

CAUSES AND CONSEQUENCES OF SEX RATIO BIAS
IN NAZCA BOOBIES (*Sula granti*)

BY

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INTRODUCTION

Nazca booby sex ratios

Tests of sex ratio (proportion of males in the population; Wilson and Hardy 2002) theory have provided some of the most prominent advances in evolutionary biology (Bull 1981, Charnov 1982, Clutton-Brock 1986, Frank 1990, Hardy 1997). Fisher (1930) deduced that, because the total reproductive value of males equals that of females (because every sexual diploid individual has exactly one mother and one father); the population sex ratio (PSR) should evolve under frequency-dependent natural selection to be 0.50 (which is also the expected PSR after meiotic production of gametes in sexual diploids). The high reproductive value of the rare sex causes selection to return any PSR bias to equality: offspring of the rare sex have better mating prospects than those of the more common sex, because the larger membership of the common sex splits the pool of mating events into smaller per capita shares than do the members of the rarer sex. Parents with a genetic predisposition to produce more of the currently rare sex will have the highest fitness as a consequence. Any tendency to produce the rare sex will be rewarded by selection and will increase in the population, causing the PSR bias to decrease. The advantage associated with producing the rare sex decreases as the PSR bias decreases. Thus, frequency dependent selection should counter PSR biases and return PSR to unity regardless of the direction of the bias. In the simplest case, parental investment in the production of sons and daughters should be equal, because a 0.50 PSR is an evolutionarily stable strategy (Birkhead and Parker 1997) that cannot be invaded by a biased allocation to offspring sexual function.

Under certain conditions the PSR may become skewed regardless of the correcting influence of Fisherian frequency dependence (Weatherhead and Teather 1991). The PSR might be altered adaptively or otherwise at any developmental stage. The primary PSR refers to variation at conception; secondary, at birth or hatching; tertiary, at the end of parental investment; and quaternary, at the time of recruitment into the adult breeding population. If one sex is more costly to rear, as in the case of sexual size dimorphism, equal parental investment in large and small offspring may be followed by greater mortality in the costly sex when food is scarce. Differential mortality of male and female offspring has been found in several species of mammals and birds (Clutton-Brock 1991).

Nazca boobies have been the focus of long-term reproductive and demographic studies in our lab. This long-lived (Anderson and Apanius 2003) pelagic seabird breeds in a large colony at Punta Cevallos on the island of Española in Galápagos, Ecuador. Details of the site are given in Anderson and Ricklefs (1987) and Apanius et al. (2008). Our lab has monitored the colony since 1984. Subsets of the population have been banded every year (except 1989) since the 1984-85 breeding season. Townsend and Anderson (2007) documented a significant male bias in the adult population that has persisted for nearly 20 years in this population. Differential mortality of adult males and females does not contribute to the PSR bias observed in Townsend and Anderson's (2007) long-term study. Therefore, the sex ratio bias must arise at an earlier (before adulthood) life history stage in the Nazca booby. Female Nazca booby adults and older nestlings are larger than males (Nelson 1978, Anderson 1993, Townsend et al. 2007, Apanius et al. 2008), and are likely to require more food per unit time (Apanius et al.

2008) and may suffer higher mortality during a food shortage. Therefore, the observed adult PSR bias could be due, in part, to excess mortality of females as nestlings or after the end of parental care, during the years spent as a juvenile and sub-adult.

Biased adult sex ratios provide valuable opportunities to test hypotheses in several areas, including sex allocation (Fisher 1930, Trivers and Willard 1973, Clark 1978, Bull 1981, Charnov 1982), mating systems (Emlen and Oring 1977, Andersson 1994, Kvarnemo and Ahnesjö 2002, Shuster and Wade 2003), and endangered species management (Clout et al. 2002). Knowledge of the ontogeny of the bias provides critical context that allows proper framing of these hypotheses. In addition, post-fledging survival is important for assessment of lifetime reproductive success (LRS). LRS is generally defined as the number of young reared successfully over an individual's lifespan (Clutton-Brock 1988, Gill 1994). In birds, LRS depends on the age of first breeding, lifespan, and cumulative annual reproductive success. Typically, the total number of nestlings raised to fledging is used to estimate LRS (Newton 1989). While fledging success is convenient to measure, it overlooks non-random mortality during the period between fledging and reproductive age. In long-lived species such as seabirds, which first breed years after fledging, estimates of LRS will be biased if offspring post-fledging mortality is high and non-random. Therefore, determination of the origin of the sex ratio bias in our population is necessary before an accurate account of relative LRS can be made.

Tracing the origin of the sex ratio bias can be accomplished through determination of the PSR at different life history stages in the Nazca booby, specifically at hatching, fledging, and appearance at the colony as an adult. Nestling and fledgling

sex must be determined with molecular techniques because the birds are sexually monomorphic at these stages. The polymerase chain reaction (PCR) can be used to amplify an intron region of the chromo-helicase-DNA binding (CHD) gene (Fridolfsson and Ellegren 1999). This intron region is shorter on the W chromosome than it is on the Z chromosome; therefore, PCR amplification of two differently sized fragments distinguishes females (the heterogametic sex) from males in most non-ratite birds (Fridolfsson and Ellegren 1999). After fledging, juvenile/sub-adult Nazca boobies spend several years at sea off the Central American and Mexican Pacific coasts (Huyvaert and Anderson 2004), before they return to the study colony as adults. Upon return, adult sexes can be distinguished by voice (Nelson 1978, Anderson 1993). Knowing the origin of the sex ratio bias is essential to test hypotheses regarding parental sex allocation and the relative reproductive value of sons and daughters and for the determination of LRS.

Divorce rates in Nazca boobies

Most birds form monogamous breeding pairs (Lack 1968), but little is known about what determines whether pair bonds are maintained or dissolved (Choudhury 1995, Ens et al. 1996). The term divorce has been used to describe cases where at least one partner has re-mated with another individual while both partners still live (Coulson 1972, Ens et al. 1993). Within monogamous species divorce rates vary widely from ~0% in waved (Harris 1973) and wandering albatrosses (Tickell 1968) to 100% in greater flamingos (Cézilly and Johnson 1995). It has been suggested that divorce should be viewed as a reproductive strategy that individuals use to maximize fitness (Coulson 1972, Ens et al. 1993, Choudhury 1995, McNamara and Forslund 1996). Thus, individuals

would be expected to divorce if the benefits outweigh the costs. Switching partners can be costly; an individual could end up with a lower quality or less compatible mate. Additionally, both males and females can lose one or more breeding seasons after divorce or loss of a mate (barnacle geese, Black et al. 1996; mute, whooper, and Bewick's swans, Rees et al. 1996; red-billed gulls, Mills et al. 1996; European blackbirds, Desrochers and Macgrath 1996; and macaroni penguins, Williams 1996). Other species re-pair within days (Cassin's auklets, Sydeman et al. 1996; oystercatchers, Ens et al. 1993). The excess of male Nazca boobies should make it easier for females to re-pair and more difficult for males to do so. For some species reproductive success increases with the length of the pair bond, perhaps due to increased familiarity between the mates (Ens et al. 1996). If familiarity is important for reproductive success, divorce may cause individuals to start over at the beginning of the process.

Theories concerning the causes for divorce abound (reviewed in Choudhury 1995, Ens et al. 1996, McNamara and Forslund 1996). Almost all have the same predictions; reproductive success should go up for the instigator of divorce compared to their success not divorcing, and reproductive success should stay the same or become reduced for the victim of divorce (Choudhury 1995, Ens et al. 1996, Dhondt 2002). Several studies have suggested that reproductive failure increases the likelihood of divorce (Choudhury 1995, Ens et al. 1996, McNamara and Forslund 1996, Dubois and Cézilly 2000). McNamara and Forslund (1996) created a "better option" model of the dependence of divorce rate on longevity, variation in mate quality, and the costs and benefits of divorce following a breeding attempt. They found that divorce rates increased with variance in mate quality and decreased with age.

Female Nazca boobies ought to have many qualified mates to choose from and a colonial existence combined with no land predators (Nelson 1978, Anderson 1993) should mean that selecting a new mate should not be very costly (in terms of finding a new suitable mate). Therefore, the divorce rate is expected to be relatively high in Nazca boobies, and females are expected to instigate the divorce.

Researchers and field assistants in Dr. David Anderson's lab have collected detailed breeding information on all banded birds breeding in the study colony since 1992 (see Huyvaert and Anderson 2004 for details of fieldwork). Mating patterns of male and female Nazca boobies can be assessed using this long-term database.

Nazca booby mate choice

Charles Darwin (1872) introduced the concept of sexual selection to explain the existence of extravagant traits, seen primarily in males, which often appear to oppose survival selection. These traits include bright coloration and/or exaggerated tail feathers seen in many bird species as well as large horns and antlers found in many grazing mammals. These traits may increase the risk of predation or are energetically costly to develop and appear to serve no obvious purpose in regard to survival. Darwin observed that many females "choose" particular males within the population as mates, creating differences in male reproductive success. Evidence of sexual selection and mate choice is common in nature (for a review see Andersson 1994). Good genes theories, including Zahavi's (1975) handicap principle and the Hamilton and Zuk (1982) hypothesis, have been developed to explain why females (and males) should practice mate choice. Good gene theories predict that potential mates should assess each other by single or multiple

traits and then select the highest quality mate available. Assessment of potential mates may be constrained by costs (time and predation risk) associated with searching for high quality mates.

Another important aspect of mate choice and sexual selection involves the operational sex ratio (OSR). The OSR is the proportional representation of males among the adults ready to mate in a population at a given moment (Emlen 1976, Emlen and Oring 1977) and is a central concept in explaining variation in sex roles and the intensity of mating competition (Clutton-Brock and Parker 1992, Andersson 1994, Kvarnemo and Ahnesjö 1996, 2002). The OSR differs from the PSR in that the OSR includes only the proportion of adults that are ready to mate, whereas the PSR includes all adults regardless of breeding status. Many sex ratio studies have only determined the PSR; however, if the OSR is 0.50, then the PSR may differ from 0.50, yet have little effect on breeding dynamics. Few studies have examined both the PSR and OSR, although some studies have determined the PSR and then inferred possible effects on male and female reproductive strategies (Cockburn et al. 2002). However, if the sexes mature at different rates or if one sex is reproductively unavailable at certain times of the breeding season then the OSR may not be skewed. A male-biased OSR is suspected for our study population (Townsend and Anderson 2007), but this has not been established empirically.

The OSR can be determined through systematic behavioral observations of all males and females present in a subsection of the main study colony that are actively seeking breeding partners. At the start of the breeding season, male Nazca boobies establish and defend nest site territories and attempt to attract females with a stretched-neck sky-point display. Other interactions between courting pairs are allopreening, gift

exchange, and parading advertisement of the feet (Nelson 1978, Anderson 1993). The OSR will be the proportion of males in the population that are in breeding condition (i.e. displaying; displaying males/displaying males and females).

Nazca Boobies are socially (Nelson 1978, Anderson 1993) and genetically (Anderson and Boag 2006) monogamous and parents share the responsibility of rearing the offspring (Townsend et al. 2007, Apanius et al. 2008). Nazca boobies do not appear (to the human eye) to have sexually dimorphic coloration or plumage, but given the extensive period (approximately six months) of parental care (Nelson 1978, Anderson 1993, Apanius et al. 2008), both males and females should benefit from choosiness in mating. If a male-biased OSR exists in our population, then females should be able to choose the best available partner from a pool of potential mates.

How females select the best available partner is an open question. Theories of mate choice are based on the premise that choosers use traits that accurately predict the potential mate's ability to produce offspring that will successfully recruit into succeeding generations (Andersson 1994, Kokko et al. 2002). The reliability of sexually selected traits is linked to life-history trade-offs that involve allocation of time and energy to parental effort vs. other fitness-related functions, like self-maintenance. This trade-off suggests that high-quality individuals are generally better able to afford investment in breeding than are low-quality individuals (Stearns 1992). To optimize reproduction, monogamous taxa with bi-parental care must choose a mate that will allow them to produce as many high-quality offspring as possible (i.e., a mate who provides good parental care (Trivers 1972) as well as "good genes" (reviewed in Andersson 1994)). Behavioral ecologists traditionally link individual quality with exaggerated secondary

sexual characteristics (reviewed in Andersson 1994) or suites of sexually dimorphic traits (Møller and Pomiankowski 1993, Endler and Houde 1995). Seabirds show notable variation in individual quality, but do not typically have sexually dimorphic plumage (Schreiber and Burger 2002). Therefore, body condition and nutritional status could be used as indicators of individual quality. Since provisioning ability is essential to breeding success in seabirds (Nur 1984, Schreiber and Burger 2002), it is commonly assumed that body mass or body condition index (BCI - usually mass corrected for body size; reviewed in Brown 1996) at the start of the reproductive cycle is an important determinant of parental performance. Individuals with greater mass for their body size are thought to be more proficient foragers (Chastel et al. 1995). Furthermore, potential mates with greater body reserves (e.g., higher BCI scores), may have more endurance during non-feeding incubation bouts (Chaurand and Wiemerskirch 1994, Olsson 1997, Gauthier-Clerc et al. 2001) and may guard young chicks for longer periods of time (Tveraa et al. 1998, Tveraa and Christensen 2002). Blood chemistry is used in veterinary medicine to assess pathological changes due to dehydration, malnutrition, trauma or disease (Fudge 1997, Harr 2002). The ability of blood parameters to resolve variation in physiological status within wild populations of breeding (and presumably healthy) birds is receiving increasing scrutiny. Generally, decreased body condition, when assessed by mass loss, is reflected in a variety of blood parameters (e.g., Jenni-Eiermann and Jenni 1994, Alonso-Alvarez et al. 2002, Forero et al. 2006, Artacho et al. 2007). Whether these hematological parameters are a more sensitive measure of potential mate quality, compared to morphological traits, has not been previously addressed. Nazca booby females are

expected to select mates in better body condition with blood chemistry profiles that reflect good nutritional status.

Body weight and skeletal measures of males can be taken at the start of the breeding season (prior to egg-laying, which typically begins in mid-October on Española) to determine BCI. Serum samples can be collected as well to determine the circulating concentrations of three hematological parameters that have sufficient background information for unambiguous interpretation of the outcomes: uric acid (UA), albumin (ALB), and immunoglobulin G (IgG). UA is the principal waste product of dietary protein catabolism in birds. Carnivorous raptors and piscivorous seabirds show a pronounced post-prandial increase in UA concentration, especially when prey is ingested in a single, large meal (Lumeij and Remple 1991, Kolmstetter and Ramsay 2000, Harr 2002). UA is expected to reflect recent foraging success. ALB is the most abundant circulating protein and is critical for maintaining a homeostatic level of colloidal osmotic pressure in the vasculature (Griminger 1976, Sturkie 1976). ALB can provide information about the long-term nutritional status of an individual (Griminger 1976, Harr 2002). IgG is the second most abundant plasma protein and provides a critical self-maintenance function by binding to and eliminating toxins, viruses, bacteria, and parasites that infiltrate mucosal barriers (Warr 1995, Hanson 1979, Lemke et al. 2004). UA and ALB concentrations can be determined through enzymatic rate reactions measured with an automated micro-plate reader, while IgG concentration can be measured after separation from other serum proteins in a polyacrylamide gel.

Overview

The focus of this dissertation is to determine how the PSR bias arose, if an OSR bias exists, and how the male-biased sex ratio affects the mating dynamics of the Nazca booby. Regardless of the cause, the adult PSR bias and the presumably biased OSR have many important consequences regarding the relative reproductive value of sons and daughters, the intensity of sexual selection, mate choice, divorce rate, and other demographic parameters such as the age of first reproduction, the sex and age specific probability of obtaining mates, and LRS.

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CHAPTER 1

Ontogenic Sex Ratio Variation in Nazca Boobies Ends in Male-Biased Adult Sex Ratio

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ABSTRACT

Knowledge of the ontogeny of the sex ratio is required for proper framing of evolutionary hypotheses regarding adaptive parental sex allocation, competition for mates, evolution of mating systems, and endangered species management. We traced the development of a male-biased adult sex ratio in Nazca Boobies by determination of the sex ratio at hatching, fledging, and at returning to join the adult population. The sex ratio at hatching and fledging did not differ from unity, while the return sex ratio was significantly male-biased. Therefore, the observed adult sex ratio bias arises after the period of parental care ends, during the subadult/juvenile stage, with important implications for mate competition.

KEY WORDS: Nazca Booby, *Sula granti*, fledgling survival, operational sex ratio, population sex ratio, sex allocation, sex-specific mortality.

