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Breeding time, health and immune response in the chinstrap penguin *Pygoscelis antarctica*

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Abstract Health status and immunocompetence have been proposed as important factors affecting individual variation in the attainment of breeding condition in birds. We studied individual variation in serological variables indicating health status (blood sedimentation rate, haematocrit, 'buffy coat' layer, proportions of different types of leucocytes) in two groups of breeding chinstrap penguins *Pygoscelis antarctica* with breeding dates 9 days apart. We sampled these individuals shortly after hatching of their young and at the end of the chick-raising period. A group of failed breeders was also sampled. Birds of both sexes were included. We also measured the T-cell-mediated immune response as indicated by an in vivo hypersensitivity response to an intradermal injection of a mitogen (phytohaemagglutinin) in early and late breeders. Sex had no significant effect on most variables. Late breeders had poorer health (more leucocytes, especially heterophils and lymphocytes) and a lower T-cell-mediated immune response than early breeders. Failed breeders were more similar to late than to early breeders. Early breeders suffered a decline in health status throughout the chick-raising period. The impact of pathogens on variation in life history traits in avian populations may be important even in extreme Antarctic environments.

Key words Antarctica · Breeding failure · Serological variables · T-cell-mediated immunity · Timing of breeding

Introduction

What determines individual variation in avian breeding time? Darwin (1871) proposed that the health and vigour of females affected both breeding date and fecundity. Perrins (1970) formally proposed that the nutritional requirements of breeding females determine clutch initiation dates. A negative trend in reproductive success and offspring survival with breeding date is almost ubiquitous in non-tropical areas (Daan et al. 1988; Brinkhof et al. 1997). Recently, several models have been proposed to explain how variation in breeding time can be maintained in the face of persistent selection for early breeding in temperate populations (Price et al. 1988; Rowe et al. 1994). One basic assumption is that the physiological condition of breeders independently determines breeding date and reproductive success, and that condition itself is not subjected to selection given its presumably low heritability (Price et al. 1988). Some of the evidence on physiological condition and timing of breeding is consistent with the condition hypothesis: of 26 food supplementation studies reviewed by Meijer et al. (1990), 15 showed a statistically significant advance in laying date when food was supplemented. Fat and/or protein stores have been shown to limit egg production in numerous species (Jones and Ward 1976; Ankney and McInnes 1978; Ankney and Scott 1980; Houston et al. 1983; Thomas 1988; Jones 1991). Although some authors dispute the importance of energy limitation in the decision when to breed (Nager and van Noordwijk 1995; Winkler and Allen 1996), body condition remains the most plausible proximate substrate of breeding decisions in birds.

Body size (Murphy 1986; Langston et al. 1990), age (Hochachka 1990; Burger et al. 1996), experience (Nager and van Noordwijk 1995) and foraging skills (Winkler and Allen 1996) may affect condition indices. However, these effects may be mediated through more proximate factors like health status and immune function. Gustafsson et al. (1994) have proposed that infectious diseases may depress body condition. Indicators of health status and fat reserves have been shown to be associated (Merilä and Svensson 1995; Svensson and Merilä 1996). In the collared flycatcher *Ficedula albicollis* Temminck, laying date apparently correlates positively with indicators of prior infection like white blood cell count,

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sedimentation rate and the presence of immunoglobulins in blood, although the data have not been presented yet (Gustafsson et al. 1994). On the other hand, nutrition and immunocompetence are positively related (Lochmiller et al. 1993), while the impact of parasites on condition is mediated by host immunocompetence (Zuk 1994). Thus, the nutritional plane would affect immunity, which in turn could affect infection risk (Gustafsson et al. 1994). A priori, we should expect a negative association of laying date with measures of immunocompetence and a positive one with indications of prior infection. There is a great need to explore these associations in an increasing number of species and environments, in order to unravel the proximate causal basis of breeding decisions.

