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BIPARENTAL CARE OF CHINSTRAP PENGUIN: MOLECULAR SEXING AND LIFE HISTORY IN THE SOUTH SHETLAND ISLANDS, ANTARCTICA

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Jaqueline Brummelhaus*, Victor Hugo Valiati[1], Maria Virginia Petry[2]

[1]Laboratório de Biologia Molecular, Universidade do Vale do Rio dos Sinos – UNISINOS, Av. Unisinos, 950, Cristo Rei, CEP 93022-000, São Leopoldo-RS, Brazil
[2]Laboratório de Ornitologia e Animais Marinhos, Universidade do Vale do Rio dos Sinos – UNISINOS, Av. Unisinos, 950, Cristo Rei, CEP 93022-000, São Leopoldo-RS, Brazil15001000
*e-mail: jaquebrummelhaus@gmail.com

Abstract: *Chinstrap penguin, Pygoscelis antarctica, breeding biology involves several distinct stages of chick rearing, in which each parent varies in their relative contribution to reproductive success. We investigated tertiary adult sex ratios using molecular sexing in breeding colonies during guard and crèche stages of chick rearing at Admiralty Bay (King George Island) and on Stinker Point (Elephant Island) in the 2010/2011 breeding season. We analyzed 52 blood samples and found this represented 31 males and 21 females. We observed that the adult sex ratio in guard and crèche stages did not vary significantly. However, we feel evidence did infer differences in relative biparental care behavior between the two stages studied. Thus, it was possible to develop a potential hypothesis of relationship between sex ratio of adults present during different stages of the breeding season by molecular biology technique with Chinstrap penguin life history and gain understanding of the importance of contributions of both sexes in raising their offspring and for attaining reproductive success.*

Keywords: Chinstrap Penguin, Guard and Crèche Stage, Biparental Care, Molecular Sexing.

Introduction

Chinstrap penguins (*Pygoscelis antarctica*) are an Antarctic species that breed during the austral summer in colonies on ice-free areas of coast, mainly on sub-Antarctic islands and the Antarctic Peninsula. In South Shetland Islands, the total breeding population was estimated to be 1.248.350 pairs (Harris, 2006). Adults return to breeding colonies from late October to early November. Both members of a given pair remain for about a month until the laying of one or two eggs is complete (Williams, 1995).

During the incubation stage, adults remain at the nest for extended periods to incubate and protect the eggs. They each spend shorter alternating periods attending the nest during the hatching and guard stages (Trivelpiece *et al.*, 1990; Williams, 1995). The guard stage may last up to 30 days after hatching. After that, chicks begin to organize themselves into loose aggregations (crèche) and chicks are left in the colony, while both parents are foraging and returning at intervals to feed their chick(s) (Conroy *et al.*, 1975a,b). After molt of chick down, the fledgling stage occurs from late February-early March. This is when chicks go to sea, leave their parents and become self-sufficient, returning again to undergo a second molt to adult plumage the following year (Williams, 1995).

The Chinstrap penguin is a monogamous species (Moreno *et al.*, 2000) and fairly equal parental investment is made by both members of the pair. Male and female participate in nest building and maintenance, taking turns to care for eggs during incubation, and guarding and feeding of chicks (Conroy *et al.*, 1975a; Moreno *et al.*, 1995, 1999; Williams, 1995; Jansen *et al.*, 2002), in a similar manner to all species that forage at sea and that breed on land they form strong pair bonds and exhibit exclusive biparental

care (Cockburn, 2006). Since both parents are essential to reproductive success, the optimum sex ratio is 1:1 for maximum Chinstrap penguin breeding success, is to be expected. The species actual sex ratio for each breeding colony represents population response to local genetic, environmental, behavioral and evolutionary factors, so it is an important parameter to understanding population life history (Seger & Stubblefield, 2002; Fargallo *et al.*, 2004; Jenouvrier *et al.*, 2010).

We investigated tertiary sex ratio (adult stage) in breeding colonies during guard and crèche stages of *P. antarctica* in Admiralty Bay (King George Island) and on Stinker Point (Elephant Island) in the 2010/2011 breeding season, by molecular sexing. Our goal was to gain a better insight into behavioral parameters of breeding success for the species.

Materials and Methods

Chinstrap penguin adults were sampled while away from their nests at Admiralty Bay (King George Island) (62°05'S; 58°23'W) and Stinker Point (Elephant Island) (61°08'S; 55°07'W), South Shetland Islands, Antarctica, in the 2010/2011 breeding season. In Admiralty Bay, we took 21 blood samples from the Chabrier Rock colony on December 30/2010 (guard stage) and ten samples in Uchatka Point colony on February 7/2011 (crèche stage). At Stinker Point, we took 21 blood samples in two breeding colonies on December 29/2010 and January 23/2011 (guard stage).

Blood samples were centrifuged, since for this study we used only red blood cells. The latter were refrigerated and stored in a sample bank at the Laboratory of Molecular Biology, Universidade do Vale do Rio dos Sinos (UNISINOS). Genomic DNA was isolated from blood samples through standard phenol/chloroform technique with digestion by proteinase K enzyme. In birds, sex identification is determined by chromosomes Z and W, which have either CHD-Z or CHD-W genes (Griffiths & Tiwari, 1993; Griffiths, 2000). The gene CHD-Z occurs in both sexes, while CHD-W occurs only in the female. A female has two different chromosomes, W and Z in her karyotype and two bands result on an electrophoresis gel in analysis, a heterogametic characteristic. The male has two copies of the single chromosome type (Z) and thus shows a single band of stain via electrophoresis (a hemizygous characteristic) (Griffiths & Tiwari, 1993; Griffiths, 2000). For molecular sexing, the CHD gene region was amplified by polymerase chain reaction (PCR) using P2 and P8 primers (Griffiths *et al.*, 1998). PCR products were analyzed by electrophoresis in 10% polyacrylamide gel and bands were visualized by ethidium bromide staining.