INTRODUCTION

Biased adult sex ratios (proportion of males in the population; Wilson and Hardy 2002) in animal populations provide valuable opportunities to test hypotheses in several areas, including sex allocation (Fisher 1930; Trivers and Willard 1973; Clark 1978; Bull 1981; Charnov 1982), mating systems (Emlen and Oring 1977; Andersson 1994; Kvarnemo and Ahnesjö 2002; Shuster and Wade 2003), and endangered species management (Clout et al. 2002). Knowledge of the ontogeny of the bias provides critical context that allows proper framing of these hypotheses. For example, a bias attributable to sex-specific mortality early in the adult lifespan disturbs the operational sex ratio (OSR; proportion of sexually receptive males in the total population of sexually receptive individuals; Kvarnemo and Ahnesjö 1996, 2002) and thus can have a strong effect on the mating system via competition for mates (Emlen and Oring 1977; Andersson 1994; Shuster and Wade 2003). Conversely, a bias arising late in the reproductive lifespan may affect the adult sex ratio but exert little selective influence on mating dynamics (Weimerskirch *et al.* 2005). Regarding sex allocation, a bias in the adult sex ratio due to mortality before the end of parental care should induce frequency-dependent selection on parents to over-produce the rare sex; a bias arising after the end of parental care should not induce such selection (Fisher 1930; Charnov 1982). The ontogeny of sex ratio bias is thus a significant point of interest.

Tracing the sex ratio through the lifespan of a bird species can be difficult and is seldom attempted, in part because the possibility of sex-specific dispersal frequently interferes with comprehensive estimates of the sex ratio at demographic mileposts, and because non-invasive sex determination of nestlings (Griffiths *et al.* 1998; Sheldon 1998;

Fridolfsson and Ellegren 1999) has only recently become available. To evaluate hypotheses regarding sex allocation, sex ratio at birth/hatching and at the end of the parental investment period/fledging have been measured for a number of species of mammals and birds (reviewed in Clutton-Brock 1986; Clutton-Brock and Iason 1986; Gowaty 1993) but only a few studies of birds (Rufous Vangas (*Schetba rufa*), Asai *et al.* 2003; Roseate Terns (*Sterna dougallii*), Szczys *et al.* 2005; Wandering Albatrosses (*Diomedea exulans*), Weimerskirch *et al.* 2005) have also documented the sex ratio after this offspring rearing period into adulthood. A biased (or balanced) sex ratio early in ontogeny may not persist into adulthood, so the sex ratio of young at the end of the parental care period may not predict the sex ratio of the adult population, highlighting the value of a comprehensive tracking of the sex ratio across the lifespan.

Townsend (2004) documented a consistently male-biased adult sex ratio (0.589, 95% CI 0.589-0.589) in a 19 yr study of Nazca Boobies (*Sula granti*) in the Galápagos Islands, suggesting more intense male-male competition for mates. Adults showed no sex-specific mortality, but age-specific mortality was not examined because the age of many of the adults was not known. Nazca Boobies have a post-reproductive lifespan (PRLS; Anderson and Apanius 2003), during which sex-specific mortality would bias the adult sex ratio but not the OSR, similar to the result for Wandering Albatrosses (Weimerskirsh *et al.* 2005). If a bias arose during the PRLS, then evolution of the mating system should be independent of the excess of adult males. To inform hypotheses regarding competition for mates given the adult sex ratio bias, identification of the timing of the appearance of the bias is required. In this paper, we use our long-term database on this population and molecular sexing of two large cohorts of chicks to trace the ontogeny

of this bias in the adult sex ratio and to determine the stage at which the male bias arises. We measured the sex ratio at hatching (secondary sex ratio), fledging (tertiary sex ratio), and early adulthood (return sex ratio, measured upon return into the colony-based adult population after several subadult years at sea).

Nazca Boobies are long-lived (Anderson and Apanius 2003) predatory birds with a low reproductive rate, raising, at most, only a single offspring per year (Humphries *et al.* 2006) from a clutch of one or two eggs (Anderson 1990). The second egg in two-egg clutches (“B-egg”) is laid several days after the “A-egg” (Anderson 1989a), and if both eggs hatch, only one chick (usually the product of the A-egg) survives siblicidal interactions shortly after hatching (Humphries *et al.* 2006). Our long-term study of the reproductive life history of Nazca Boobies has revealed several strengths of this species as a model for research on sex ratio evolution. Colonial breeding (Anderson 1993) permits collection of large sample sizes, providing substantial statistical power. Adults and nestlings have been banded in most years since 1984-85, giving a total of 1960 birds banded as adults and 8822 as nestlings (many of whom survived to adulthood). Unlike many seabirds, adults are sexually dimorphic (in voice) and can be easily sexed (Nelson 1978). The colony attendance patterns of banded adults yield high encounter probabilities (ranging from 0.82 to 0.90 for males and 0.76 to 0.86 for females, not significantly different; Townsend 2004) during our annual band-resight surveys (Huyvaert and Anderson 2004), giving estimates of sex-specific survival with low error. Finally and of particular importance, both natal philopatry and adult nest site fidelity are essentially 100%, so a permanent absence from the breeding colony reliably indicates death (Huyvaert and Anderson 2004). We know of no studies that have traced the

ontogeny of the sex ratio in a highly philopatric species which lacks the confounding influence of dispersal. Of the few studies available, females disperse to breed in the Rufous Vanga (Asai *et al.* 2003), up to 42% of juvenile Roseate Terns are estimated emigrate to other sites (Lebreton *et al.* 2003), and 23% of Wandering Albatross juveniles disperse to breed in non-natal colonies (Inchausti and Weimerskirsh 2002).

METHODS AND STUDY SITE

We calculated the secondary sex ratio of 918 and 1236 hatchlings in the 2001-02 and 2002-03 breeding seasons, respectively, based on analyses of blood samples collected from hatchlings at our study site at Punta Cevallos, Isla Española, Galápagos Islands (details in Anderson and Ricklefs 1987 and Huyvaert and Anderson 2004). We also calculated the post-siblicide offspring sex ratio by excluding all hatchlings lost during brood reduction, leaving 692 and 915 hatchlings in the two seasons, respectively. In these two years, we monitored 1410 and 1582 nests, respectively, on a daily basis, marking A- and B-eggs on the day of laying. We took blood samples within 24 hrs of hatching from the brachial or femoral vein by venipuncture, collecting the blood on a filter paper tab, and preserving it in 70% ethanol. In 2001-02 we failed to collect blood samples from five hatchlings due to errors and from eight who disappeared before sampling. In 2002-03, the corresponding figures were five and three. In 2001-02 we arrived at the colony after the start of hatching, and the 18 chicks already present, plus an unknown (but small) number that had already died and disappeared, were excluded from all analyses. Genomic DNA was isolated from blood samples by phenol/chloroform extraction (Sambrook *et al.* 1989). Sex of the nestlings was determined by PCR

amplification of an intron region of the CHD gene (Fridolfsson and Ellegren 1999). We validated this sexing technique with blind testing of 100 known-sex adult Nazca Boobies. Of the 2259 sex determinations attempted, 55 (2.4%) failed to amplify due to DNA degradation or low DNA content of the sample (28, or 1.2% of the total) or for unknown reasons (27, or 1.2% of the total). Our study does not include the determination of the primary (at fertilization) sex ratio because molecular identification of embryonic sex is problematic (Arnold *et al.* 2003) and would require sacrificing hundreds of eggs within a national park.

We calculated the tertiary sex ratio in these same two years by monitoring the sexed offspring during the daily nest checks, determining either the date of death or the date of fledging. Nazca Booby offspring remain at or near the nest site until they can fly, and are usually seen at the nest site at lower frequency after becoming volant.

Approximately 15 days before fledging, chicks finish replacing their original downy plumage with pennaceous feathers. Since 1992-93, we have used daily nest checks to note the age at which chicks reached the “1% down” developmental stage, at which only 1% of the original down still remained. Since little mortality occurs after the 1% down stage (Humphries *et al.* 2006), we estimated fledging success using the number of chicks reaching this stage. To assess the breeding conditions in 2001-02 and 2002-03, we compared the number of nests initiated and the number of offspring fledged in those years with those of the previous nine years in all nests in the “Study Area,” a subsection of the breeding colony in which all nests are monitored in every year since 1992.

After fledging, Nazca Booby fledglings vacate the colony; band returns indicate that the juvenile/sub-adult period of several years is spent at sea off the Central American

and Mexican Pacific coasts (Huyvaert and Anderson 2004). They return to Punta Cevallos as pre-breeding adults and are first detected in the annual band resight survey, conducted at the beginning of each breeding season (detailed in Huyvaert and Anderson 2004). Thereafter, these adults are seen regularly at Punta Cevallos, and we refer to this switch from a pelagic existence to a colony-centered existence as “return.” We estimated the return sex ratio as the representation of males (determined by voice; Nelson 1978) among all individuals that were observed as adults in the colony during either the annual band resight survey (Huyvaert and Anderson 2004) or during yearly nest monitoring of breeders (described in Anderson and Ricklefs 1987 and Huyvaert and Anderson 2004), for the cohorts that fledged during the breeding seasons beginning in 1984-87 and 1992-95 (eight cohorts). Ongoing annual band resight surveys began in 1984 and comprehensive nest monitoring began in the 1992-93 breeding season. Adults rarely appear in the colony for the first time after reaching the age of eight or nine (T. Maness, unpublished data); therefore, most of the fledglings that survived the juvenile period had returned and were regular colony attendants when this study was conducted (the youngest cohort was 10 years old).

Statistical Analyses

Empirically determined sex ratios were compared using G tests, and empirical and predicted sex ratios were compared using χ^2 Goodness of Fit tests.

We examined sex- and age-specific variability in chick survival probabilities (S) by developing “known fate” models implemented in Program MARK (White and Burnham 1999). Using daily nest check data, we constructed individual encounter

histories for all chicks showing whether (a) the chick was alive at the beginning of a given interval, (b) the chick died during an interval, or (c) the chick fledged during an interval, after which time it was censored. All individuals entered the study upon hatching, which we set to be the first interval in all cases (an “even entry” design), although hatching occurred over periods of 128 and 175 days, in 2001-02 and 2002-03, respectively. We developed an *a priori* set of four models incorporating the effects of chick sex (g: male or female), 10d age group (a), and their interaction.

We based model selection and ranking on relative differences of the Akaike Information Criterion (AIC) following Burnham and Anderson (2002). We used adjusted AIC values (AIC_C) to account for small sample sizes, although the ratio of n to K (number of model parameters) was sufficiently large in most cases (>40 ; Burnham and Anderson 2002) to justify use of AIC. In such cases, inferences using AIC and AIC_C strongly tend to converge (Burnham and Anderson 2002, p. 66). Also following Burnham and Anderson (2002), any competing models with AIC_C differences (ΔAIC_C) < 2 were considered to have similar explanatory power. In 2001-02, monitored nestlings were followed until they had not been seen for 21 days after apparently fledging, but in 2002-03 only until fledging. Elsewhere we have shown that little mortality occurs after fledging and before vacating the colony (Humphries *et al.* 2006). As a further test of the assumption that sex-specific mortality is not important between fledging and vacating the colony, we used the longer encounter histories from 2001-02 in two separate analyses: one for the period hatching to fledging (matching that of 2002-03 in extent), and another for the period hatching to vacating the colony.

All statistical tests except mark-recapture modeling were performed with Statistica software (kernel release 5.5; Statsoft Inc. 1999).

RESULTS

Molecular Sexing

Of known-sex adults, all 50 male and 50 female adults were correctly sexed from their blood samples.

The secondary sex ratio (all hatchlings) was not different from 0.50 in either 2001-02 (0.488, 95% C.I. 0.456-0.520; $\chi^2_1 = 0.53$, n.s.) or 2002-03 (0.474, 95% C.I. 0.446-0.502; $\chi^2_1 = 3.31$, n.s.). Similarly, the post-siblicide sex ratio (hatchlings, excluding brood reduction victims) was not different from 0.50 in either 2001-02 (0.490, 95% C.I. 0.453-0.527; $\chi^2_1 = 0.28$, n.s.) or 2002-03 (0.474, 95% C.I. 0.442-0.507 $\chi^2_1 = 2.41$, n.s.). The post-siblicide sex ratio was not different from the secondary sex ratio in 2001-02 ($G_1 < 0.001$, n.s.) or in 2002-03 ($G_1 < 0.001$, n.s.). In 2001-02, the tertiary sex ratio (0.490, 95% C.I. 0.428-0.552; N=245) was not different from the post-siblicide sex ratio ($G_1 = 0.00$, n.s.), or from 0.50 ($\chi^2_1 = 0.10$, n.s.). In 2002-03, the tertiary sex ratio (0.482, 95% C.I. 0.447-0.517, N=788) was also not different from the post-siblicide sex ratio ($G_1 = 0.100$, n.s.), or from 0.50 ($\chi^2_1 = 0.971$, n.s.).

Nestling Survival

In the Study Area (see Methods), the 2001-02 and 2002-03 breeding seasons were years of below (245 fledglings; 35% post-siblicide survival rate) and above average (788

fledglings; 86% post-siblicide survival rate) breeding productivity, respectively, compared to the previous nine years (Fig. 1).

Model ranking using AIC_C in both years gave little support for an influence of nestling sex on survival to fledging (Table 1). The model likelihood of the best model that included a nestling sex effect (g) in 2001-02 (S(g*a)) indicated that it received essentially no support relative to the best model overall, which included no sex effect (S(a); Table 1). The model likelihood of the best model including a sex effect in 2002-03 (also S(g*a)) indicated that the data supported the best model (S(a)) approximately 11.1 times ($= 0.0897^{-1}$; Table 1) more. Given the low support for the second ranked models (those including sex effects), use of “real parameter estimates” from those models for visual representation of the survival schedules of males compared to females was not advised (Burnham and Anderson 2002). Instead, we used the ratio of the actual number of nestlings surviving (or fledging during) a 10-d age class to the number entering the class to show survival schedules (Fig 2).

Return Sex Ratio

The return sex ratio of four of the eight cohorts was significantly male biased ($\chi^2_1 \geq 5.35$, $P < 0.02$ for the 1986-87, 1987-88, 1992-93, and 1994-95 cohorts), and did not differ from a balanced sex ratio for the other four cohorts (Fig. 3). The overall return sex ratio was 0.575 (95% C.I. 0.551-0.598), and was significantly male biased (Fig. 3; $\chi^2_1 = 36.06$, $P < 0.001$).

DISCUSSION

We have shown that the male-biased adult sex ratio in our study population cannot be accounted for by male-biased hatching and/or fledging sex ratios in our study population and that no sex-specific mortality occurred during the nestling period (Fig. 2, Table 1). The two breeding seasons in which these parameters were measured were of below average (first year) and above average (second year) reproductive performance (Fig. 1). Parents might adaptively alter the sex of their offspring according to current environmental conditions, or sex-specific mortality might occur in poor food years; the fact that the sex ratio was balanced at hatching and fledging in both of these contrasting years provides evidence that the sex ratio at these stages is not sensitive to inter-annual environmental variation in Nazca Boobies. The overall return sex ratio, from eight cohorts, was significantly male-biased at a value similar to the overall adult sex ratio (Townsend 2004), due to detectable deficits of females in four of the cohorts. These data indicate that the male biased adult sex ratio in our study population arises after the period of parental care ends, during the juvenile/subadult stage between fledging and return to the colony. This bias cannot be attributed to sex-specific emigration (Huyvaert and Anderson 2004). Instead, the deficit of female recruits is apparently a consequence of sex-specific post-fledging mortality.

Sex differences in mortality of newly independent animals could have a variety of causes. In many bird and mammal species, males exhibit higher juvenile mortality, presumably because their larger body size leads to higher energy requirements, which they fail to satisfy while they are learning to become independent (Clutton-Brock *et al.* 1985). Adult female Nazca Boobies are 16% heavier than males (Nelson 1978),

indicating the possibility of higher energy requirements, which could lead to higher mortality rates while learning to forage. If this were true, then the general quality of environmental conditions early in life should predict mortality based on this cause, but only partial correspondence exists between the indicators of breeding conditions in Fig. 1 and the eventual return sex ratios from those cohorts shown in Fig. 3. El Niño-Southern Oscillation (ENSO) conditions affect food supply around our study colony and depress breeding performance (Anderson 1989b), and three of the four cohorts with a male bias at return experienced conditions of the 1987-88 or 1992-93 ENSO events within a year of fledging (Fig. 3). Sub-adult females might have experienced more food stress than males did during these events, accounting for the deficit of females at return in these cohorts, but the strongly male-biased 1994-95 cohort, which did not experience ENSO conditions, departs from this pattern.

Alternatively, members of the larger sex may fledge at a disadvantage if parents meet their requirements less well than those of the smaller sex, which would exacerbate problems associated with post-independence inexperience. Townsend (2004) found that the asymptotic mass of Nazca Booby male nestlings was 105.8% that of mean adult male mass found by Anderson (1993), but the asymptotic mass of female nestlings was only 99.4% of typical adult female mass in the 2000-01 breeding season. In addition, asymptotic mass of females was more variable than that of males. This result suggests that female, but not male, nestlings were food-stressed to a degree that their growth was compromised. Hylton *et al.* (2006) found no correlation between body condition and subsequent post-fledgling mortality in the slightly size dimorphic Wood Stork (*Mycteria americana*), although they observed excess mortality of the larger sex (males) after

fledging. The authors acknowledge, however, that their results may have been confounded by their inability to monitor body condition late in the nestling period; condition was assessed 3-4 weeks prior to fledging. Assessment of the condition of offspring just prior to independence and comparative survival analyses are needed to fully assess the influence of fledging condition on sex-specific mortality. Dispersal has also been linked to increased mortality risk in mammals and birds (Gaines and McClenaghan 1980; Clarke *et al.* 1997; Yoder *et al.* 2004), but is unlikely to account for higher mortality of juvenile females in Nazca Boobies given their virtually complete natal philopatry (Huyvaert and Anderson 2004).