A decrease in several components of fitness with breeding date has been noticed in seabirds breeding in temperate areas (Perrins 1966; Parsons et al. 1976; Hedgren 1981; Wanless and Harris 1988). Extreme environments with short breeding seasons like Antarctica may be expected to exhibit even clearer trends, given the presumably high costs of breeding late. Negative effects of late breeding on reproductive success have been reported in Adélie penguins *Pygoscelis adeliae* Hombron and Jacquinot (Taylor 1962; Spurr 1975; but see Davis and McCaffrey 1986) and gentoo penguins *P. papua* Forster (Bost and Jouventin 1991; but see Williams 1990). A multiyear study of breeding chinstrap penguins *P. antarctica* Forster in the South Shetlands revealed that late hatching leads to depressed growth and earlier crèching and fledging (Viñuela et al. 1996). A subsequent experiment demonstrated that these trends were due to a reduced investment level by parents in late-hatched chicks, presumably due to the increasing need of preparing for the annual moult as the season advances (Moreno et al. 1997). Depressed growth is strongly associated with mortality for recently independent young (Moreno et al., in press). Given the deleterious consequences of late breeding for adults, we may expect that breeding delays are the consequence of physiological restrictions operating at the beginning of the season. These restrictions based on depressed condition may also be detected later, given that breeding effort is not likely to allow a quick recovery.

The aim of this study was to examine differences in condition between early and late breeders in a population of chinstrap penguins during the chick-raising phase. We also included a sample of failed breeders in order to test if breeding failure is also related to depressed condition. Antarctic penguins appear to be relatively free of infectious diseases (Clarke and Kerry 1993), although there is serological evidence of infection with a number of avian diseases found elsewhere (Gardner et al. 1997). In our population, no evidence of haematozoa could be detected (Merino et al. 1997). However, helminths are prevalent in our study population (personal observation) and bacterial and viral infections in Antarctic penguins cannot be discounted (Clarke and Kerry 1993). As measures of condition-related health state and immune function, we focussed on serological measures (Gustafsson et al. 1994) and tests of cell-mediated immunocompetence (Lochmiller et al. 1993). We sampled individuals both soon after hatching and shortly before fledging of the chicks in order to discern changes in health status and immunocompetence throughout the presumably costly chick-raising phase. A depressed condition of late breeders while raising chicks may be due to a carry-over of differences before laying given a common reproductive effort of early and late breeders. Failed breeders should show values closer to late than to early breeders if failure as well as delayed breeding is related to initial health status and immunocompetence.

Materials and methods

Study species

The chinstrap penguin breeds in large colonies in the area of the South Shetlands and the Scotia Sea (Williams 1994). It is the latest-laying penguin in this area (Trivelpiece et al. 1987). The modal clutch is two eggs and the most frequent broods consist of two chicks. After a 1-month incubation period, when both parents take turns in covering the eggs, chicks remain on the nest for approximately another month while being protected by one of the parents (Lishman 1985; Moreno et al. 1994; Viñuela et al. 1996). After this so-called guard phase, chicks are left on their own by the parents and aggregate to form crèches. Both parents return to the colony only to feed their chicks during this crèche phase, until they become independent at 50–60 days of age (Viñuela et al. 1996).

Study area and sampling

The study was conducted on the large chinstrap penguin colony of Vapour Col on Deception Island, South Shetlands (63°00′ S, 60°40′ W) during the austral summer of 1996–1997. In one large nest aggregation (roughly 1000 nests) we selected randomly two groups of 30 nests according to hatching date (15 December and 24 December, respectively). The mean hatching date of an independent randomly selected sample of 33 nests was 19 ± 2.4 (SD), (range 14–27 December). All selected nests contained two recently hatched chicks. Nests were marked with numbered sticks. Adults guarding chicks on the nest were sampled shortly after hatching (initial sampling: 17–20 December for early nests and 26–30 December for late nests). They were immobilized for the immunocompetence test and the extraction of blood samples from foot veins. Flipper length, bill length and bill depth were measured according to Amat et al. (1993). Adults could be sexed according to a discriminant function based on bill measurements (Amat et al. 1993). They were banded with standard metal flipper bands (34 × 17 mm, Lambournes). Chicks were kept warm in a stuffed bag during the interval, and parent and chicks returned to the nest together. No parent deserted due to the manipulation. Only one adult from each pair was sampled.

