ich diskutiere die Möglichkeit, dass die Abwesenheit der von „Life-history“- und „Sex-allocation“-Theorie vorhergesagten „Trade-offs“ durch den langsameren „Pace-of-life“ tropischer und subtropischer Vogelarten, im Vergleich zu Arten aus der gemäßigten Zone, erklärt werden kann.

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Finally, I want to thank my supportive family. My father, Horst Edler, is unfortunately not around anymore to see me bringing my thesis to an end, but without his encouragement, pride in me, support, and love I would not have started in the first place. My mother Sigrid Edler and her husband Andreas Thomas not only made sure I had clothes on my back and got properly fed when I ran out of money, they also always gave me the feeling I am doing the right thing. The Friedrich family took me in on my way to and from Africa, picking me up at the airport no matter what time. I always enjoyed their hospitality and I am deeply in dept for the guidance of my aunt Monika Friedrich. My sister Britta Streit and her husband Thorsten listened patiently when I was boasting about my progress or moaning about all the delays. Big thanks to all my other aunts, uncles, cousins, and my grandma for their loving support…

…and to Booga, for still being around after all these years.

Curriculum vitae

Personal information

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Private address	Meesjenweg 4 26215 Wiefelstede-Nuttel Germany Tel: +49(0)4402 916 0 951
Date/location of birth	9th of July 1972, Leonberg, Germany
Nationality	German

University education

since 10/2002	PhD studies at the Carl-von-Ossietzky-University Oldenburg/Germany Titel of thesis 'Immunocompetence, testosterone and breeding success in red bishop (*Euplectes orix*)'
11/1995 – 10/2001	Biology; Technical University Munich/Germany; from 05/98 majored in zoology
Degree:	Diploma (2.1) Titel of diploma thesis "Blutparasiten, Immunokompetenz und Fortpflanzungserfolg beim Oryxweber *(Euplectes orix)*"

Field studies and teaching experience during PhD-studies

10/2005 – 11/2005	Field studies in the Addo Elephant National Park/South Africa
05/2005	Supervision of practical course 'Behaviour and information processing'
10/2004 – 03/2005	Field studies in the Addo Elephant National Park/South Africa
05/2004	Supervision of practical course 'Behaviour and information processing'
10/2003 – 03/2004	Field studies in the Addo Elephant National Park/South Africa
11/2003 – 12/2003	Supervision of field studies of diploma student
05/2003	Supervision of practical course 'Behaviour and information processing'
10/2002 – 03/2003	Field studies in the Addo Elephant National Park/South Africa

Repeated employment as scientific assistant and researcher in the research group 'Zoophysiologie und Verhalten', Carl-von-Ossietzky-University Oldenburg/Germany

Grants during the PhD

09/2005	Wolfgang-Schulenberg-Scholarship of the University Oldenburg
08/2004	Travel grant of the 'Universitätsgesellschaft Oldenburg' (UGO)
01/2004 – 03/2004	Scholarship of the 'Deutscher Akademischer Austauschdienst' (DAAD)

Conferences during the PhD

2007	Conference of the European Ornithological Union, Vienna, Austria. Talk: EDLER R, GOYMANN W, SCHWABL I, FRIEDL TWP 'An experimental test of the immunocompetence handicap hypothesis in male red bishops (*Euplectes orix*)'. Session chair 'Behaviour, hormones and colouration'.
2005	98. Jahresversammlung der Deutschen Zoologischen Gesellschaft, Bayreuth, Germany. Co-author of talk by TWP Friedl: EDLER R, FRIEDL TWP 'Immunocompetence of extrapair and within-pair young in the polygynous red bishop (*Euplectes orix*)'.

2005 XXIX. International Ethological Conference in Budapest, Hungary.
Poster:
EDLER R, GOYMANN W, SCHWABL I, FRIEDL TWP
'The influence of testosterone on behaviour and immunocompetence in male red bishops (*Euplectes orix*)'.

2004 10th International Behavioral Ecology Congress in Jyväskylä, Finnland.
Poster:
EDLER R, FRIEDL TWP
'Extrapair paternity and offspring immunocompetence in the polygynous red bishop (*Euplectes orix*)'.

2003 96. Jahresversammlung der Deutschen Zoologischen Gesellschaft, Berlin, Germany.
Poster:
EDLER R, FRIEDL TWP
'Viability and immunocompetence in nestlings of the red bishop (*Euplectes orix*): effects of brood size and nestling sex'.

2002 1st European Conference on Behavioural Biology, Münster, Germany.
Poster:
EDLER R, FRIEDL TWP, KLUMP GM
'Immunocompetence and reproductive performance in the polygynous red bishop (*Euplectes orix*)'.

Oldenburg, den 20.09.07

Ralf Edler

Oldenburg, den 20. September 2007

Erklärung gemäß § 10 Absatz (2) Punkt b) der Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg vom 11.12.2003

Die vorgelegte Arbeit wurde selbständig verfasst und es wurden nur die angegebenen Hilfsmittel benutzt. Kapitel 2 der vorgelegten Arbeit befindet sich unter dem Titel *"Within-pair young are more immunocompetent than extrapair young in mixed-paternity broods of the red bishop"* bei Animal Behaviour in Druck (siehe Literaturliste), alle anderen Teile der Doktorarbeit sind noch nicht veröffentlicht.

Ralf Edler

Literaturliste

Edler R, Friedl TWP (2007) Within-pair young are more immunocompetent than extrapair young in mixed-paternity broods of the red bishop. Anim Behav, in press.