The male bias in our population's sex ratio arises before the age of breeding, and so is expected to affect the OSR. If true, then mating system theory predicts more competition for mates among males than among females, a higher opportunity for selection on males, and a higher variance in male reproductive success (Emlen and Oring 1977; Andersson 1994; Shuster and Wade 2003). Our results provide the critical context in which to frame hypotheses regarding parental sex allocation and mate choice in this species and insight into the causes of bias sex ratios in birds. To our knowledge, our study is the most complete documentation of the ontogeny of the sex ratio across the lifespan for any highly philopatric wild bird species.

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Table 1. AIC_C rankings of known fate models developed in Program MARK. Survival probabilities (S) were modeled as constant (.) over age intervals, as a function of age (a), and as a function of sex (g), incorporating interactions of these factors. The two sets of models fit for 2001-02 test the assumption that truncation of the encounter history at the fledging stage does not affect model selection and ranking.

Model	AIC_C	ΔAIC_C	AIC_C weight	Model likelihood	Number of parameters
<i>2001-02, hatching until fledging</i>					
S(a)	1213.0	0	1.00000	1	15
S(g*a)	1243.7	30.7	0.00000	0.0000	30
S(.)	1271.7	58.8	0.00000	0.0000	1
S(g)	1273.7	60.8	0.00000	0.0000	2
<i>2001-02, hatching until last seen</i>					
S(a)	2690.0	0	0.99960	1	20
S(g*a)	2705.7	15.6	0.00040	0.0004	40
S(.)	2925.2	235.4	0.00000	0.0000	1
S(g)	2927.2	237.1	0.00000	0.0000	2
<i>2002-03, hatching until fledging</i>					
S(a)	1303.0	0	0.91771	1	13
S(g*a)	1307.5	4.8	0.08229	0.0897	26
S(.)	1387.4	84.7	0.0	0.0	1
S(g)	1389.0	86.3	0.0	0.0	2

Figure 1. Number of nests initiated, and number of chicks reaching the 1% down developmental stage (see text), in the Study Area (a representative area of the Punta Cevallos Nazca Booby colony) over an 11-year period. The two years in which secondary and tertiary sex ratios were estimated are indicated in bold. “92” refers to 1992-93 breeding season, etc.

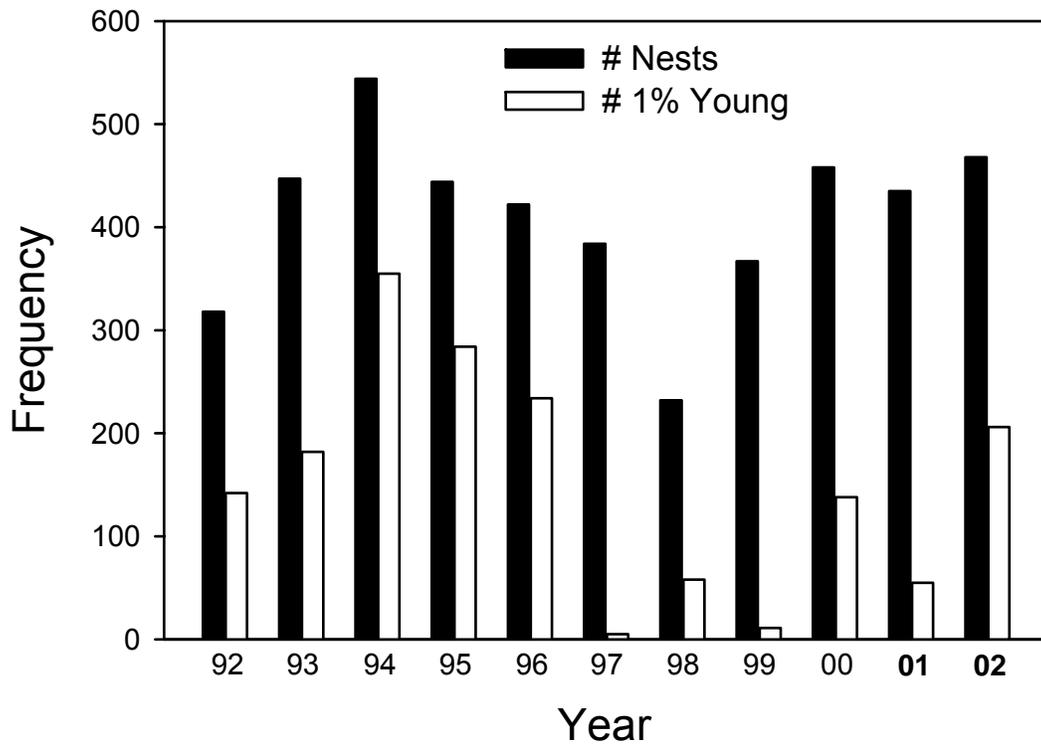


Figure 2. Proportion (\pm binomial 95% C.I.) of male and female offspring that enter a 10d age class and also survive that age class in 2001-02 (A) and 2002-03 (B).

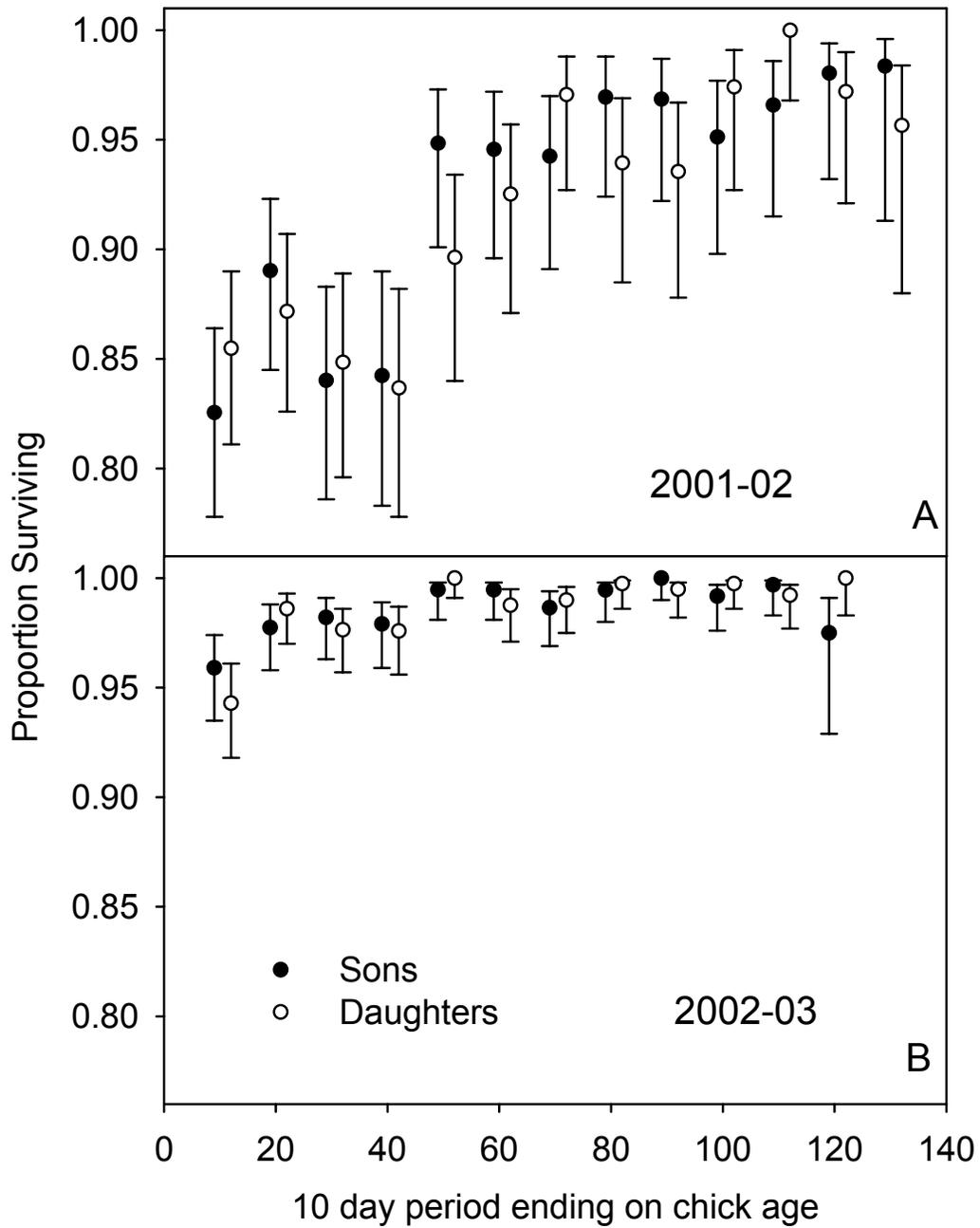
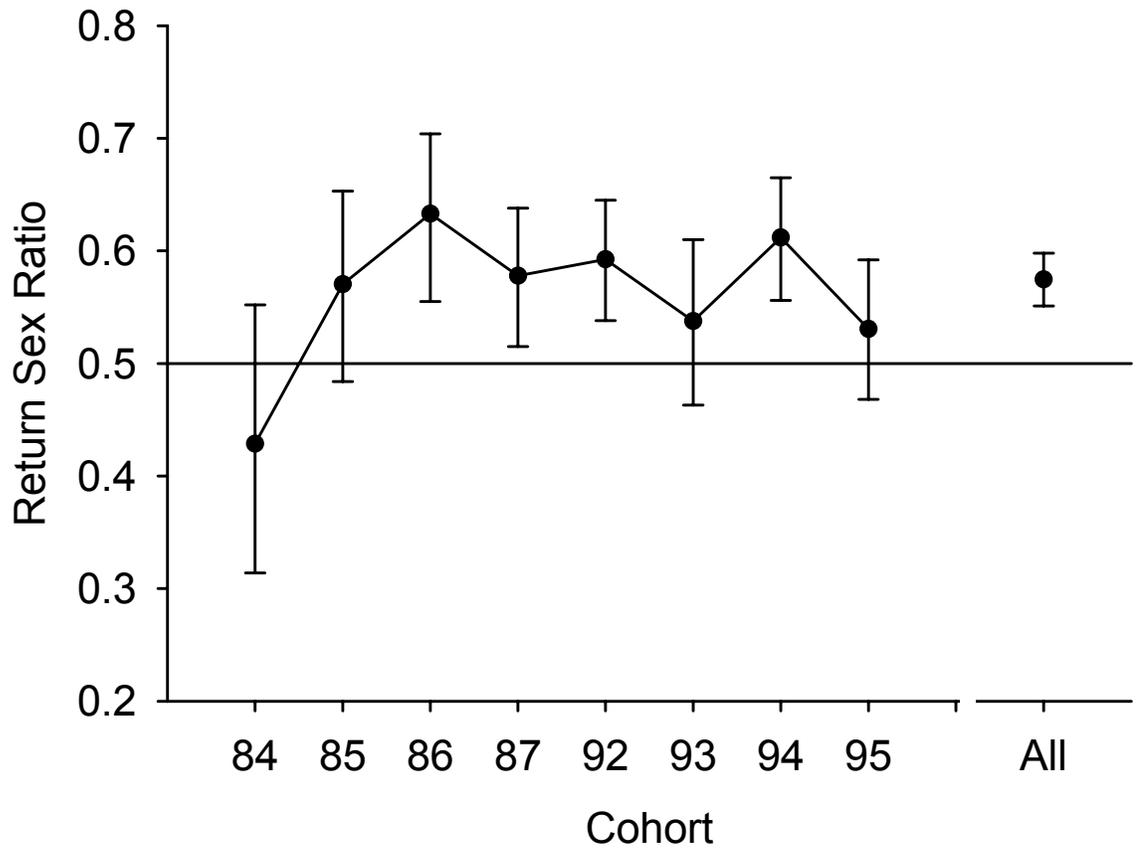


Figure 3. Return sex ratio (\pm binomial 95% C.I.) of all birds in selected cohorts that returned to join the adult, colony-based population. “84” refers to 1984-85 breeding season, etc. “All” is the overall return sex ratio determined from all eight cohorts.



CHAPTER 2

Serial Monogamy and Sex Ratio Bias in Nazca Boobies

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ABSTRACT

Biased operational sex ratios (OSRs) can drive sexual selection on members of the over-represented sex via competition for mates, causing higher variance and skew in reproductive success (RS) among them if an individual's quality is a persistent characteristic. Alternatively, costs of reproduction may degrade breeding performance, creating the opportunity for members of the limiting sex to switch mates adaptively, effectively homogenizing variance and skew in RS among the sex in excess. We tested these two contrasting models in a male-biased population of the Nazca booby (*Sula granti*) with demonstrated costs of reproduction with data on total RS over a 14-year period. Variances and skews in RS were similar, and males changed from breeder to non-breeder more frequently than females. Under the persistent individual quality model, females should mate only with high quality males, and non-breeding males should seldom enter the breeding pool, yet 45% of non-breeding males (re)entered the breeding pool each year on average. Many Nazca booby females apparently exchange a depleted male for a new mate from the pool of current non-breeder males. Our evidence linking serial monogamy to costs of reproduction is novel and suggests selection on female mating preferences based on an interaction between at least two life-history components (OSR and reproductive effort).

Keywords: mate choice; operational sex ratio; cost of reproduction; serial monogamy; divorce; multi-state mark-recapture models

INTRODUCTION

A biased operational sex ratio (OSR; relative ratio of individuals ready to mate: males/(males + females); Enders 1993; Kvarnemo & Ahnesjö 1996; 2002) should drive sexual selection on members of the over-represented sex via competition for mates, and can logically be expected to cause higher variance and skew in reproductive success (RS) than in the limiting sex (Emlen & Oring 1977; Andersson 1994; Shuster & Wade 2003). For example, as the limiting sex in a male-biased OSR, females should have more opportunity than males do to choose among competing potential mates, and when females consistently recognize and mate with high quality males, and reject low quality males, they induce a higher variance and skew in mean mating success of males. Members of the limiting sex presumably have easy access to potential mates, so even the least competitive members may have mating successes similar to those of the most competitive members (Kvarnemo & Ahnesjö 2002; Shuster & Wade 2003). Numerous theoretical and empirical studies (Andersson 1994) as well as recent experimental manipulation of the sex ratio during a single breeding season (Jones *et al.* 2004; 2005; Mills *et al.* 2007) have supported this idea.

If the pattern of RS seen in a single season is to persist over the lifetimes of iteroparous organisms, then the mating pool must consistently exclude the same individuals of the sex in excess. This assumes that quality is a persistent characteristic of individuals, as proposed in optimal divorce/re-mating strategy models (McNamara & Forslund 1996, Dubois *et al.* 2004). Under a persistent individual quality model, high quality members of the sex in excess tend to remain “in the game”, and low quality members tend to remain excluded. This pattern is expected especially in long-lived

species where reproductive output may improve with age and/or experience (see Clutton-Brock 1988 for a review; Wooller *et al.* 1992; Forslund & Pärt 1995; Anderson & Apanius 2003; van de Pol *et al.* 2006) and with the length of the pair bond (Black *et al.* 1996; Pyle *et al.* 2001; van de Pol *et al.* 2006).

Alternatively, breeding quality may not be a persistent trait of individuals, but may change throughout the lifespan, declining in old age (see Clutton-Brock 1988 for a review; Anderson & Apanius 2003; van de Pol *et al.* 2006), with temporary infection (although susceptibility to infection has been proposed as a fixed trait; Hamilton and Zuk 1982), or with reproductive effort (Reid 1987; Sæther *et al.* 1993; Jacobsen *et al.* 1995; Weimerskirch *et al.* 1995; Pyle *et al.* 1997; Erikstad *et al.* 1998; Golet *et al.* 1998; 2004; Kalmbach *et al.* 2004; Townsend & Anderson 2007). In this situation, an individual could switch mates adaptively if its current mate's quality dropped below the value of those in the unmated pool (Ens *et al.* 1996; Dubois *et al.* 2004). Divorce is also expected to increase in frequency with increasing availability of unpaired individuals (see Choudhury 1995 for a review). With a biased OSR and temporally variable breeding quality, members of the over-represented sex might rotate in and out of the breeding pool, via the effect of choice exerted by the other sex.

