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Sex differences in cell-mediated immunity in the Magellanic penguin *Spheniscus magellanicus*

Juan Moreno[1], Jaime Potti[2], Pablo Yorio[3] & Pablo García Borboroglu[3]

[1] *Museo Nacional de Ciencias Naturales-CSIC, Departamento de Ecología Evolutiva, J. Gutierrez Abascal 2, E-28006 Madrid, Spain (e-mail: jmoreno@mncn.csic.es)*
[2] *Departamento de Biología Animal. Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain*
[3] *CENPAT-CONICET, Bv. Brown S/N, Puerto Madryn, Chubut, Argentina*

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Males in domestic and laboratory populations usually display a lower immunocompetence than females due to differential action of steroid hormones, but information for natural populations is scant. Here we show that male Magellanic penguins *Spheniscus magellanicus* had a significantly lower cell-mediated immunity at hatching of their chicks than females. This was assayed through the phytohemagglutinin injection assay, a standard method of measuring T-cell-dependent immunocompetence in birds. Breeding phenology affected condition at laying and cell-mediated immunity at hatching for males, with higher values for late-breeding males. In females, condition at laying declined with breeding date, with no link with immunocompetence at hatching. Early breeding males may incur date-dependent costs in terms of condition and subsequent immunocompetence.

Introduction

It is well established that sexual dimorphism exists within the immune system of poultry (Norton & Wira 1977, Marsh 1992) and laboratory mice (Astorquiza *et al*. 1987, Gaillard & Spinedi 1998). Although there are many exceptions (e.g. Klein *et al*. 1998), males in birds and mammals generally show weaker immune responses to a variety of antigens (Schuurs & Verheul 1990, Zuk & McKean 1996). Male birds have also consistently smaller immune organ masses than females (Møller *et al*. 1998). These sexual differences are mediated to some degree by the immunosuppressive effects of testosterone (Cohn 1979, Grossman 1985, 1989, Olsen & Kovacs 1996, Gaillard & Spinedi 1998). The level of sex steroids also seems to play an important role in the production and development of the immunocompetent cells (Marsh 1992). Sex differences in immunocompetence may cause the observed higher prevalences of infections and parasites in males (Alexander & Stimson 1988, Poulin 1996, Zuk & McKean 1996), with potential ecological and evolutionary implications (Zuk 1990, Møller *et al*. 1999).

Competition for early arrival at the breeding grounds is supposed to be stronger for males than for females, as males try to control resources necessary for successful reproduction in order to attract mates or to pair with the females in best condition (Darwin 1871). This has led to the prevalent earlier arrival of males in migratory birds. This competition for the best territories may lead to higher costs in terms of nutrition for early arriving males than for late males (Kokko 1999). These costs could be also expressed in terms of immunocompetence, although the possiblity remains unchecked. Females have to produce eggs after arrival, and only those in good condition may be able to accumulate the necessary reserves early in the breeding season (Drent & Daan 1980). Thus, we should expect a negative association of female condition at laying with breeding date, which could be reflected in a similar phenological trend in immunity.

Although the evidence of sex differences in immunocompetence is well established in domestic animals and laboratory settings, there is still scarce evidence about these inequalities in wild populations. In the present study, we analyse possible differences in a population of Magellanic penguins *Spheniscus magellanicus* with respect to the T-cell-dependent immune response to phytohemagglutinin (PHA), and relate it to gender and body condition. We also explore the effects of phenology on immunocompetence, given the differential costs of early arrival at the breeding grounds in the two sexes.

Material and methods

Magellanic penguins breed along the South American coast and nearby islands on both the Atlantic and Pacific oceans. Breeding colonies in Argentina occur from Península Valdés (42°S), Chubut, to the Beagle Channel (55°S), Tierra del Fuego (Yorio *et al*. 1998). They have a seasonal breeding schedule, arriving at the colony to start breeding in the Chubut area in late August and early September. Males arrive on land from a few days to a month earlier than females, and both sexes fast during the settlement and laying period. They lay two eggs in early to mid October. Males and females take turns incubating eggs during an incubation period of 40 days. The study was conducted on a large Magellanic penguin colony on Isla Vernacci Norte (45°11´S, 66°31´W), located near the mouth of Caleta Malaspina, Golfo San Jorge.