On 8 January, 20 randomly selected failed breeders occupying empty nests were sampled and measured. On 18 January–6 February, banded adults were sampled opportunistically when one adult of a marked nest was found in the colony feeding the chicks (final sampling). No immunization test was performed on adults at this stage. The mean age of chicks at the time of the final sampling of early parents was 36.9 ± 4.4 ($n = 16$), while it was 37.2 ± 5.2 ($n = 9$) for late pairs ($F = 0.02$, $P = 0.89$). For a few individuals, we were able to obtain measurements both after hatching and while feeding large chicks.

Serological measurements

As a measure of health state, we employed four clinical screening methods which are known to detect disease processes: red blood cell sedimentation rate (SDR), packed red blood cell volume (haematocrit) (PCV), white blood cell count (WBC) as indicated by measurement of the 'buffy coat' layer (Wardlaw and Levine 1983) and the proportion of different white blood cells obtained from blood smears. The first three methods were performed on fresh blood.

Two blood samples were collected to obtain the repeatability of the serological measurements (Table 1). SDR is a diagnostic method based on the fact that the pace of red blood cells through plasma is enhanced by increased levels of one of the major acute-phase proteins (fibrinogen) and immunoglobulins. High SDRs are indicative of many acute and chronic diseases (Sharma et al. 1984). Blood was collected in heparinized capillary tubes which were placed vertically for 3 h under ambient conditions (around 5°C). Then the length of the capillary containing plasma and the total length of the occupied capillary was measured with a magnifying glass to the nearest 0.05 mm. SDR was calculated as the length of the capillary tube containing plasma in mm corrected for differences in sample volumes by dividing it by the total occupied volume. As the SDR is slower when the blood contains more erythrocytes, we regressed the SDR on the haematocrit (see below) and used residuals from this regression model as a direct estimate of SDR corrected for the amount of red blood cells (Soler et al., in press).

PCV measures the relative amount of red blood cells in the total blood volume [i.e. red blood cell volume/(red blood cell volume + plasma volume)]. Low values are indicative of acute and chronic diseases or may reflect nutritional deficiencies of some minerals or proteins (Coles 1997). Bacterial infections as well as blood and gastrointestinal parasitism may also cause lowered PCV (Harrison and Harrison 1986). PCV was measured after 8 min centrifugation with a portable Bayer Diagnostics minicentrifuge M1101.

The height of the buffy coat layer is indicative of the number of white blood cells in a sample (WBC; Wardlaw and Levine 1983). An elevated WBC is diagnostic of acute or chronic infections (Harrison and Harrison 1986; Gustafsson et al. 1994). The height of the buffy coat layer was measured with a magnifying glass, after centrifugation, to the nearest 0.05 mm, and corrected by dividing it by the total blood volume (Merilä and Svensson 1995).

Leucocytes are part of the immune system and the number and proportions of different types of leucocytes reflect the health status of individuals (Fox and Solomon 1981). The most frequently occurring type of leucocyte in most avian species is the lymphocyte, the second most common being the heterophil (Sturkie 1986), but some avian species have more heterophils than lymphocytes (Lucas and Jamroz 1961; Hawkey et al. 1985). The three other types (eosinophils, basophils and monocytes) occur only in low numbers (Sturkie 1986). The typical response to infectious diseases in birds is an increase in the number of heterophils and lymphocytes (Wilson and Wilson 1978; Ross et al. 1979; Davis 1981; Hawkey et al. 1985; Averbeck 1992). Blood samples were smeared on individually marked microscope slides, air dried and fixed in absolute ethanol for 5 min and stained with Giemsa pH 7.2 for 30 min. All smears were examined by the same person at ×1000 and the proportions of different types of leucocytes following Hawkey and Dennet (1989) were obtained from examination of a total of 100 leucocytes (Dufva and Allander 1995). Simultaneously, the number of red blood cells was counted (11 011 ± 3574). By calculating the number of leucocytes per 10 000 red blood cells, we obtained the relative amount of different white blood cell types in relation to total cell volume. To obtain the repeatability of these proportions, ten smears were recounted (Table 1).