In this paper we consider the interaction of the sex ratio and costs of reproduction in the mating system of Nazca boobies (*Sula granti*). Our study population at Punta Cevallos, Isla Española, Galápagos Islands has shown a consistent male bias (0.589, 95% CI 0.589-0.589 (a highly precise point estimate); Townsend & Anderson 2007), arising during the post-fledging sub-adult period (Maness *et al.* 2007), over the course of a 21 year ongoing study. Sex-specific costs of reproduction may override the effect of OSR

on mating system through increased mortality risk or increased need to recover from breeding (“time out;” Clutton-Brock & Parker 1992) in the sex providing more parental care (Kokko & Monaghan 2001). Although costs of reproduction exist in Nazca boobies, they do not appear to be sex-specific. Mothers and fathers incur similar survival costs of reproduction (Townsend & Anderson 2007); during breeding, the sexes lose similar amounts of mass and neither sex exhibits decreased immune-mediated self-maintenance (Apanius *et al.* 2008). Behavioral evidence indicates that all adults present in the breeding colony attempt to enter the breeding pool (Maness & Anderson 2008). Therefore, the adult sex ratio of our study population apparently reflects the OSR, both are male-biased, and confounding effects of differential costs of reproduction (i.e., Kokko & Monaghan 2001) on evolution of the mating system apparently do not exist.

Even in monogamous species like the Nazca booby, a long-lived pelagic seabird with obligate bi-parental care and no extra-pair fertilization (Anderson & Boag 2006), we expect an unbalanced OSR to induce a greater variance and skew in RS in the over-represented sex, if the same competitive individuals of that sex consistently acquire mates of the limiting sex. However, male quality might not be persistent, given the known costs of reproduction borne by breeders (Townsend & Anderson 2007), so we also considered an alternative model of mate choice, serial monogamy (*sensu* Baeyens 1981), in which females change mates adaptively between consecutive breeding efforts. Under this model, based on transient individual quality rooted in costs of reproduction, females exchange a depleted male for one that has not recently bred. Females using this strategy exploit their status as the limiting sex, and as the larger sex (females are 16% heavier; Anderson 1993) that can control social interactions, to maximize the parental contribution

of their mate. Ideally, a female should switch mates before her mate's performance begins to decline in order to maximize her reproductive output. Under this model, male variance in reproductive success might not exceed that of females, despite the sex ratio bias, because the rotation in mating imposed on males by females would tend to homogenize reproductive success among males. In this study we tested both this "rotation" model and the "persistent individual quality" model.

Given the observed male-biased OSR, the model of persistent individual quality makes several predictions: (1) males should have higher variance and (2) skew in RS than females, and (3) breeding state transition probabilities (breeder to non-breeder) should be similar between the sexes because "winner" males and females should remain in the breeding category while "loser" males should remain non-breeders. A female may switch mates between consecutive breeding attempts, but her new mate should come from the current pool of high quality breeders. Following the same logic, (4) the number of years individuals breed consecutively (breeding bout length) should be similar among breeding males and females. The rotation model predicts the opposite on all four points, with males having a similar or lower variance and skew in RS, higher breeding state transition probabilities, and shorter breeding bout lengths. Additionally, the rotation model predicts that the RS of divorced males (an inverse proxy for condition) in the year prior to divorce should be higher than that of retained males if a successful breeding attempt induces higher costs of reproduction than does a failed attempt.

MATERIAL AND METHODS

(a) *Variance and skew in RS*

To test predictions about variance and skew in RS we calculated the mean, variance, and skew in the total number of fledglings produced by each known-age adult from each of eight hatch-year cohorts breeding in the 14 breeding seasons from 1992-93 through 2005-06 (see Huyvaert & Anderson 2004 and Townsend & Anderson 2007 for details of fieldwork and study site). Adults in these cohorts fledged during the breeding seasons beginning in 1984-87 and 1992-95. The oldest birds were 21 years old at the end of the study while the youngest were 10 years old. Successful reproduction is apparently rare after approximately 20 years of age (Anderson & Apanius 2003), so these estimates do not reflect lifetime RS for longer-lived individuals in the younger cohorts. Also, we did not begin collecting comprehensive RS data until the 1992-93 breeding season, so we do not have the early breeding history of the oldest cohorts. Because cohorts differed in number of years available for reproduction and the mean and variance of RS were positively correlated (figure 1 in electronic supplementary material), a Wilcoxon matched pairs test was used for within-cohort comparisons of standardized variances (variance divided by the squared mean; also known as the opportunity for selection (I); Crow 1958; Wade 1979; Wade & Arnold 1980; Shuster & Wade 2003) of males and females. Recent studies (Jones *et al.* 2005; Mills *et al.* 2007) have supported the use of the opportunity for selection based on Bateman's principle (Bateman 1948) as an index of mating competition.

Reproductive skew was compared using the B index (Nonacs 2000). The B index calculates the observed variance and then subtracts the expected variance if every group

member had an equal probability of gaining a resource (e.g., mate). B values of zero indicate randomly distributed resources, while significantly positive or negative values indicate more skewed or more equally shared resources, respectively. We used the Skew Calculator 2003 (Nonacs 2003a) to determine B index values, their 95%CI and level of statistical significance.

Much debate exists in the sexual selection literature over the best measure of inequality (Kokko *et al.* 1999; Nonacs 2000, 2003b; Fairbairn & Wilby 2001; Jones *et al.* 2005; Mills *et al.* 2007), leading Kokko *et al.* (1999) to advocate the use of multiple measures. Most measures correlate with each other (Nonacs 2003b; Mills *et al.* 2007), but the B index was reliable under a wide range of assumptions and allows the comparison of groups of different sizes and productivities (Nonacs 2003b). We calculated 12 additional inequality measures (detailed in table 1 in electronic supplementary material) and the Spearman rank correlations between all 13 inequality indices (table 2 in electronic supplementary material). Frequency distributions of RS of males and females within cohorts are presented in figure 2 in electronic supplementary material.

(b) *Breeding state transition probabilities*

We examined sex-specific variability in breeding state (non-breeder or breeder) transitions using multi-state mark-recapture model selection implemented in Program MARK (Cooch & White 2005). Individual encounter histories were established for the four oldest cohorts for a 13 year period (1992-2004). In these encounter histories, individuals could occupy one of three categories in a particular year: not seen, non-

breeder, or breeder. The 2005-06 breeding season was not included in this analysis because we monitored only successful breeding events in this year. Candidate models included survival (s) probability, resight (p) probability, and breeding state transition (ψ) probability parameters; each could remain constant (.) or vary by group (g), sex (r), or year (t). We used Akaike's Information Criterion (AIC) for model selection and ranking (Burnham & Anderson 2002). In practice, we used QAIC_C, a version of AIC incorporating adjustment of the variance inflation factor, based on an estimate of median c-hat ($\hat{c} = 1.19$; Cooch & White 2005). Models with the lowest QAIC_C values were assumed to better explain variation in the data.

(c) *Breeding bout lengths*

Breeding bout lengths were determined for the same individuals over the same 13 year period used to test breeding state transition probabilities; individuals which never bred were excluded. A bout length was calculated as the number of consecutive years an individual bred (incubated 1+ eggs for any length of time). If an individual had two or more breeding bouts during the 13 year period, then the mean bout length was used for that individual. The total number of breeding bouts was also determined. Overall male and female mean bout lengths and total number of breeding bouts were compared with separate t-tests.

(d) *RS of divorced and retained males*

RS within year (X) was determined for subsequently divorced or retained males (in year X+1) in the four oldest cohorts over a nine year period (1992-2001). Males were

classified as retained (those that bred with the same female in years X and X+1) or divorced (bred with different females in years X and X+1 or males that rotated out of the breeding pool for one or more years and then returned to the breeding pool). Individuals which never bred were excluded. To allow divorced males the opportunity to resume breeding again, only males (retained or divorced) that survived to year X+4 were included in the analysis; hence, retentions and divorces from the last four breeding seasons were excluded. Many males could not be categorized as retained or divorced because they bred with unbanded females, so mean RS for all uncategorized males was determined as well. Mean RS (fledglings/male/year; a mean proportion) of retained, divorced, and all other males was compared with an ANOVA because assumptions of normality and homogeneity of variance were met. Additionally, divorced males were further subdivided into six categories by non-breeding gap length (0, 1, 2, 3, ≥ 4 years, still out) to determine the proportion of males falling into each category.

(e) *Statistical Analyses*

Most statistical tests were performed using Statistica (v. 6.1 Statsoft Inc., Tulsa, OK, USA), except MARK analyses were performed using Program MARK (White & Burnham 1999; Cooch & White 2005).

RESULTS

(a) Variance and Skew in RS

Standardized variance (I) in RS of males and females did not differ ($Z = 0.980$, $p = 0.327$), although the data suggested an age trend; males had higher values at younger ages while females had higher values at older ages (figure 1).

Following correction with the false discovery method (Benjamini & Hochberg 1995; Curran-Everett 2000) for multiple comparisons, the B index values indicated that male RS was more skewed than would be expected from a random process (values significantly greater than zero) in seven of eight cohorts while female RS was more skewed in five of the eight cohorts (figure 2). Female RS tended to be less skewed in younger cohorts. No significant difference in reproductive skew between males and females was found in any cohort (figure 2). Therefore, one sex did not exhibit more reproductive skew than the other, although inspection of the distributions for males and females suggests a tendency for higher skew in females, not males (figure 2 in electronic supplementary material).

(b) Breeding state transition probabilities

Model ranking using QAIC_C gave support for an influence of sex on breeding state transition probability (table 1). The model likelihood of the best model that did not include a sex effect on breeding state transition probability ($\psi(g^*t)$) indicated that it received essentially no support relative to the best model overall, which included a sex effect ($\psi(r^*g^*t)$); table 1). The difference between the two best models (ΔQAIC_C) was less than 2, so these two were considered to have similar explanatory power (Burnham &

Anderson 2002). Both top models included a sex effect as well as group (g) and year (t) effects on breeding state transition probability. Real parameter estimates derived from weighted averaging of these two models showed that males had higher breeder to non-breeder transition probabilities than females did in 11 out of 12 years; 95% CIs indicated significant differences in eight of the 12 years (figure 3). Females had higher non-breeder to breeder transition probabilities than males did in 10 out of 12 years; 95% CIs indicated a significant difference in five of the 12 years (figure 3 in electronic supplementary material). Transition probabilities were year-dependent, but the mean non-breeder to breeder transition probability for males was $0.452 (+/-0.161\text{MSE})$; thus, few males should be excluded from breeding at some point during their lifetimes. The sex ratio bias of our population suggests that approximately 33% of males should be non-breeders under the persistent individual quality model, but only 8.2% (95% CI = 5.6-11.8%) of males never bred based on estimates from our mark-recapture models.

(c) *Breeding bout lengths*

Breeding bout length was significantly shorter in males (males = 3.44 ± 0.12 years, $n = 282$, females = 4.70 ± 0.22 years, $n = 201$; $t = -5.35$, $p < 0.0001$; figure 4). Males also had significantly more breeding bouts than females (males = 1.85 ± 0.05 , females = 1.65 ± 0.05 ; $t = 2.59$, $p = 0.01$). Breeding bouts were ended for most birds by poor environmental conditions in 1999-00 (see high breeder to non-breeder transition probabilities in figure 3), truncating the bouts of many females, but not males, at 6 years (figure 4). These females had bred continuously since the beginning of the study. As a

result, our estimates of bout length and number probably understate the continuity of breeding of females more than males.

(d) *RS of divorced and retained males*

The mean RS of retained (0.416 ± 0.097), divorced (0.412 ± 0.060), and all uncategorized (0.403 ± 0.092) males in the year prior to divorce/retention did not differ ($F_{2,24} = 0.006$, $p = 0.994$). Most divorced males resumed breeding after one year of non-breeding and few males obtained a new mate in the year of divorce (figure 5).

DISCUSSION

Males did not exhibit the higher variance and skew in RS predicted by the persistent individual quality model of mate selection in our male-biased study population. Instead, these values were homogenized in males because males rotated in and out of the breeding pool, as indicated by their higher breeder to non-breeder transition probabilities (figure 3), greater than zero non-breeder to breeder transition probabilities (figure 3 in electronic supplementary material), shorter and more numerous breeding bouts (figure 4), and short periods outside the breeding pool (figure 5). Non-breeding males had nearly a 50% chance to (re)enter the breeding pool per year, and few males were excluded from breeding completely. Since females bred more regularly than males (figures 3 and 4), some females must switch mates between successive breeding attempts to continue their longer breeding bouts, and we found that approximately 38% of Nazca booby pairs divorce each year (Maness & Anderson unpub. data).

Nazca booby males could voluntarily skip breeding attempts as some Procellariiform seabirds do (Bradley *et al.* 2000, Dobson & Jouventin 2007); however, evidence obtained from a behavioral study conducted in the 2003-04 breeding season on this Nazca booby population (Maness & Anderson 2008) clearly refutes this idea. Every Nazca booby male present in a subsection of the “Study Area” (detailed in Townsend & Anderson 2007) in the 2003-04 breeding season performed mate attraction behaviors (described in Nelson 1978). Of 111 males that bred in the same area the prior season (2002-03), 22 became non-breeders in 2003-04; 13 of these non-breeders were seen displaying in 2003-04, while the remaining nine individuals have not been seen in the four yearly censuses (described in Huyvaert & Anderson 2004) conducted since 2002-03. Therefore, 100% of males breeding in 2002-03 and present in 2003-04 attempted to obtain a mate by performing mate attraction behaviors, while 100% of 2002-03 breeders not seen in 2003-04 are mostly likely deceased.

Alternatively, costs of reproduction provide a basis for serial monogamy, imposed by females. Assuming that breeding induces “fecundity” costs of reproduction (as well as the demonstrated survival costs (Townsend and Anderson 2007)) in male Nazca boobies, females could mate-switch adaptively, replacing a temporarily degraded male for a current non-breeder in better condition. Females, but not males, would have this option in a male-biased population. Typically, female Nazca boobies in our study population simply leave the nest site held by their current mate and join another elsewhere (Maness & Anderson 2008). In such a mating system, few males would mate consistently, and few males would be excluded from the mating pool, even under a male-biased OSR. Our data support this interpretation. Since females breed more often than

males, one might expect that they would suffer higher mortality rates, but Townsend and Anderson (2007) found similar mortality rates between the sexes. This apparent contradiction may be explained by sex differences during the non-breeding season, when females vacate the breeding colony (presumably the site of negative density-dependent effects). In contrast, most males remain, defending nest sites that they will use in future breeding seasons, so males may have less opportunity to recover condition between breeding seasons (D. J. Anderson, unpub. data).

This evidence linking serial monogamy to sex ratio bias is novel and suggests selection on female mating preferences based on an interaction between at least two life-history components: OSR and reproductive effort. A within-sex, variance-based measure of sexual selection, like the opportunity for selection (Crow 1958; Wade 1979; Wade & Arnold 1980; Shuster & Wade 2003) indicates that Nazca booby males do not appear to be under stronger sexual selection than females are, despite the biased OSR. Yet, males had more trouble securing a mate each breeding season as indicated by behavioral observations as well as breeder to non-breeder transition probabilities (figure 3) and females have good reason to remain choosy. Our novel findings suggest that the use of variance-based measures of intensity of selection may only be correct when restricted to a single breeding season.

Mate rotation influenced by OSR may explain divorce in successful pairs, an area seldom addressed in divorce theory. Most birds (90% of species; Lack 1968) are socially monogamous, but far fewer species form persistent pair bonds (Black 1996). Selective factors favoring mate fidelity include obligate bi-parental care (Williams 1966; Lack 1968; Emlen & Oring 1977) and the “mate familiarity effect” (improved joint

reproductive performance from experience with each other; Black 1996). Long lifespan is also positively associated with high mate fidelity (Ens *et al.* 1996). From these considerations, long-lived seabirds are expected to form persistent pair bonds, and most do (Ens *et al.* 1996): albatrosses form some of the longest pair-bonds known in any animal taxon (Jouventin *et al.* 1999, Tickell 2000). Still, variation exists in divorce rate, even between different populations of the same long-lived species (Ens *et al.* 1996).

Many hypotheses regarding divorce fall under the “better option” model, whereby individuals may leave a partnership to obtain a higher quality mate, and thus to improve their reproductive success (reviewed in Choudhury 1995 and Ens *et al.* 1996). The rotation model suggests that divorced males should be more successful than retained males because higher costs of reproduction may be incurred by successful breeders. Most hypotheses regarding divorce predict worse performance in divorced males (see Choudhury 1995 and Ens *et al.* 1996): in Nazca boobies, prior performances of divorced and retained males were similar. The finding that RS was not higher in divorced males, as expected under the rotation model, may be explained by several possibilities which are not mutually exclusive: females might follow a mixture of divorce strategies; more than one year of successful breeding may be needed for some males to become depleted; and successful breeding may not induce higher costs than attempted breeding, especially if the nest failed late in the breeding period (Townsend & Anderson 2007) detected this effect for survival costs of reproduction). It should be noted that most hypotheses regarding divorce do not predict equal performance between divorced and retained mates either.