The study was conducted during the austral spring and summer of 1999–2000. We randomly marked and studied a sample of 156 nests. At every nest, we banded adults with stainless steel flipper bands during egg laying. We weighed adults with a 6 kg (50-g increments) spring scale and estimated mass to the nearest 10 g. We measured bill length and depth with digital calipers to the nearest 0.1 mm, and flipper and foot lengths (from the bend in the tarsus to the end of the middle toe nail) with a ruler to the nearest 1 mm. Within pairs, we sexed birds based on bill size and the behavioural pattern of nest relief. Males have longer and thicker bills (Scolaro 1978, Boersma *et al*. 1990) and females take the first incubation spell (Yorio & Boersma 1994). We used a sample that included birds that had been measured and weighed during egg-laying to assess body condition. Body condition was estimated as the residuals of a linear regression of mass on flipper length ($F_{1,296} = 158$, $P < 0.001$, $r^2 = 0.35$).

The skin test provides a measure of the proliferative response of circulating T-lymphocytes to an injected mitogen. Phytohemagglutinin (PHA) has long been known for its mitogenic properties and the PHA injection test has been routinely used in poultry science (Goto *et al.* 1978). The main cellular response observed 6 to 12 hours after injection consists of a prominent perivascular accumulation of T-lymphocytes followed by macrophage infiltration (Goto *et al.* 1978). The PHA skin test is considered as a useful method to evaluate thymus-dependent function (Goto *et al.* 1978), and has been routinely used as an assay of T-lymphocyte cell-mediated immune function in studies of poultry (Tsiagbe *et al.* 1987, Cheng & Lamont 1988, Lochmiller *et al.* 1993). It is being increasingly used also in ecological studies (Saino *et al.* 1997, Sorci *et al.* 1997, Moreno *et al.* 1998, Moreno *et al.* 1999), given its benign character compared with other methods used to evaluate immunocompetence. Typically in birds, one wing web is challenged with PHA, while the opposite is injected with phosphate buffered saline (PBS), and the difference between the swellings attained during 24 h is used as response. We have used the simplified protocol proposed by Smits *et al.* (1999), which avoids the injection of PBS in the opposite structure as a control. The advantages of eliminating the PBS injection are a decrease by half in the handling time of the birds (and thereby in stress), a reduction in the probability of errors when injecting into thin structures such as wing or foot webs, and a decrease in the coefficient of variation that is due to measurement inaccuracies (Smits *et al.* 1999).

A subsample of adult penguins were injected intradermally in the right external foot web with 0.1 ml of a solution of 5 mg PHA in 1 ml of PBS (Sigma Chemical Co.) at hatching of their chicks. The thickness of each foot web was measured with a digital spessimeter with constant pressure (Mitutoyo 7/547, Tokyo, Japan) to the nearest 0.01 mm at the injection site before and one day (25% of individuals) or two days after the injection. Always the same researcher took three measurements of the foot web on each occasion to calculate repeatabilities. The interval between measurements depended on the presence/absence of the injected individuals the day after injection. There was no significant difference in inflammation according to inter-sample period (females: $F_{1,40} = 0.0002$, $p = 0.99$; males: $F_{1,29} = 2.64$, $p = 0.115$). We have included inter-sample period when analysing sex differences.

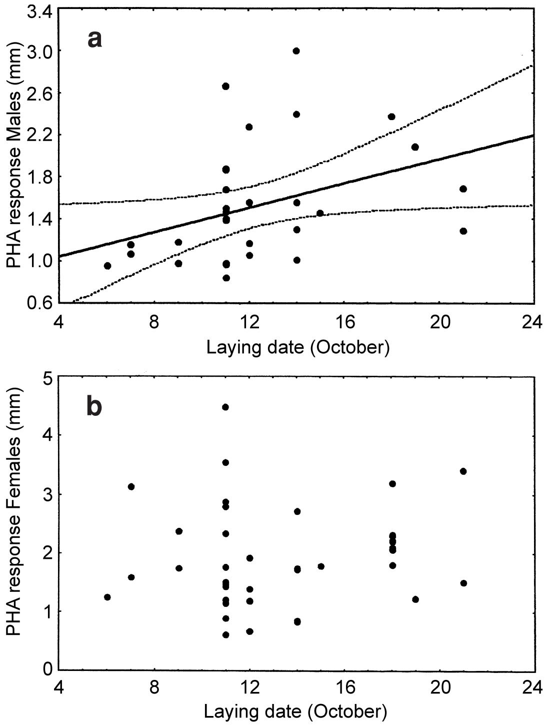

Fig. 1. Associations of response to injection of phytohemaglutinin in the foot web (inflammation in mm due to a cell-mediated immune response) with laying date for (**a**) males ($r_{29} = 0.37$, $p = 0.025$) and (**b**) females ($r_{40} = 0.123$, $p = 0.44$).