Cell-mediated immunity

A phytohaemagglutinin-P (PHA) injection assay (Cheng and Lamont 1988) was used to evaluate the in vivo T-cell-mediated immune response of penguins. Birds were injected intradermally in the right external foot web with 0.1 ml of 1 mg/ml phytohaemagglutinin-P (Sigma) in PBS solution. The external web in the other foot (control) was injected with 0.1 ml of PBS (Tsiagbe et al. 1987). The thickness of each foot web was measured with a digital calliper (to the nearest 0.01 mm) at the injection site before and 1 day (27.9 ± 6.9 h) after the injection. Three birds were sampled after 2 days instead of 1. As there was no significant difference in inflammation according to intersample period ($F_{1,33} = 0.23$, $P = 0.63$), we have included these individuals in analyses.

The cell-mediated immune response was estimated as the change in thickness of the right foot web from the day of injection until the following day minus the change in thickness of the left foot web during the same period (Lochmiller et al. 1993). The same researcher took five measurements of each web on each occasion, and the mean was used in subsequent analyses. These measurements allowed us to calculate the repeatability of foot web thickness (Table 1). Tests were only performed during the posthatching sampling period, as some of these individuals were still guarding the chicks on the nest 24 h after injection. However, some had been relieved in the interval and could not be measured again.

Statistical analyses

The repeatability of measurements was calculated as the intraclass correlation coefficient based on variance components derived from a one-way analysis of variance (Becker 1984; Lessells and Boag 1987; Falconer 1989). For repeatable measurements, the average for each sampling was used. Sex of the individual has been included as a cofactor in all analyses given the potential impact of sex differences on haematological values and immunocompetence. Parametric tests have been used whenever their assumptions were met. Because PCV was not normally distributed, it was log-transformed to achieve normality. Sample sizes of different serological measures differ due to clotting in some capillaries, breakage and other technical problems. All *P*-values refer to two-tailed tests. If not otherwise stated, means are presented together with their SD.

Table 1 Repeatability (intraclass correlation coefficient derived from ANOVA) of health status and immune response variables between measurements (*T-cell response*), capillary tubes (*SDR*, *PCV*, *WBC*) or cell counts (white blood cell types) (*SDR* blood sedimentation rate, *PCV* packed cell volume or haematocrit, *WBC* white blood cell count or thickness of the buffy coat layer)

Measurement	Number of repeated measurements	Repeatability	*F*	*df*	*P*
SDR	2	0.76	7.5	82,83	<0.0001
PCV	2	0.74	6.7	87,88	<0.0001
WBC	2	0.67	5.1	77,78	<0.0001
Heterophils	2	0.77	7.6	9,10	0.0019
Lymphocytes	2	0.57	3.6	9,10	0.0282
Monocytes	2	0.50	3.0	9,10	0.0505
Eosinophils	2	0.22	1.6	9,10	0.2462
Basophils	2	−0.04	0.9	9,10	0.5425
T-cell response	5	0.88	39.2	32,132	<0.0001

Results

Serological measurements

SDR, PCV and WBC were significantly repeatable (Table 1). For cell counts in blood smears, only the frequencies of the two most common white blood cell types, namely heterophils and lymphocytes, were significantly repeatable (Table 1). Only these two cell types will be further analysed.

We tested for differences with respect to sex and breeding period in a two-way ANOVA with interaction (Table 2). As sex had only a significant effect on the amount of lymphocytes in the final sampling and there were no significant interactions with breeding period, data for the two sexes are presented jointly (Table 3). Neither initial PCV nor SDR differed between early and late breeders (Tables 2, 3). Breeders had slightly higher PCV values than those reported in breeding adults of the same population in another year (51.0; Merino and Barbosa 1997). However, there was a highly significant difference in initial WBC between early and late breeders (Table 2), with late breeders showing higher values (Table 3). The relative amount of heterophils and lymphocytes also differed between early and late breeders (Table 2), with late breeders having higher amounts of both types of white blood cells (Table 3). Final SDR was significantly higher for late than for early breeders, while there were no differences in PCV, WBC and relative counts of heterophils and lymphocytes (Tables 2, 3). The only difference between sexes was in the relative amount of lymphocytes in the final samples (Table 2), with females having higher relative amounts than males (30.4 ± 14.4, $n = 12$ vs. 19.9 ± 7.6, $n = 9$).