If costs of reproduction are ubiquitous in iteroparous organisms, as suggested by life history theory (Stearns 1992), then any bias in the OSR should allow the members of the limiting sex the opportunity to improve their reproductive output through mate rotation. This implies that offspring provisioning ability, rather than persistent genetic quality (or experience), may be more important to potential mates in some instances, particularly in altricial species with prolonged parental care. If the genetic quality of potential mates varies little among individuals, then the degrading effects of reproductive effort may make switching mates more beneficial than staying with an experienced mate. Van de Pol *et al.* (2006) found that pairs of oystercatchers (*Haematopus ostralegus*) which had been together for many years (>10 years) had lower reproductive success than did newly formed pairs. Individuals of both sexes whose mates were experimentally removed, even ones with pair-bonds in excess of 10 years, improved their reproductive output after pairing with a new mate. The authors concluded that divorce would seem to be advantageous for either member of very old pairs (van de Pol *et al.* 2006), yet few pairs actually separate (Ens *et al.* 1993; Heg *et al.* 1993; Heg *et al.* 2003; van de Pol *et al.* 2006). We suggest that the territorial requirements of this population (Ens *et al.* 1992; Heg *et al.* 1993; Ens *et al.* 1995) create an effectively even OSR, regardless of any bias in the adult sex ratio, because few openings in the breeding pool exist. Leaving a mate will likely lead to non-breeding status (van de Pol *et al.* 2006). Mate changing in oystercatchers may, thus, be constrained by their even OSR, such that they cannot take advantage of re-pairing with a new or refreshed mate whose condition has not declined with recent reproductive effort. Comparisons of standardized variances in reproductive success between the sexes and divorce rates in other monogamous species with biased

sex ratios are needed to assess the generality of the novel rotation re-mating strategy found in Nazca boobies.

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Table 1. QAIC_C rankings of multi-state models developed in Program MARK. Survival probabilities (s), recapture probabilities (p), and breeding state transition probabilities (ψ) were modeled as constant (.) or were allowed to vary as a function of sex (r), group (g; breeder/non-breeder), time (t; year), or by incorporating interactions (*) of these factors.

Model	QAICc	ΔQAICc	QAICc Weight	Model Likelihood	Parameters	Qdev
s(g) p(g) ψ (r*g*t)	5277.19	0.00	0.57	1.00	52	2087.35
s(g) p(r*g) ψ (r*g*t)	5277.71	0.53	0.43	0.77	54	2083.77
s(g) p(g*t) ψ (r*g*t)	5296.56	19.38	0.00	0.00	74	2061.30
s(r*g*t) p(r*g*t) ψ (r*g*t)	5314.67	37.48	0.00	0.00	140	1940.06
s(g) p(r*g*t) ψ (r*g*t)	5327.55	50.37	0.00	0.00	98	2042.16
s(g) p(g*t) ψ (g*t)	5375.90	98.71	0.00	0.00	50	2190.16
s(g) p(g) ψ (g*t)	5835.04	557.85	0.00	0.00	28	2694.18
s(g) p(r*g*t) ψ (r*t)	5861.85	584.66	0.00	0.00	74	2626.59
s(.) p(.) ψ (.)	6238.87	961.68	0.00	0.00	3	3148.41

Figure 1. Standardized variances in reproductive success of males and females within a hatching cohort. The trend (not significant) toward higher values for young males and older females may be explained by younger males having fewer breeding attempts than females (Maness & Anderson, unpub. data), amplifying the contrast in RS between successful and unsuccessful males, and by the rotation of mating partners by females homogenizing variance in RS among older males.

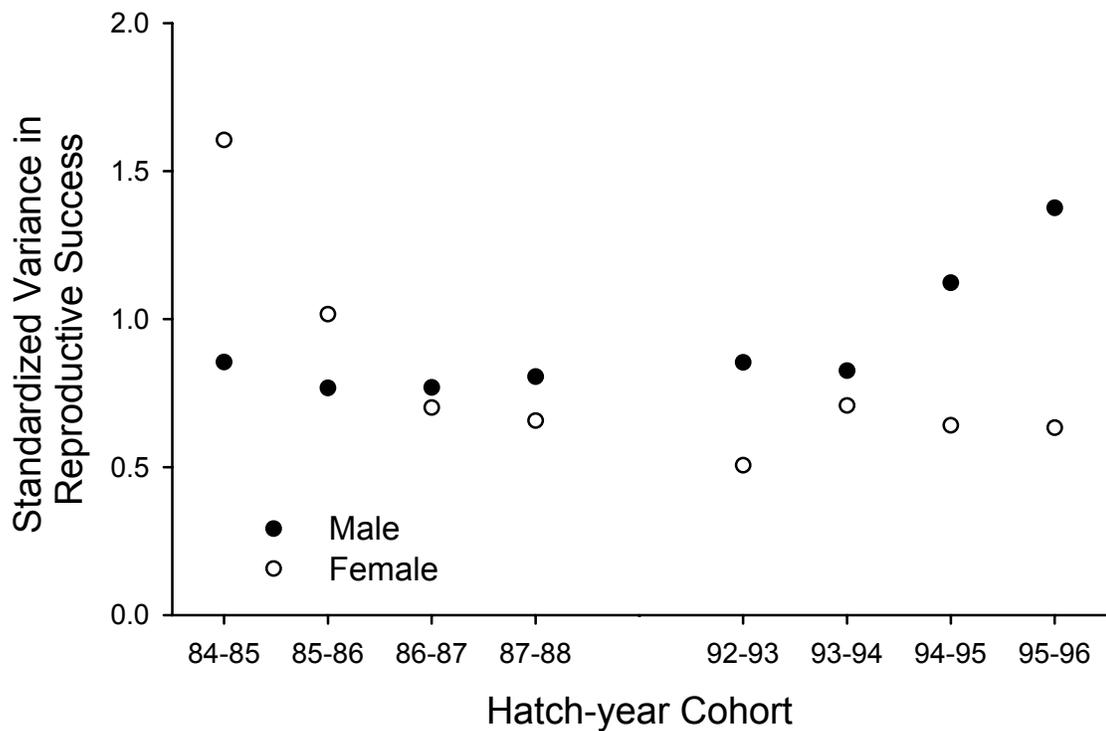


Figure 2. Relationship between male and female reproductive skew (B Index, Nonacs 2000) within a hatching cohort. Despite a male sex ratio bias, no difference in reproductive skew was found between the sexes. Error bars represent 95% CI. The dashed line indicates the B value for randomly distributed reproduction.

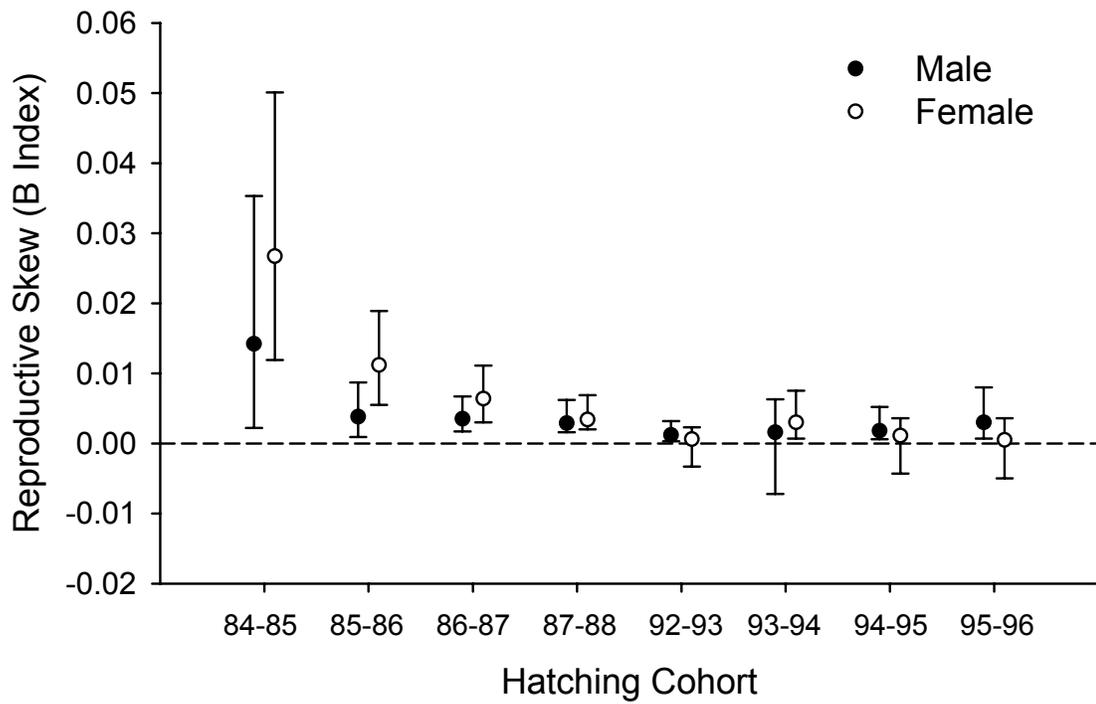


Figure 3. Real parameter estimates of breeder to non-breeder transition probabilities of males and females modeled over a 13 year period. Males had significantly higher transition probabilities in most years. Probabilities increased dramatically for both sexes after the breeding failures during the strong El Niño-Southern Oscillation event of 1997-98. Most birds did not breed during in 1999-2000, but females that did breed in that season were more likely than males were to become non-breeders the following year. Error bars denote 95% CI.

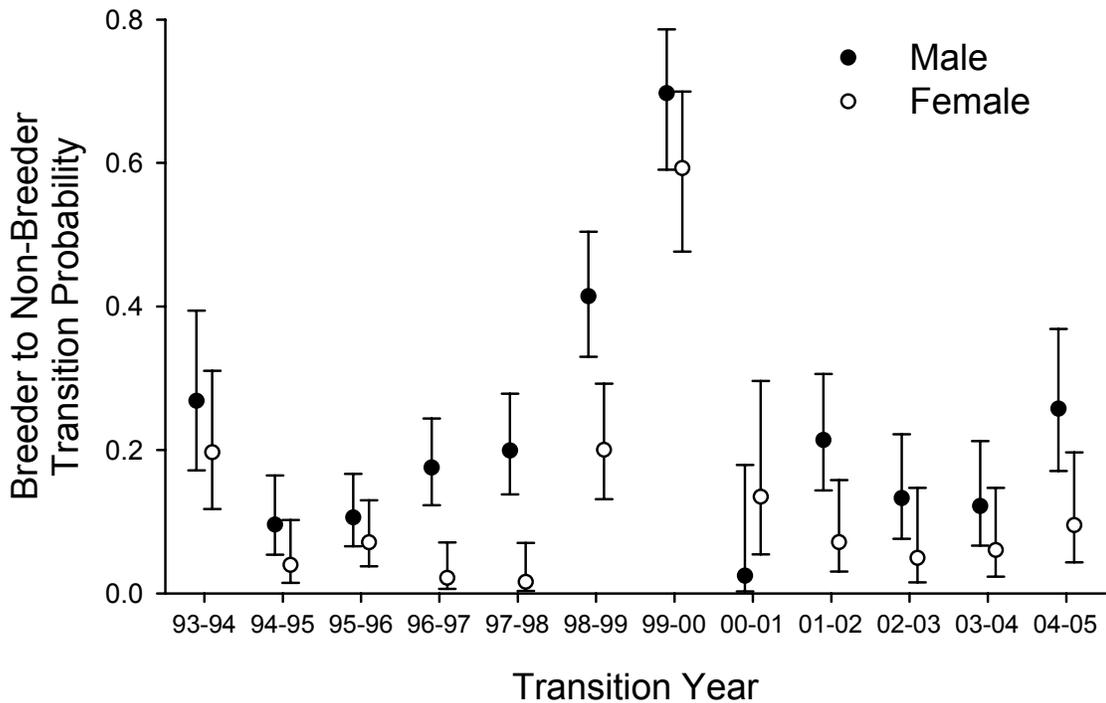


Figure 4. The mean number of years male and female Nazca boobies bred consecutively (bout length) shown as a proportion. Females had significantly longer breeding bout lengths than males did ($t = -5.35, p < 0.0001$).

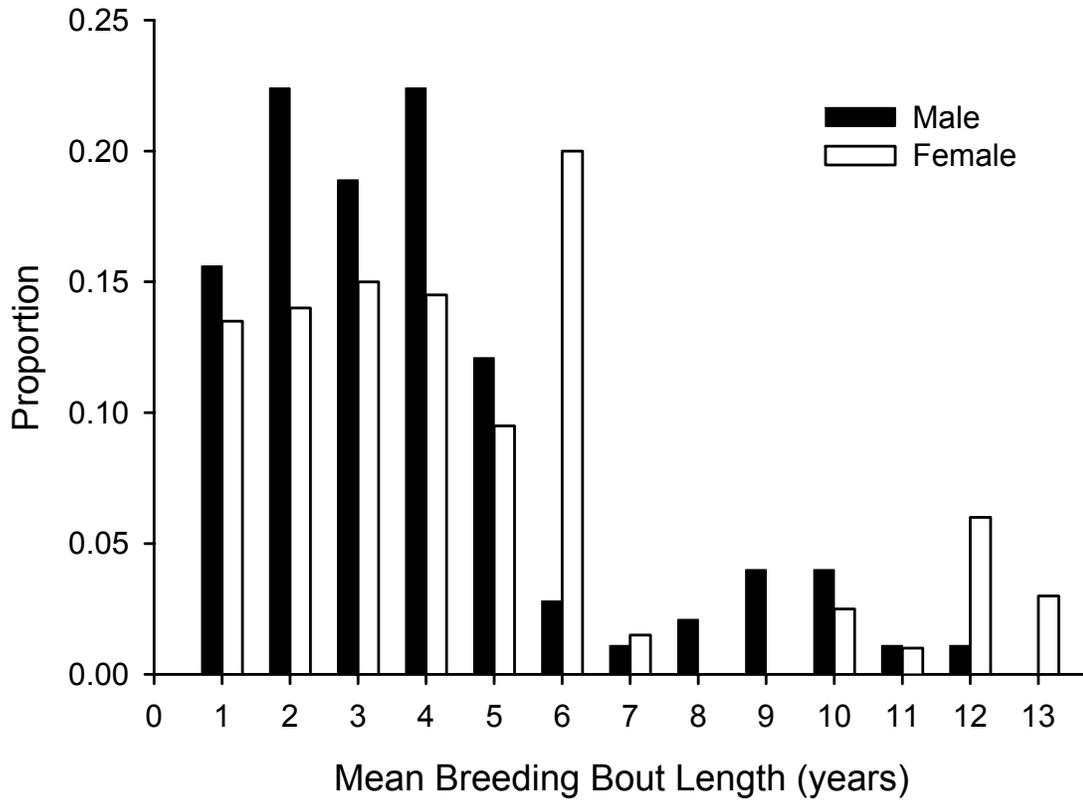
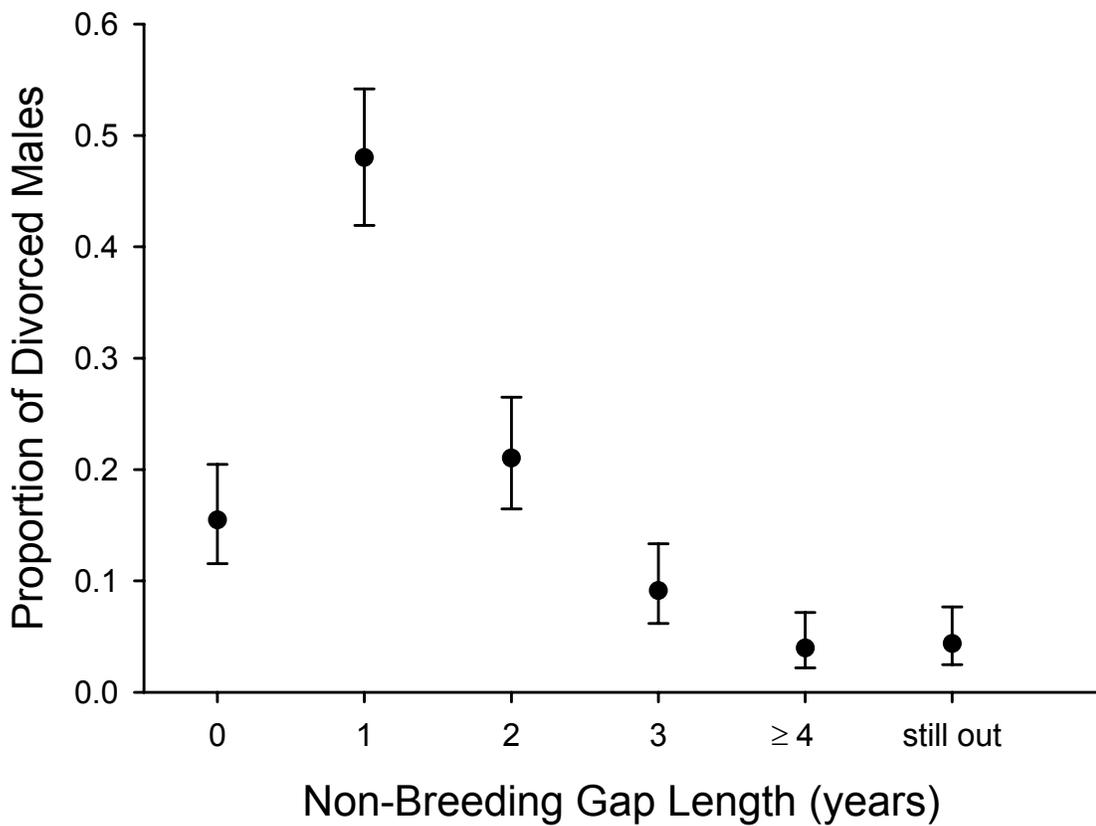
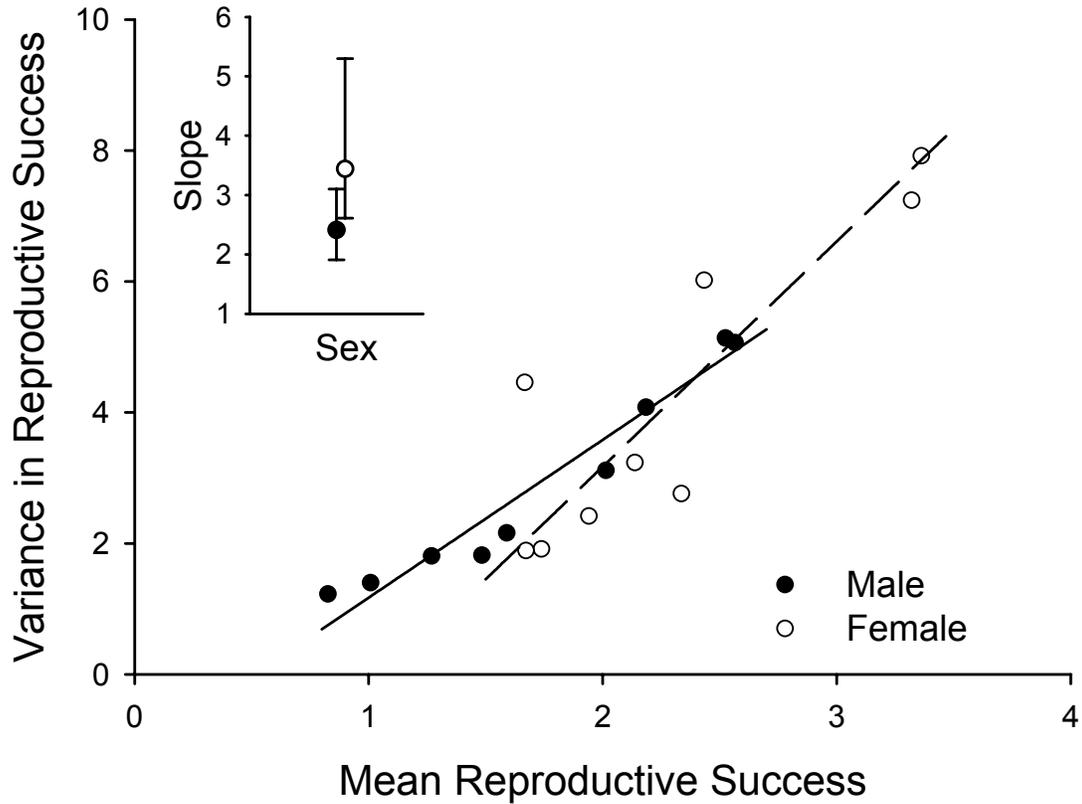