Sex ratio was calculated for each date and breeding colony. The predicted hypothesis of existence of a balanced male/female sex ratio on different dates (different rearing stages) and separate breeding colonies was tested through chi-square (χ^2) analysis by Systat 13 software.

Results

We analyzed 52 samples and found 31 males and 21 females. The PCR amplification showed a single band of about 270 base pairs (pb) for males, while females showed two bands of about 370 and 270 pb (Figure1).

For Chabrier Rock breeding colony, in Admiralty Bay, we found a sex ratio equal to 0.52 ($\chi^2 = 0.05$, $p > 0.05$) (10 males and 11 females) for dates that correspond to the chick guard

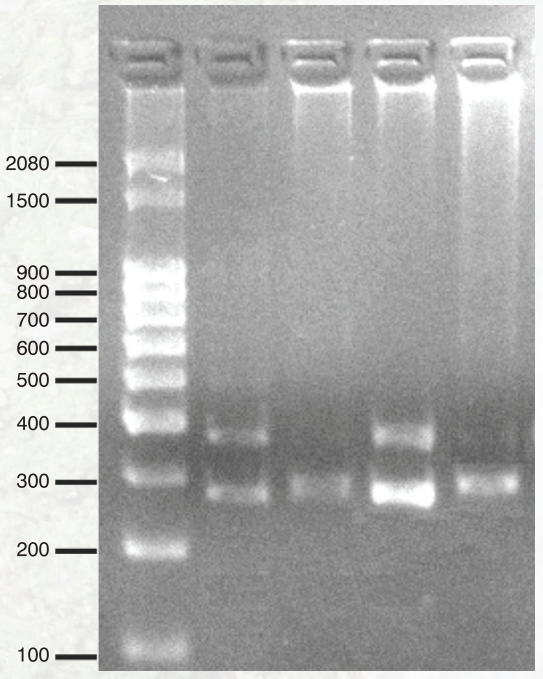

Figure 1. PCR products of P2-P8 primers on DNA from female (about 370 and 270 pb) and male (about 270 pb) of *Pygoscelis antarctica* and molecular weight (100 bp DNA ladder - Invitrogen, Carlsbad, CA).

stage. In Uchatka Point breeding colony (Admiralty Bay) we found the sex ratio of 0.8 ($\chi^2 = 3.6$, $p > 0.05$) (08 males and 02 females) for dates that correspond to the crèche stage. In Stinker Point, Elephant Island, we found a sex ratio of 0.6 for December 29/2010 ($\chi^2 = 0.4$, $p > 0.05$) (06 males and 04 females) and 0.54 for January 23, 2011 ($\chi^2 = 0.09$, $p > 0.05$) (06 males and 05 females). These dates correspond to the guard stage. For guard and crèche stages, sex ratio did not differ significantly from the expected ratio of males to females 1:1.

Discussion and Conclusion

Our results show that sex ratios for Chinstrap penguin adults present at the breeding colony during chick rearing in guard stage did not deviate from the expected ratio of 1:1. This information confirms biparental care of offspring during one of the most critical reproductive stages. Data collected in December and January correspond to chick hatching and guard stages. During this period, adults alternate turns between foraging and chick care every ½ day to 1 day, thus both parents have the opportunity to feed and bring prey for chicks (Conroy *et al.*, 1975b; Williams, 1995; Meyer *et al.*, 1997; Jansen *et al.*, 2002). Chinstrap penguins feed their offspring soon after hatching, which determines the need for the adults to exchange foraging and protection duties at short intervals during this life phase (Conroy *et al.*, 1975b; Croxall & Furse, 1980) and helps explain the sex ratio observed during chick hatching which we observed in December 2010. During incubation stage (36-40 days), females alternate turns with males at incubating eggs, in periods of three-ten days, spending a longer time at sea for feeding after a prolonged non-feeding courtship period in which the couple remained in colonies to ensure mating and posture (Conroy *et al.*, 1975a,b; Williams, 1995). However, the sex of offspring may affect the duration of foraging trips during the guard phase, as in *Pygoscelis adeliae*, indicating that adults can adjust their level of parental investment differently according to the sex of offspring (Beaulieu *et al.*, 2009).

During the crèche stage, the sex ratio difference observed was larger, but not significant, probably due to small sample size. However, this implies that at least one pair member spends more time in the colony. This is similar to what occurs at the beginning of the incubation stage for this penguin species, when exchange intervals are of longer duration. Observations from past studies similarly report that individual adults remain longer at sea to feed before returning to feed chicks during the crèche stage (Conroy *et al.*, 1975b; Jansen *et al.*, 2002), which explains the greater variation of sex ratio of adults sampled in our study in February 2011.

For Chinstrap penguins, quality of biparental care is essential for reproductive success, because successful rearing of offspring is subject to many factors, such as parent's inexperience, lack of adequate thermal protection and consequent egg and chick losses (Conroy *et al.*, 1975a,b; Trivelpiece *et al.*, 1990; Williams, 1995). In addition, there are important reasons for biparental care, among them the need to obtain food from the sea over long distances and interspecific predation, in that some species of birds specialize on preying on the nests of others, as argued by Cockburn (2006). Through use of molecular techniques to determine sex of *P. antarctica*, it was possible to examine possible relationships between adult sex ratios present for different stages of the breeding season with the specie's life history and improve the understanding of the importance of contributions of both sexes in raising their offspring to achieve reproductive success.

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