Failed breeders showed significantly higher WBC values and relative amounts of lymphocytes than breeders (Tables 3, 4). The difference in WBC was due to the significant difference between failed and early breeders, as failed and late breeders did not differ with respect to WBC (Tables 3, 4). However, failed breeders had higher relative amounts of lymphocytes than both categories of breeders (Tables 3, 4). No differences between failed breeders and breeders were detected in SDR or PCV (Tables 3, 4). Males had lower SDRs than females (Table 4; -0.25 ± 0.75, $n = 34$ vs. 0.22 ± 0.77, $n = 38$).

When comparing initial and final samples for the individuals which could be sampled twice, we found significant differences in WBC when including the

Table 2 Results of two-way ANOVA on the effects of breeding date and sex on serological variables (see Table 1 for abbreviations)

	df	Breeding date		Sex		Interaction	
		F	*P*	*F*	*P*	*F*	*P*
Initial sampling							
SDR	1,52	1.48	0.23	3.57	0.06	0.01	0.90
PCV	1,51	2.37	0.13	1.23	0.27	1.19	0.28
WBC	1,49	17.0	0.0001	0.05	0.83	0.82	0.37
Heterophils	1,35	9.57	0.0039	2.43	0.13	1.76	0.19
Lymphocytes	1,35	5.39	0.026	1.47	0.23	0.01	0.92
Final sampling							
SDR	1,20	6.05	0.02	1.12	0.30	1.52	0.23
PCV	1,20	0.50	0.48	0.26	0.62	0.26	0.62
WBC	1,21	1.27	0.27	0.19	0.67	4.11	0.06
Heterophils	1,17	0.00	0.99	1.26	0.28	1.03	0.32
Lymphocytes	1,17	0.23	0.64	6.92	0.02	0.00	0.99

Table 3 Serological measures (means ± SD) for early, late and failed breeders. Number of individuals in parentheses (see Table 1 for abbreviations)

	Early breeders	Late breeders	Failed breeders
Initial sampling			
SDR	0.0064 ± 0.73 (30)	−0.15 ± 0.72 (26)	0.23 ± 0.99 (16)
PCV	55.3 ± 3.6 (30)	54.5 ± 3.1 (26)	54.0 ± 3.35 (19)
WBC	0.22 ± 0.10 (29)	0.35 ± 0.11 (25)	0.34 ± 0.08 (17)
Heterophils	42.2 ± 15.2 (22)	56.0 ± 16.7 (17)	48.2 ± 11.6 (16)
Lymphocytes	26.4 ± 12.9 (22)	38.0 ± 13.8 (17)	65.6 ± 14.5 (16)
Final sampling			
SDR	−0.296 ± 0.79 (15)	0.49 ± 0.71 (9)	
PCV	52.7 ± 3.0 (15)	53.1 ± 3.1 (9)	
WBC	0.42 ± 1.6 (16)	0.35 ± 1.4 (9)	
Heterophils	57.2 ± 20.3 (14)	55.8 ± 22.3 (7)	
Lymphocytes	25.6 ± 12.7 (14)	26.7 ± 14.2 (7)	

Table 4 Comparisons of serological variables of breeders with failed breeders, and of failed breeders with early and late breeders (see Table 1 for abbreviations)