Figure 5. The number of divorced males, expressed as a proportion, categorized by non-breeding gap length determined from cohorts that fledged from the 1984-85 to the 1987-88 cohorts over a nine year period (1992-2001). “0” indicates that divorced males bred with a new female with no interruption in breeding status. “Still out” indicates that the divorced male was still alive (in 2005-06), but had yet to resume breeding at the time of this study. Most divorced males resumed breeding after a gap of one year. Error bars indicate 95% CI.



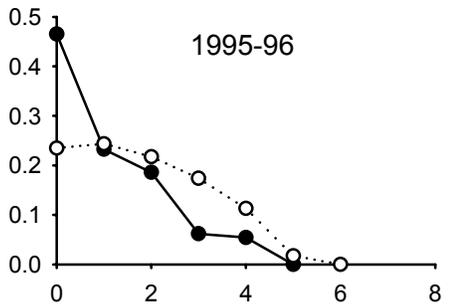
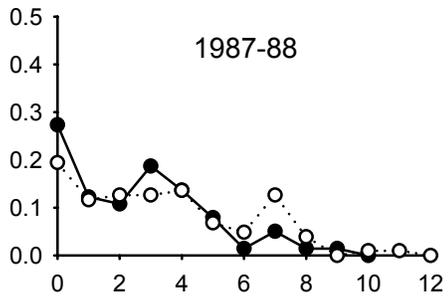
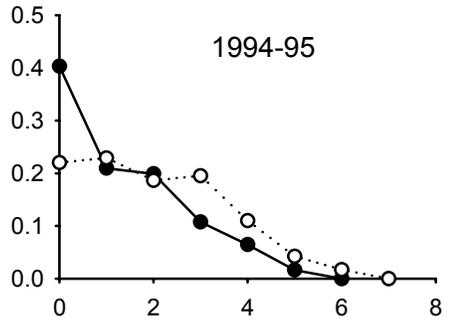
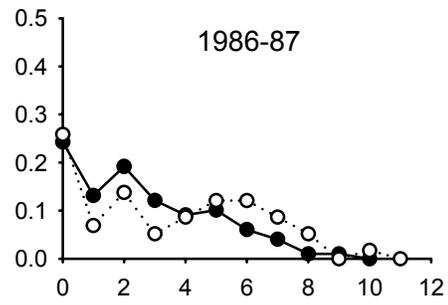
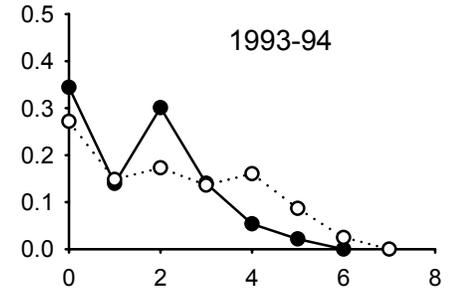
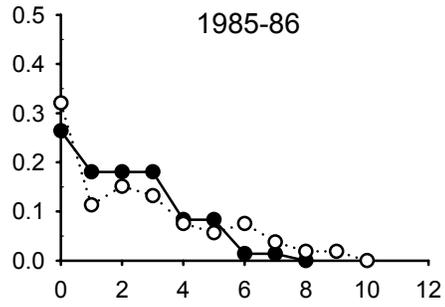
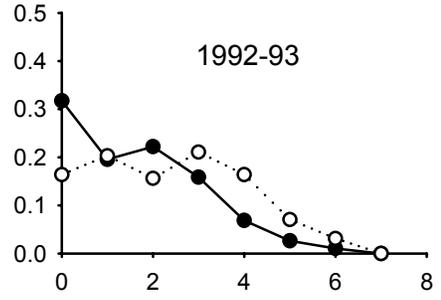
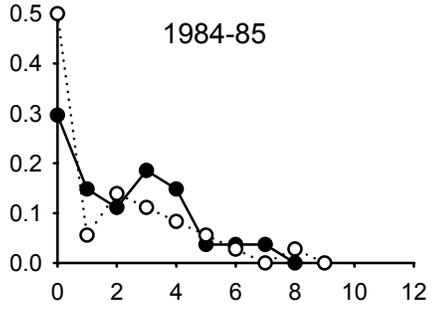
APPENDIX

Supplementary figure 1. Relationship between mean reproductive success and variance in reproductive success of eight hatching cohorts over a 14 year period as determined by reduced major axis regression performed in SAS (v. 9.1.3, Cary, North Carolina, USA). Regression equations: females, $y = 3.44x - 3.71$; males, $y = 2.41x - 1.24$. Insert shows regression slopes and their 95% CIs.

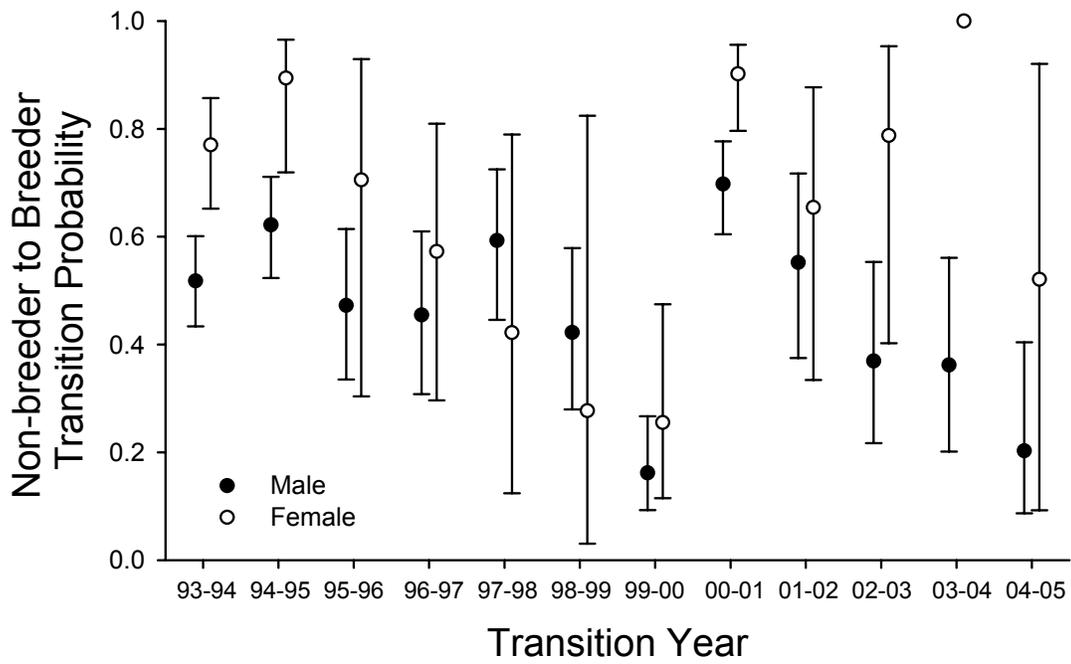


Supplementary figure 2. Frequency distributions of total reproductive success for male and female Nazca boobies from eight different hatching cohorts summed across the 14 breeding seasons from 1992 to 2005. X-axis in each panel is total reproductive success (total number of fledglings produced) while each Y-axis is proportional frequency within sex. The hatching cohort is given in the title of each panel.

● Male
○ Female



Supplementary figure 3. Real parameter estimates of non-breeder to breeder transition probabilities of males and females modeled over a 13 year period. Females had significantly higher transition probabilities in five of the years. Males had an overall probability of 0.452 of becoming a breeder despite the male-biased adult sex ratio. Error bars denote 95% CI. Error estimates are large for females because few females fail to breed each year.



Supplementary table 1. Values for 13 inequality measures of reproductive success in male and female Nazca boobies from eight different hatching cohorts. S index, corrected S index (S_c), effective number index (S_3), monopolization index (Q) iterative skew index (λ), Morisita index (I_δ), standardized Morisita index (I_p), and binomial skew index (B) are detailed in Nonacs 2003b and calculated with the Skew Calculator (Nonacs 2003a). Equations for Gini coefficient (G), moment skewness (g_1), index of dispersion (D), and coefficient of variation (CV) are given in Kokko *et al.* 1999. The opportunity for selection or standardized variance (I) is described in the text (Materials and Methods).

Cohort	Sex	S	S_c	S_3	Q	λ	I_δ	I_p	B	G	g_1	D	CV	I
1984-85	Male	0.31	0.22	0.47	0.015	0.12	1.39	0.5022	0.014	0.52	0.63	1.87	0.92	0.85
	Female	0.51	0.38	0.63	0.028	0.13	1.98	0.5059	0.027	0.66	1.20	2.62	1.26	1.60
1985-86	Male	0.27	0.15	0.44	0.004	0.05	1.28	0.5007	0.004	0.49	0.63	1.55	0.88	0.77
	Female	0.33	0.26	0.51	0.012	0.07	1.60	0.5041	0.011	0.56	0.85	2.47	1.01	1.02
1986-87	Male	0.25	0.18	0.43	0.004	0.03	1.35	0.5012	0.004	0.49	0.67	1.87	0.87	0.77
	Female	0.26	0.23	0.41	0.007	0.05	1.37	0.5022	0.006	0.48	0.29	2.21	0.83	0.70
1987-88	Male	0.28	0.21	0.45	0.003	0.02	1.41	0.5011	0.003	0.50	0.72	1.87	0.88	0.81
	Female	0.20	0.17	0.40	0.004	0.03	1.35	0.5013	0.003	0.46	0.53	2.15	0.82	0.66
1992-93	Male	0.32	0.15	0.46	0.001	0.02	1.22	0.5002	0.001	0.51	0.67	1.23	0.95	0.85
	Female	0.17	0.07	0.34	0.001	0.02	1.08	0.3539	0.001	0.40	0.23	1.07	0.72	0.51
1993-94	Male	0.35	0.15	0.45	0.002	0.36	1.15	0.3640	0.002	0.50	0.50	1.10	0.94	0.83
	Female	0.28	0.18	0.42	0.003	0.04	1.24	0.5005	0.003	0.48	0.33	1.27	0.84	0.71
1994-95	Male	0.40	0.17	0.53	0.002	0.02	1.34	0.5005	0.002	0.57	0.82	1.24	1.10	1.12
	Female	0.22	0.09	0.39	0.001	0.02	1.13	0.4565	0.001	0.45	0.48	1.19	0.86	0.64
1995-96	Male	0.47	0.16	0.58	0.003	0.04	1.39	0.5005	0.003	0.61	1.02	1.41	1.40	1.38
	Female	0.24	0.07	0.39	0.001	0.03	1.06	0.1850	0.001	0.45	0.36	0.88	0.79	0.63

Supplementary table 2. Spearman rank order correlations between the 13 indices of inequality described in supplementary table 1.

Significant correlations ($p < 0.05$) are in bold. Most indices were highly correlated.

Index	S	S _c	S ₃	<i>Q</i>	λ	<i>I</i> _{δ}	<i>I</i> _p	B	<i>G</i>	<i>g</i> ₁	D	CV	<i>I</i>
S	1.000	0.465	0.944	0.351	0.418	0.500	0.283	0.362	0.929	0.738	0.235	0.926	0.938
S _c	0.465	1.000	0.544	0.858	0.568	0.885	0.899	0.848	0.568	0.444	0.865	0.379	0.559
S ₃	0.944	0.544	1.000	0.504	0.371	0.668	0.465	0.508	0.994	0.882	0.409	0.968	0.997
<i>Q</i>	0.351	0.858	0.504	1.000	0.687	0.853	0.956	0.998	0.516	0.431	0.909	0.366	0.501
λ	0.418	0.568	0.371	0.687	1.000	0.453	0.525	0.702	0.368	0.126	0.471	0.300	0.362
<i>I</i> _{δ}	0.500	0.885	0.668	0.853	0.453	1.000	0.906	0.836	0.688	0.691	0.894	0.532	0.674
<i>I</i> _p	0.283	0.899	0.465	0.956	0.525	0.906	1.000	0.945	0.490	0.474	0.974	0.319	0.471
B	0.362	0.848	0.508	0.998	0.702	0.836	0.945	1.000	0.518	0.421	0.896	0.372	0.505
<i>G</i>	0.929	0.568	0.994	0.516	0.368	0.688	0.490	0.518	1.000	0.885	0.438	0.959	0.997
<i>g</i> ₁	0.738	0.444	0.882	0.431	0.126	0.691	0.474	0.421	0.885	1.000	0.474	0.874	0.888
D	0.235	0.865	0.409	0.909	0.471	0.894	0.974	0.896	0.438	0.474	1.000	0.279	0.418
CV	0.926	0.379	0.968	0.366	0.300	0.532	0.319	0.372	0.959	0.874	0.279	1.000	0.962
<i>I</i>	0.938	0.559	0.997	0.501	0.362	0.674	0.471	0.505	0.997	0.888	0.418	0.962	1.000

CHAPTER 3

Mate rotation by female choice and coercive divorce in Nazca boobies (*Sula granti*)

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ABSTRACT

The formation and dissolution of mating pair-bonds have been fruitful areas of investigation for evolutionary biologists. Adaptive mate choice has been the focus of most research on pair dissolution. However, recent work has shown that an important cause of pair dissolution can be involuntary forced divorce, where intruder(s) oust one or both partners. Previously, we presented evidence that, in a male-biased population, females exchanged a reproductively depleted male for a new "refreshed" mate. This "mate rotation" model of divorce could be driven by female choice, forced divorce by unpaired males, or by males forgoing reproduction to recover condition. Here, we examine these alternatives with behavioral and demographic data from our long-term study of banded Nazca boobies. The mate rotation pattern of divorce appears to be driven by a combination of two circumstances involving a female's abandonment of a previous partnership. In some cases, the female appeared to cooperate with a male intruder, causing her former partner to leave the nest. In other cases, the female abandoned her former mate and joined a new male at another nest site. Recent studies that have disentangled age and breeding experience from pair-bond length have shown that an initial increase in reproductive output correlated with pair-bond length, but after a period of time together the reproductive success of the pair declined. Given the assumed ubiquity of the cost of reproduction, divorce after a lengthy pair bond would seem to be advantageous for one or both mates when fresh potential partners are available.