Results

Our measurements of foot-web thickness were significantly repeatable (Initial foot-web measurements: $r = 0.69$, $F_{72,218} = 7.6$, $p < 0.0001$; final foot-web measurements: $r = 0.86$, $F_{72,218} = 19.9$, $p < 0.0001$).

Immunocompetence was positively associated with laying date for males, but not for females (Fig. 1). Males showed significantly lower levels of immunocompetence at hatching of their chicks than females (1.52 ± 0.56 versus 1.92 ± 0.70, $F_{1,68} = 5.46$, $p = 0.022$) when controlling for inter-sample period ($F_{2,68} = 0.97$, $p = 0.33$, Interaction: $F_{12,68} = 0.97$, $p = 0.33$) and laying date as covariate ($F_{1,68} = 2.86$, $p = 0.096$). Immunocompetence was positively associated with body condition at laying for males ($r = 0.42$, $F_{1,29} = 6.42$, $p = 0.017$), but not for females ($F_{1,40} = 0.03$, $p = 0.87$). Late-breeding males were in better condition at laying than early males ($r = 0.21$, $F_{1,139} = 6.45$, $p = 0.012$). The relationship was in the opposite direction for females ($r = -0.31$, $F_{1,153} = 16.2$, $p < 0.001$).

Discussion

The present results confirm in a natural population previous evidence about sex differences in immunocompetence in birds and mammals obtained in laboratory studies (Grossman 1985, 1989). Males had significantly lower levels of cell-mediated immunity at a stage of breeding where reproductive costs should not be operating fully (before the prolonged and presumably costly nestling stage). Thus, we should not expect sex differences in immunity to be due to differential parental effort in caring for nestlings (*see* Moreno *et al.* 1999 for evidence of effects of parental effort on cell-mediated immunity). Moreno *et al.* (1998) did not find a significant difference in cell-mediated immunity between males and females in the chinstrap penguin *Pygoscelis antarctica* at the same stage of breeding. The effect size of sex was significantly lower in that study ($r = 0.16$ vs. $r = 0.29$, $F_{1,39} = 5.65$, $p = 0.022$), suggesting an interspecific difference. The lower cell-mediated immunity in male Magellanic penguins may have implications for their capacity to resist attacks by parasites and therefore on survival (Zuk & McKean 1996, Møller *et al.* 1998, Soler *et al.* 1999, Tella *et al.* 2000).

There were also striking differences between males and females in the association of immunocompetence with breeding date. While late-breeding males showed a stronger response than early males, there was no effect of date for females. Late males were also in better condition at laying than early males, while there was the opposite tendency for females. Males arrive on average two weeks earlier than females at the breeding grounds (Boersma *et al.* 1990, Williams 1995) and may suffer consequently a more stressful prelaying stage without feeding than females. Competition for early arrival in migratory birds may lead to a negative association of costs with date of territory occupancy (Kokko 1999). Only high quality males may be able to sustain these costs of early arrival (Möller 1994). On the other hand, late-breeding females may delay laying because of their poor condition (Drent & Daan 1980). For females, competition for early arrival may not be as strong as for males, as they do not have to compete for breeding territories as strongly as males. Their later arrival may reduce costs in terms of condition. Also, for females the prebreeding period may not be sufficiently protracted to affect condition at laying. For males, breeding date may affect condition, while for females the causality would go in the opposite direction.

The effects of a ‘cascading’ competition among males for early territory occupancy (Kokko 1999) may be seen in the significant effects of prebreeding condition on immunity 40 days later. It has been shown in several species that nutritional condition is positively correlated with cell-mediated immunity (Sorci *et al.* 1997, Tella *et al.* 2000). Why this should only be apparent for males remains an open question. One possibility is that females may suffer marked changes in cell-mediated immunity during laying associated to a surge in estrogen production. Female sex hormones apparently enhance humoral immunity while inhibiting cell-mediated responses (Alexander & Stimson 1988, Grossman 1989). These changes could disrupt the link between condition at laying and cell-mediated immunity at hatching of the chicks. It may also imply that the prolonged prebreeding fasting of males may have long-term consequences for their subsequent capacity to survive pathogen-attacks (Saino *et al.* 1997, Soler *et al.* 1999). Competition for early arrival in males may imply costs not sustained by females.

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