	df	Breeding status		Sex		Interaction	
		F	*P*	*F*	*P*	*F*	*P*
Failed breeders vs. breeders							
SDR	1,68	2.13	0.15	10.4	0.002	2.94	0.09
PCV	1,93	0.01	0.90	0.00	0.95	0.10	0.76
WBC	1,90	6.68	0.01	0.00	0.93	0.02	0.89
Heterophils	1,51	0.00	0.96	0.01	0.94	0.41	0.53
Lymphocytes	1,51	64.9	0.000	0.44	0.51	1.43	0.24
Failed breeders vs. early breeders							
SDR	1,42	0.71	0.40	8.46	0.006	2.10	0.15
PCV	1,45	1.14	0.29	0.58	0.45	1.66	0.20
WBC	1,41	18.8	0.000	0.11	0.75	0.15	0.70
Heterophils	1,34	1.58	0.22	0.02	0.88	0.17	0.68
Lymphocytes	1,34	71.5	0.000	0.16	0.69	0.79	0.38
Failed breeders vs. late breeders							
SDR	1,38	3.46	0.08	8.40	0.006	1.68	0.20
PCV	1,41	0.28	0.60	0.17	0.68	0.01	0.91
WBC	1,36	0.004	0.95	0.26	0.61	0.21	0.65
Heterophils	1,29	4.15	0.05	1.53	0.23	3.16	0.09
Lymphocytes	1,29	30.0	0.000	0.07	0.80	0.48	0.49

Table 5 Comparison of initial and final samplings of the same individuals for the whole sample and for early breeders (paired-sample *t*-tests). Data for late breeders were too few for analysis (see Table 1 for abbreviations)

	t	*P*	*N*
Total sample			
SDR	−0.69	0.496	24
PCV	0.72	0.474	31
WBC	−2.41	0.022	31
Heterophils	−0.10	0.334	17
Lymphocytes	1.27	0.223	17
Early breeders			
SDR	1.34	0.200	15
PCV	2.41	0.030	15
WBC	−3.79	0.002	15
Heterophils	−1.66	0.123	13
Lymphocytes	0.11	0.915	13

whole sample (Table 5). When only including early breeders for which the sample was large enough, PCV and WBC differed between samples (Table 5). Early breeders had lower PCV and higher WBC values at the end than at the beginning of the chick-raising period (Table 3).

Cell-mediated immunity

Our measurement of foot web thickness was significantly repeatable (Table 1). Only 35 individuals could be tested given the high probability of birds having been relieved from nest duties between visits to the colony. When comparing early and late breeders controlling for sex, early breeders showed a significantly higher foot web index (0.87 ± 0.47, $n = 19$) than late breeders (0.60 ± 0.29, $n = 16$; $F_{1,30} = 4.56$, $P = 0.041$), while sex ($F_{1,30} = 0.81$, $P = 0.37$) and the interaction term ($F_{1,30} = 0.27$, $P = 0.60$) were not significant.

Discussion

Ill health has been implicated as a factor which may delay the onset of breeding (Gustafsson et al. 1994). The importance of parasites in mediating life history traits in avian populations has lately received well deserved attention (Möller 1996). In this context, Gustafsson et al. (1994) referred to unpublished data showing significant associations between laying date and SDR, WBC and the proportion of heterophils, indicating that disease might cause later breeding. However, there is another link between health status and breeding condition, namely that mediated by the immune system. One common factor that characterizes all classes of immune function is that their operation requires resources that the host might otherwise have used for some other function (Sheldon and Verhulst 1996). Indirect evidence that immune function is costly to hosts is the common observation that poor nutrition is associated with disease (Keymer and Read 1991; de Lope et al. 1993) and with lower levels of cell-mediated immune function (Gershwin et al. 1985; Lochmiller et al. 1993). Thus, the requirements for breeding may compete with immune function, leading to an association of immunocompetence with breeding date (Gustafsson et al. 1994). Furthermore, brood size (Saino et al. 1997) and breeding date (Sorci et al. 1997) seem to affect the immunocompetence of nestlings, indicating that the immune system is involved in reproductive trade-offs affecting present reproductive success. Although serological variables and

cell counts in blood smears can give information about health status (Zuk 1996), they do not give explicit information about immunocompetence. The PHA intradermal reaction, a T-lymphocyte-dependent response, has been well researched and has been shown to be a reliable indicator of in vivo cellular immunity in birds (Goto et al. 1978; McCorkle et al. 1980; Lochmiller et al. 1993). Thus, we used serological variables as indicators of health status and the PHA intradermal reaction protocol as an independent measure of immune response. If breeding date was affected by health status and/or immunocompetence, we expected late breeders to be in poorer health and/or have a depressed immune response compared with early breeders.