Keywords: cost of reproduction; divorce; mate choice; mate rotation; Nazca booby; pair-bond; serial monogamy; sex ratio bias; *Sula granti*

INTRODUCTION

The variability observed in bird mating systems, especially the prominence of monogamy and extended pair bonds (Black 1996), has long been a focus of interest to evolutionary biologists (Lack 1968). Recent work has emphasized the termination of mating partnerships, providing a complement to the older and larger literature on pair formation (reviewed in Andersson 1994). Divorce, the disruption of the pair bond without death, gives insight into important evolutionary topics including sexual selection and mate choice. The frequency of divorce varies considerably between species, from 0% in waved albatrosses, *Phoebastria irrorata* (Harris 1973) to 98% in greater flamingos, *Phoenicopterus roseus* (Cézilly & Johnson 1995), as well as between different populations of the same species (reviewed in Ens et al. 1996).

Divorce may be imposed on the pair and so be non- or maladaptive; possibilities include chance events leading partners to lose contact (Choudhury 1995; Ens et al. 1996), asynchronous arrival at the breeding area (Dhondt & Adriaensen 1994; Naves et al. 2006), and intruder(s) forcing divorce by ousting one or both partners (Taborsky & Taborsky 1999, Jeschke et al. 2007). Alternatively, divorce may reflect adaptive abandonment by either partner to un-do its previous mate choice. Most adaptive hypotheses fall under the “better option” model (reviewed in Choudhury 1995 and Ens et al. 1996), whereby a partner leaves because of incompatibility (Coulson 1972), to obtain a higher quality mate (Ens et al. 1993), to correct errors in mate choice (Johnston & Ryder 1987), or to obtain a mate whose condition has not been impaired by recent reproductive effort (Cézilly & Johnson 1995; Maness & Anderson 2007). Adaptive divorce could also result from one pair member, but not both, resting to recover breeding

condition. This pattern of divorce might be expected in species with frequent “time-outs”, such as long-lived birds (e.g., Jouventin & Dobson 2002; Dobson & Jouventin 2007), or among low-quality individuals (Mougin et al. 1997; Cam et al. 1998, Bradley et al. 2000). These hypotheses have been evaluated at the ultimate level (reviewed in Choudhury 1995 and Ens et al. 1996; Heg et al. 2003; Jeschke et al. 2007), but few have added a proximate perspective by also investigating the behaviors leading to the divorce event.

The probability of divorce may be conditioned by the operational sex ratio (OSR; Emlen & Oring 1977) of the population of interest (Choudhury 1995). Considering a male-biased population, males (the abundant sex) have fewer breeding opportunities than females do and so should be less likely than females to divorce voluntarily; a female has a pool of unpaired males available, facilitating mate switching if her mate’s condition declines below that of members of the non-breeding pool (Cézilly & Johnson 1995; Maness & Anderson 2007); and pairs may be more likely to be victims of forced divorce imposed by unpaired males (López-Sepulcre & Kokko 2005). Our previous work on a male-biased population (Townsend & Anderson 2007; Maness et al. 2007) of Nazca boobies (*Sula granti*) showed that divorce was common and that males enter and exit the breeding pool more frequently than females do (Maness & Anderson 2007). Those results and others indicated a female mating preference based on costs of reproduction and enabled by the ready availability of unpaired, high-condition males, consistent with a model of adaptive “mate rotation” that maximized the physiological condition of the female’s current mate (Maness & Anderson 2007).

Many studies have suggested that females are the predominant initiators of divorce in birds (reviewed in Cézilly et al. 2000). Our previous study suggested that female Nazca boobies drive divorce and that males are involuntarily rotated out of pairings by female choice; however, we could not rule out some other mechanisms underlying the divorce patterns we observed (Maness & Anderson 2007). Here, we test divorce hypotheses using data on pre-breeding behaviors, pairing histories, and reproductive consequences of pairing history of our study population. Divorce in Nazca boobies could be driven by adaptive mate rotation involving female choice, by males choosing non-breeding status to recover condition, or by forced divorce imposed on the pair by one of the many non-breeding males in our population (approximately one third of all adult males lack mates at a given moment; Maness & Anderson 2007). Jeschke et al. (2007) found evidence that forced divorce may be more common than previously thought.

If the adaptive mate rotation model operates in our population, then 1) prior to divorce, a female choosing to leave her former partner should sample the behavior of her old mate, plus that of one or more other males before opting for a new mate; 2) the RS of divorcing females should be high prior to, and not decline after, divorce; 3) divorced males should attempt to retain their mate or to attract a new one because they divorced involuntarily; and 4) the RS of male “victims” of female choice should be high prior to, and decline after, divorce. The prediction that divorce should follow a successful breeding attempt contrasts with a meta-analysis demonstrating a negative association between breeding success and mate retention (Dubois & Cézilly 2002). We do not suggest that unsuccessful males should be retained preferentially under mate rotation;

successful and unsuccessful males should be retained if a better alternative is not available. However, successful breeders are more likely to be in poorer condition than recently non-breeding males, given the demonstrated survival costs of reproduction (Townsend & Anderson 2007) and mass loss during breeding (Apanius et al. 2008) in this species, and females should select against these successful breeders, (i.e., upgrade mates). Previously, we tested the prediction that male victims of divorce have higher RS than do retained males in the year preceding divorce and found no difference in these estimates (Maness & Anderson 2007). However, the operation of more than one factor causing divorce could have confounded this test conducted at the population level. Accordingly, a more specific prediction of the mate rotation model regarding male RS, replacing 4) above, is that 4) RS of males divorced *due to female choice* should be high prior to, and decline after, divorce.

If males choose temporary non-breeding status, then these males 1) should not attempt to breed; and 2) might abandon their nest sites.

If divorce is forced upon a Nazca booby pair by a non-breeding male, then 1) one or both members of the pair should resist (with aggression against the incomer) the take over; 2) aggressive behavior of the current male in a pair should predict his success in mate retention; 3) RS of males that lose their nest site should be high prior to divorce (leading to poor condition and thus poor fighting ability) and low after divorce; and 4) the RS of only the incomer should improve post-divorce (Jeschke et al. 2007) and the RS of the female victim of forced divorce may decline.

METHODS

Behavioral Observations

Nazca boobies are socially and genetically monogamous (Anderson & Boag 2006), long-lived pelagic seabirds (Anderson & Apanius 2003) with bi-parental care and similar parental roles (Anderson & Ricklefs 1992; Apanius et al. 2008). The population breeds seasonally at our study site at Punta Cevallos, Isla Española, Galápagos Islands, with most egg-laying between October and January, and most fledging completed by June. At the beginning of each breeding season, male Nazca boobies defend nest sites, where they exclude other males and perform mate attraction behaviors (Nelson 1978). To determine behaviors preceding divorce (or mate retention) and to identify behaviors females could use to assess potential mates, all resident birds (defined below) in a subsection of the colony known as the “mini-area” were observed during the pre-breeding season, starting 30 August 2003, through the egg-laying period of 2003. The “mini-area” is the northern third of the “study area”, which is the northern half of sub-colony 1 (Huyvaert & Anderson 2004; Apanius et al. 2008 give a detailed description). Egg-laying began in mid-October and continued until February of 2004, but observations ended on 21 December 2003, when 95% of all clutches had been established. Since most birds vacate the colony during the heat of midday (Anderson & Ricklefs 1992; Anderson et al. 2004), birds were observed from 06:00 to 09:00 and 15:00 to 18:00. All resident birds in the mini-area were fitted with metal as well as numbered plastic bands. The sex of adult Nazca boobies can be determined easily by voice (Nelson 1978). Males were given black plastic leg bands, while females had grey bands. The numbering on each plastic band was large enough to read easily by eye or with binoculars from approximately 20m (the

maximum distance observers were from the birds). We noted 26 specific behaviors (see Electronic Supplementary Material) of males and females at the beginning of the 2003-04 breeding season. Our treatment of behavior types followed those of Van Tets (1965) and Nelson (1978), except as noted in the Electronic Supplementary Material.

The mini-area was divided into four observation subsections (A, B, C, and D), each of which contained approximately 75 birds that were present regularly. Each subsection was observed on a 6-day rotating schedule: on day 1, section A was observed from 0600 to 0900 hours and section B from 1500 to 1800 hours; on day 2, section C was observed from 0600 to 0900 hours and section D from 1500 to 1800 hours; no observations were performed on day 3; on day 4, section B was observed from 0600 to 0900 hours and section A from 1500 to 1800 hours; on day 5, section D was observed from 0600 to 0900 hours and section C from 1500 to 1800 hours; no observations were performed on day 6; then the cycle started over again at day 1. The same two observers performed every observation session. The first observer dictated a bird's behavior, its plastic identification number, its location (nest number; every nest site in this area has a permanent metal identification number), and the identity of any birds it interacted with; the second observer recorded the dictated information and noted the time. The observers sat on natural rock formations (see Fig. 1 of Apanius et al. 2008) or a raised wooden platform, both of which afforded a clear view of most birds in the observation area and were one or more meters from the nearest nest site. If the view of a bird or its nest site was continuously obstructed by the rocky terrain, the bird's observable behaviors were noted (particularly its interactions with neighbors), but these birds were omitted from all analyses. Given that individuals in our population are indifferent to humans, we assumed

that our presence caused no disturbance to the birds. As a precaution, the observers were in position 15min before each observation period began to allow the birds any necessary acclimation to their presence. The observers performed one 6-day practice rotation prior to beginning actual data collection and had no knowledge of the previous pairing patterns of the birds. Approximately two weeks after data collection began the observation hours were reduced to 0600 to 0800 hours and 1600 to 1800 hours because most birds vacated the colony before 0900 hours and did not return until after 1600 hours. The observation hours were further reduced to 0600 to 0700 hours and 1700 to 1800 hours after 6 December 2003 since most clutches had been established by that date and interactions of interest were largely restricted to these hours. We were also present in the mini-area for several additional hours each day in the course of conducting other demographic studies and opportunistically noted aggressive interactions between individuals and injuries sustained from fights. These behaviors were not included in the factor analysis described below.

Several different Nazca booby behaviors appear to convey similar messages to a receiver. For example, yes/no head wagging, wing-flailing, and jabbing are all used in territoriality (Van Tets 1965; Nelson 1978). Therefore, many behaviors were expected to correlate with one another. We used factor analysis to reduce the 26 recorded behaviors to a smaller set of factors for use in later analyses. We used principal components analysis (PCA; SPSS, ver.15.0; SPSS, Inc., Chicago, IL, USA) for this data reduction, using a covariance matrix as input, rather than a correlation matrix, because we wanted to compare factor structures between different groups of birds (Kim & Mueller 1978). Logistic regressions compared factor structures between different groups of males

(breeders vs. non-breeders and retained vs. divorced males). Akaike's Information Criterion corrected for small sample sizes (AICc) was used for logistic regression model selection and ranking (Burnham & Anderson 2002). Since AICc converges to AIC with large sample sizes, AICc should be employed regardless of sample size (Burnham and Anderson, 2002). We also report Bayesian Information Criterion (BIC) values as an alternative model selection tool. The BIC penalizes free parameters more strongly than does AIC (Schwarz 1978).

We recorded the behaviors of 160 resident males and 107 resident females, over a total of 318 hours. Birds present during observation periods for more than 500 minutes (2 S.D. from the mean) were considered resident birds; birds present less than the cut-off time were considered visitors and were excluded from all analyses. Two resident females did not breed in 2003-04, but did form exclusive bonds with a social partner. These females and their social partners were excluded from all analyses that determined predictors of mating success. Only the behaviors that males performed prior to the subpopulation's initiation of breeding (the first egg was laid in the mini-area on 16 October 2003) were considered for factor analysis, because an unpaired male's behavior may change as the likelihood of obtaining a mate diminishes as fewer females remain available for breeding. We calculated the frequency of each of the 26 behaviors that a male performed and the total time that a male was present over the truncated observation period. The 26 recorded behaviors were likely to factor well (KMO test = 0.784; Bartlett's approximate $\chi^2_{325} = 2768.95$, $P < 0.0001$). Five components, explaining 85.0% of the variance in the data set, were extracted. A Varimax rotation with Kaiser Normalization was used to determine component loadings.

We named the five components Mate, Territorial, Prospecting, Aggressive, and Advertising, based on the behaviors that loaded onto them (see Table 1 for the behaviors and their component loading scores). Two behaviors, displaced and fight, did not load highly onto any factor, probably because they were relatively rare behaviors. These behaviors were left in the analysis because removing them did not appreciably change the outcome of the PCA and they were subsequently assigned to the factors they correlated with most. Two other behaviors, garden and circle flight, loaded onto two factors each. Garden correlated with Advertising and Territorial components, reflecting the dual nature of this behavior. Males may garden to make their symbolic nests more attractive to females (Advertising) and they also pick up and rearrange nest material during conflicts with neighbors (Territorial). Circle flight correlated with Mate and Territorial components. Males perform circle flights to establish and maintain territories (Nelson 1978). In this study, males also performed circle flights repeatedly to bring gifts to potential mates or preceding mutual jabs with a potential mate.

The five behavioral components and total minutes present were used as fixed effects in a logistic regression (“R” open-source software by R-project, ver.2.6.1; <http://www.r-project.org>) with mating success (105 breeding and 53 non-breeding males) as the binary outcome variable. All predictors were entered using a block entry method. The omnibus test of model coefficients was significant ($\chi^2_6 = 76.55, P < 0.0001$) and the model was a good fit (-2LL = 125.043; Hosmer and Lemeshow $\chi^2_8 = 1.71, P = 0.99$; AICc = 139.79; BIC = 160.48); however, total minutes present was not a significant predictor ($P = 0.91$) and was dropped from the model. The final model correctly classified 80.4% of males (-2LL = 125.044; Hosmer and Lemeshow $\chi^2_8 = 1.71, P = 0.99$;

AICc = 137.60; BIC = 155.42). Significant positive predictors of male mating success were Mate, Territorial, and Advertising behaviors, while Prospecting behavior was a significant negative predictor and Aggression was not significant (Table 2A). All males were used in this analysis, including those retained as mates from the previous season. Females who retain their mate may use different behavioral criteria than do divorcing females when deciding with whom to mate because they have prior experience with the male (Dubois et al. 2004); therefore, a second logistic regression was performed with retained males left out of the model. As in the previous logistic regression, the five behavioral components and total minutes present were used as fixed effects with mating success (43 breeding and 53 non-breeding males) as the binary dependent variable. Again, total minutes present was not a significant predictor ($P = 0.90$; AICc = 107.43; BIC = 124.10) and was dropped from the model. The omnibus test of model coefficients was significant ($\chi^2_5 = 36.87$, $P < 0.0001$) and the model was a good fit (-2LL = 92.174; Hosmer and Lemeshow $\chi^2_7 = 8.77$, $P = 0.27$; AICc = 105.28; BIC = 119.56) and correctly classified 80.2% of males. Thus, the two regression approaches produced nearly identical results with significant positive predictors of mating success being Mate, Territorial, and Advertising behaviors, Prospecting was a significant negative predictor, and Aggressive behaviors were not significant (Table 2B). We used these aggregating behavioral components to test predictions of various divorce models below.

Reproductive Success of Male and Female Categories

To examine predictions regarding pre- and post- divorce RS, we categorized male and female Nazca boobies using a modification of the partnership categorization of Jeschke et al. (2007): *retained* (partnership remains intact), *stayer* (focal bird divorces and retains its nest site), *mover* (focal bird divorces and leaves its nest site), and *incomer* (focal bird acquires both a nest site and a new partner). Pairs in which one member died were not divorces and were omitted from all analyses. We calculated RS in the years prior to and after divorce, or in years without divorce (see flow diagrams in Figs. 1 and 2). To separate males and females into the four partnership categories, we examined our database of reproductive histories of 469 banded adults in the “study area” (see above). Nest sites in this area have been marked with permanent nest numbers since 1995. We restricted our investigation to the breeding seasons beginning in 2000 through 2004 because 1) we could not track nest takeovers prior to 1995, 2) many adults became non-breeders for several years after the strong El Niño-Southern Oscillation event of 1997–1998 (Maness & Anderson 2007), and 3) the size of the banded population was larger during these years than during earlier years of our work, facilitating the tracing of mating patterns of recognizable birds. The proportion of birds banded in the study area was higher than in the rest of our study site, avoiding analytical problems associated with pairs including an unbanded (and so unidentifiable) bird.

Pairs of birds were followed for three years (the mean breeding bout length for males; Maness & Anderson 2007). Year X was the year prior to the divorce (or no divorce if retained), year X+1 was the year immediately after divorce, and year X+2 was the final year we followed the fate of pairs. The RS of each bird was determined for each

of these points in a breeding sequence. Since the extra pair fertilization rate of Nazca boobies is estimated as 0% (Anderson & Boag 2006) and RS is binary for Nazca boobies, (they raise at most, one offspring per year; Humphries et al. 2006), within-year RS between categories was compared using log-linear analysis of frequency tables (Statistica, v. 6.1; Statsoft, Inc., Tulsa, OK, USA). Repeated measures log-linear analyses (SPSS, ver.15.0; SPSS, Inc., Chicago, IL, USA) were used to assess whether within-category RS improved or declined after yr X. To avoid pseudo-replication, RS was considered separately by sex and partnership category.