Pygoscelid penguins breeding in Antarctica have to compress a complete breeding cycle into the short Antarctic summer. Selection for early breeding should be very strong, obviously balanced by selection for avoidance of adverse climatic conditions in spring (Moreno et al. 1997). Any delay in the start of breeding may retard the onset of the postbreeding moult, with possible negative repercussions on adult survival (Moreno et al. 1997). If selection for early breeding is expected in pygoscelid penguins, how is variation in breeding date maintained? Ainley et al. (1983) have shown that young female Adélie penguins tend to lay slightly later on average than older birds, so age may be involved. Individual variation in laying date may also have a genetic basis: some individuals lay consistently early and others late, relative to the average for the population (Spurr 1975; Williams 1994). In our study population, the breeding date of pairs is significantly and highly repeatable between years ($r = 0.89$, $F_{36,5} = 17.2$, $P = 0.002$; Viñuela et al. 1996). This repeatability may be due to an underlying genetic basis for laying date (van Noordwijk et al. 1981) or to other effects which similarly affect the breeding decisions of each individual between years. Health status and immune response may be consistent for individuals if immunocompetence itself has a genetic basis (Briles et al. 1977; Lamont et al. 1987; Gustafsson et al. 1994; Hedrick 1994; Saino et al. 1997).

We have explored if serological indicators of health status and T-cell-mediated immunity differed between two samples of breeders with a 9-day difference in the hatching dates of their broods (the range of hatching dates in the population was 2 weeks). Given that measurements were obtained when the breeding season was well advanced, one could suggest that late breeding itself may cause disease if late breeders are exposed to more vectors or have more difficulties in finding food due to a seasonal decline in food availability. However, the dense colonial breeding of chinstrap penguins (Carrascal et al. 1995) and the large overlap in the breeding seasons of both groups of breeders make this suggestion unrealistic. Breeding failure as well as delayed breeding may also be related to health status and immunocompetence. Accordingly, we should expect that failed breeders should have indications of prior infection. We have therefore included a sample of failed breeders still occupying territories in the colony in our serological study, expecting them to be more similar to late than to early breeders.

The results support our prediction that health status and immunocompetence may be involved in the breeding date variation observed among individuals of the same breeding colony. Late breeders had more white blood cells, indicative of more exposure to infectious diseases (Wilson and Wilson 1978; Rose et al. 1979; Davis 1981; Hawkey et al. 1985; Averbeck 1992) than early breeders. This was expressed both as the height of the buffy coat layer (Gustafsson et al. 1994) and as counts of heterophils and lymphocytes in blood smears (Zuk 1996). They also had a weaker T-cell-dependent immune response to PHA injection, indicating depressed immunocompetence (Lochmiller et al. 1993). Failed breeders were more similar to late than to early breeders in WBC, and had very high relative amounts of lymphocytes. Given that failed breeders were not subjected to the stresses of reproduction when sampled, these differences cannot be attributed to their status. It seems more appropriate to attribute their breeding failure to their health status. Why only lymphocytes showed an increase remains a question for further study. There was a deterioration in the health status of breeders, especially early breeders, during the course of the presumably costly chick-raising phase. This was manifested as lowered PCV and increased WBC. The fact that reproductive effort may increase parasitization (Norris et al. 1994; Richner et al. 1995; Möller 1996) or depress immune function (Deerenberg et al. 1997) is receiving increasing attention as a plausible mechanism for reproductive trade-offs (Sheldon and Verhulst 1996). Our study indicates that there may be detectable effects of reproductive effort even in a non-manipulative situation.

Most animals in most environments are exposed to parasites and infections given the huge diversity of parasitic organisms (Price 1980). Parasites have the potential of imposing severe selective pressures on their hosts (Haldane 1949; Clarke 1979; Hamilton 1980; Lehmann 1993; Möller 1996). Thus, the latter require an immune system that allows them to fend off the omnipresent pathogens, and the immune response is therefore a physiological universal that affects all other aspects of the organism's biology. Here, we have demonstrated that delayed breeding and breeding failure are associated with poor health and a depressed immune response in an avian population breeding in the extreme Antarctic environment, where levels of pathogens may be predictably lower than in temperate or tropical habitats.

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