To minimize the number of statistical tests performed, omnibus log-linear analyses of partnership category (four levels) by RS_{yrX} (two levels) by RS_{yrX+1} (two levels) frequency tables were analyzed separately by sex to test for category by RS_{yrX} , and category by RS_{yrX+1} , interactions. Tabulations of category by RS_{yrX} by RS_{yrX+2} (two levels) were analyzed similarly. If the model fit was adequate and the maximum likelihood (ML) χ^2 of two-way interactions was significant, then individual contrasts of interest were examined. Effect sizes were determined by odds ratios. The false discovery method (Benjamini & Hochberg 1995, Curran-Everett 2000) was used to adjust the critical value when multiple comparisons were performed. All hypotheses tested below were directional, so one-tailed tests were performed in all cases.

RESULTS

Mate rotation predictions:

Twenty-four of the 87 males (27.6%) that bred the year before (2002-03) the behavioral observations were conducted were divorced at the beginning of the 2003-04

breeding season. Fourteen (58.3%) of these divorces involved pairs that successfully fledged a chick in 2002-03.

Nineteen (79.2%) divorces were attributable to female choice. Fourteen of these 19 involved mover females. Eight of the 14 movers performed Mate behaviors (see Methods) with her old mate and one or more other males before settling with a new mate at a new nest site, consistent with prediction 1); six of these fledged a chick in the previous year. Six mover females did not interact with their previous partners and spent no time at their old nests; instead, these females performed Mate behaviors with one or more males before settling at a new site with a new male. Only one of these six movers produced a fledgling with her old mate in the previous year. The remaining five of the 19 divorces were all preceded by successful breeding in the previous year and involved stayer females. In two of these five cases, the female only interacted aggressively toward her old mate and assisted the incoming male in defending their nest site from the previous year from her old partner. One of these incoming males was defeated and driven away by the old male, but then the female joined with the incoming male to defeat and drive away her old partner. In the remaining three cases, the female performed Mate behaviors with her old mate and with the incoming male while her original mate was absent, presumably foraging. The female then became aggressive toward her old mate and assisted the incoming male in defending the nest site from her old partner.

Five of the 24 divorces (20.8%) could not be attributed exclusively to female choice. Two of these divorces had no clear instigator and were perhaps cases of mutual divorce because neither member of the pair spent time at their old nest sites, the pair members did not interact (except for one brief aggressive encounter), and no incoming

birds took over their old sites. All males and both females in these two divorce cases obtained new mates in 2003-04. One of these five divorces appeared to be due to asynchronous arrival of the pair members because the original male re-mated before his old mate returned from non-breeding absence; she was unusually late, arriving 60 days later than the median arrival date for females. One of the five divorces could have been due to chance events or forced divorce because the pair's nest site was occupied by a new pair before either member of the old pair returned from non-breeding absence, and both former residents had aggressive encounters with the incoming pair. The last of these five divorces was consistent with a forced divorce because an incoming male defeated and chased away the original male before the original female returned from non-breeding absence. The female paired with the incomer and so this divorce could arguably be attributed to female choice because she could have chosen to pair with her old mate (a non-breeder in 2003-04) at his new nest or to pair with a new male at a new site rather than staying with the incomer. However, we did not treat this divorce as a case of female choice.

Inconsistent with prediction 2) of mate rotation, females divorcing in 2003-04 were not more likely to have fledged a chick (RS = 0.63, 95%CI = 0.41 – 0.81) in the preceding year than females that retained their mates (RS = 0.48, 95%CI = 0.36 – 0.61, $\chi^2_1 = 1.22$, $P = 0.14$), but the statistical power of this test was low (0.304) due to the small number of females divorcing in the one year analysis. Prediction 2) received partial support in the larger multi-year log-linear analysis. The female category by RS_{yrX} by RS_{yrX+1} frequency table produced a well fit model ($\chi^2_4 = 5.14$, $P = 0.27$) with significant two-way interactions ($\chi^2_7 = 15.77$, $P = 0.027$). Female stayers had significantly higher

RS_{yrX} than did retained females ($\chi^2_1 = 4.54, P = 0.016$, adjusted $\alpha_{crit} = 0.017$, odds ratio = 1.19; Fig. 2), but female movers did not have higher RS_{yrX} than did retained females ($\chi^2_1 = 0.12, P = 0.36$; Fig. 2). The RS_{yrX} of stayer and mover females combined (all divorcing females, $RS_{yrX} = 0.47, 95CI = 0.38-0.57$) was not different from the RS_{yrX} of retained females ($RS_{yrX} = 0.40, 95CI = 0.35-0.46; \chi^2_1 = 1.48, P = 0.11$). No difference was found in the RS_{yrX+1} between any of the female categories ($\chi^2_3 = 3.76, P = 0.29$). The log-linear analysis of female category by RS_{yrX} by RS_{yrX+2} frequency table produced a well fit model ($\chi^2_4 = 2.30, P = 0.68$) with marginally significant two-way interactions ($\chi^2_7 = 13.89, P = 0.053$). Stayer females had marginally higher RS_{yrX+2} than retained females ($\chi^2_1 = 4.10, P = 0.022$, adjusted $\alpha_{crit} = 0.017$, odds ratio = 3.16; Fig.2), but mover females did not have higher RS_{yrX+2} than retained females ($\chi^2_1 = 1.13, P = 0.144$). All divorced females (movers and stayers; $RS_{yrX+2} = 0.53, 95CI = 0.39-0.66$) did not have higher RS_{yrX+2} than retained females ($RS_{yrX+2} = 0.51, 95CI = 0.44-0.58; \chi^2_1 = 0.08, P = 0.39$). Repeated-measures log-linear analysis revealed that RS did not change for any female category across either time span ($LR_1 < 3.14, P > 0.04$, adjusted $\alpha_{crit} = 0.006$). As predicted, some (stayer), but not all, divorced females had higher RS prior to divorce than those females choosing to retain their partners. Also, the RS of divorcing females did not decline post-divorce.

All of the divorced males performed Advertising behaviors during the pre-breeding period; those that attracted a female to their nests performed Mate behaviors with them; none willingly abandoned their old sites; and none appeared to try to drive their old mate (or potential new mates) from their nest site, supporting prediction 3). The RS values for male “victims” of divorce in 2003-04 are the same as those reported above

for female choice, inconsistent with prediction 4) because these male victims were not more likely to have fledged a chick the prior year than retained males (but see analysis of larger sample size below).

No divorces that occurred during the observation year could be attributed exclusively to male choice, and only two divorces could not be attributed at least partly to female choice (a female arriving late, and a pair displaced by another pair), so we made the simplifying assumption that all divorces in the multi-year analysis were results of female choice. Inevitably, without behavioral observations in all years, a small minority of divorces will be classified incorrectly with this assumption. The multi-year log-linear analysis of male category by RS_{yrX} by RS_{yrX+1} frequency table produced a well fit model ($\chi^2_4 = 5.89$, $P = 0.21$) with significant two-way interactions ($\chi^2_7 = 50.93$, $P < 0.0001$). Mover males had significantly higher RS_{yrX} than retained males ($\chi^2_1 = 5.59$, $P = 0.009$, odds ratio = 2.26; Fig. 1), but stayer males did not ($\chi^2_1 = 0.04$, $P = 0.42$). All divorced males combined (movers and stayers) had similar RS_{yrX} ($RS = 0.47$, 95CI = 0.38-0.57) to retained males ($\chi^2_1 = 1.48$, $P = 0.11$). Repeated-measures log-linear analysis revealed that the RS of mover and stayer males was significantly reduced one year after divorce ($LR_1 = 14.07$, $P < 0.001$, odds ratio = 0.12 and $LR_1 = 6.06$, $P = 0.007$, odds ratio = 0.40, respectively), but not reduced two years after divorce with respect to year X ($LR_1 = 1.159$, $P = 0.28$ and $LR_1 = 0.111$, $P = 0.74$, respectively). Prediction 4) received mixed support because some, but not all, divorced males had higher RS in the previous year than retained males and the RS of divorced males was significantly reduced the year after divorcing.

Male time out predictions:

All males that were divorced at the beginning of the 2003-04 breeding season established nest site territories and performed Advertising behaviors during the pre-breeding season inconsistent with prediction 1). Only two males appeared to willingly give up their previous nest sites and both of these males obtained new mates at new sites, inconsistent with prediction 2). Moreover, 58.3% of divorced males obtained new mates in 2003-04.

Forced divorce predictions:

Six divorced males lost their nest sites to an incoming male and one lost his nest to an incoming pair (described above) in 2003-04. All seven of these males attempted to resist the nest take-over: they all had aggressive encounters with the incoming male and/or their former mates prior to losing their nests, consistent with prediction 1). As described above, the six former partners of these males did not appear to resist the incoming males. Instead, these females were aggressive toward their former mates, indicating that males but not females were forced to divorce in these cases. Although Aggressive behavior was not a significant predictor of male mating success, retained males had marginally higher component scores for Aggressive behaviors (Welch $F_{1, 35.4} = 3.65$, $P = 0.032$, adjusted $\alpha_{crit} = 0.025$), than those males that lost their nest sites. But retained males did not have higher component scores for Territorial behaviors (Welch $F_{1, 7.9} = 1.53$, $P = 0.13$), providing mixed support for prediction 2). (Welch tests (SPSS, ver.15.0; SPSS, Inc., Chicago, IL, USA) were performed because the sample size (events per parameter) was too small to adequately estimate logistic regression coefficients of the

PCA behavioral components (Peduzzi et al. 1996)). The males who lost their nests to incomers had significantly higher RS in 2002-03 (RS = 1.0, 95CI = 0.63 – 1.0) than did retained males (RS = 0.48, 95CI = 0.36 – 0.61; $\chi^2_1 = 6.74$, $P = 0.009$, $\phi_2 = 0.31$; Cramer's coefficient (Zar 1999, pg. 403) is reported here because the odds ratio cannot be calculated when a proportion = 1.0), consistent with prediction 3). Only one male that was forced from his former nest site obtained a new mate in 2003-04, so RS dropped for this group of males (RS = 0.14, 95CI = 0.03 – 0.53; repeated measure analysis of contingency tables and effect size cannot be calculated because of zeros in one column of the table) which is also consistent with prediction 3). Incomer males had significantly lower RS_{yrX} than all other male categories ($\chi^2_1 > 12.69$, $P < 0.001$, odds ratio < 0.07 ; Fig. 1). The log-linear analysis of male category by RS_{yrX} by RS_{yrX+2} frequency table produced a well fit model ($\chi^2_4 = 5.14$, $P = 0.27$) with significant two-way interactions ($\chi^2_7 = 21.06$, $P = 0.004$). Incomer males had significantly higher RS_{yrX+2} than all other male categories ($\chi^2_1 > 3.99$, $P < 0.046$, odds ratio > 4.55 ; Fig. 1). The RS of incomer males significantly improved in both years after their partner's divorce (yr_X to yr_{X+1} : $LR_1 = 15.25$, $P < 0.001$, odds ratio = 36.85 and yr_X to yr_{X+2} : $LR_1 = 18.02$, $P < 0.001$, odds ratio = 77.50; Fig. 1). Incoming males were the only category, male or female, to significantly improve their RS post-divorce (or no divorce, if retained), which is consistent with prediction 4). However, the RS of stayer females did not decline after the forced divorce which is inconsistent with prediction 4).

DISCUSSION

Empirical and theoretical assessments of pair formation, divorce, and re-mating strategies have been fruitful areas of investigation for evolutionary biologists (see Choudhury 1995 and Ens et al. 1996 for reviews), but the importance of involuntary forced divorce as a mechanism of pair dissolution has been overlooked and under-investigated (Jeschke et al. 2007). Non-breeding adults have been postulated to be a buffer against population fluctuation (Durell & Clarke 2004), but recent theoretical work has suggested that these “unemployed” (Anderson et al. 2004) individuals can reduce population growth, perhaps through harassment of breeders and disruption of established pair-bonds (López-Sepulcre & Kokko 2005; Jeschke et al. 2007). In the case of Nazca boobies, non-breeding adults regularly attack unattended chicks in our study colony and the resulting injuries can lead to substantial nestling mortality from subsequent blood-feeding by landbirds (Anderson et al. 2004). Non-breeding males in this study also harassed incubating females; sometimes the female stopped incubation to chase these males away (TJM pers. obs.). Given the excess of adult males in our population and their interference with normal breeding activities, non-adaptive forced divorce is a plausible cause of pair disruption in addition to adaptive mate rotation, and instances of forced divorce have been reported in other species (Ens et al. 1993; Dhondt et al. 1996; Williams & McKinney 1996; Taborsky & Taborsky 1999; Heg et al. 2003; Jeschke et al. 2007). We know of no other study before ours that has systematically investigated the behavior of pairs and non-breeding adults prior to divorce to reveal the proximate cause(s).

Our behavioral observations provided evidence for chance events, forced divorce, and female choice against successful (mate rotation) as well as unsuccessful (better option) males as likely causes of divorce in our population. Two instances of divorce could not be classified but were consistent with both parties instigating the divorce (error in mate choice or incompatibility). Individuals of differing quality or breeding status may pursue different divorce strategies (Choudhury 1995); therefore, it is likely that multiple causes of divorce exist within a population, as we have concluded in this study. This pattern could be fairly common, but only a few studies have documented this phenomenon to date (Ens et al. 1993; Cockburn et al. 2003; Heg et al. 2003).

We found marginal support for mate rotation predictions among stayer females, whose gain in RS compared to that of retained females approached significance. Mover females did not have higher RS prior to divorce than did retained females, inconsistent with prediction 2) of the mate rotation model, and behavioral observations suggested that these females divorced as predicted by the better option model and not the mate rotation model. Mover females came largely from unsuccessful pairs, and most did not visit their old nest sites or interact with their old partners, suggesting that they had decided to avoid their old partners some time before we began our observations. Thus, reproductive histories and behavioral observations indicate that female choice against prior mates can follow the mate rotation model in the case of successful pairs and can follow the better option model in the case of failed pairs. It is possible that the male partner in unsuccessful pairs (all stayers in our observational study) could be the instigator of divorce. We find this unlikely because many divorced males became non-breeders in the year following divorce, and RS of divorced males decreased significantly, at least in the

year immediately following divorce, while mover females' RS did not. In divorces involving successful pairs, the females tended to interact (perform Mate behaviors) with their old mate and one or more other males before pairing with a new male, perhaps indicating that the decision to upgrade mates required more time and information than in the case of previously unsuccessful pairings.

We did not find evidence of adaptive departure of males from the breeding pool to recover condition because all divorced males advertised their availability to potential mates, none willingly gave up their nest sites (except for the two cases of mutual divorce), and none attempted to drive their old mate or potential new mates from their nest sites.

Divorced males that moved from their old nest sites defended their nest sites from the incoming male and former mate prior to being forced away, consistent with forced divorce by outside males in cooperation with pair females. Incoming males were the only category of males that improved their RS significantly after divorce (Fig. 1). This outcome is predicted by both the mate rotation model of divorce and the forced divorce scenario because females should switch to previously non-breeding males under mate rotation and unemployed males should most easily oust depleted (= successfully breeding) males under forced divorce. Collectively, our results suggest that mate rotation was usually accomplished by forced divorce. Divorce generally had a negative effect on male RS, particularly in the year immediately following divorce, because many mover and stayer males failed to obtain a new mate that year. Most divorced males return to breeding status after one or two years of non-breeding (Maness & Anderson 2007), and most mover males would then become incomers, so it could be argued that divorce

benefits these males as well. However, in all seven cases (100%) of an incoming male taking a mover male's nest site, the males had aggressive interactions that led to bleeding lacerations for both males and often (five out of seven cases) the stayer female also had aggressive interactions with her former mate which occasionally led to injury to the stayer female (TJM pers. obs.). Also, a male that did not have an established nest site was highly unlikely to obtain a mate, as indicated by the negative logistic regression coefficient of Prospecting behaviors. In summary, we found no evidence to support voluntary ceding of the breeding attempt by the mover male to the incomer.

Instead, behavioral observations suggest that, in most cases, the divorce appears to be a new type of forced divorce, one that is forced upon a successful male by his old mate and the incoming male in the context of adaptive mate rotation. Females have good reason to join with the incomer because the RS of the female stayers, the supposed "victims" of forced divorce, was higher than any other female category prior to divorce and remained highest two years after the divorce (Fig. 2). Males that lost their nest sites to an incoming male were less aggressive in the pre-breeding season than retained males were, although this behavior was not a significant predictor of overall male mating success. Other behavioral criteria, such as Mate, Prospecting, Territorial, and Advertising behaviors, may be used in females' mate choice decisions, perhaps because highly aggressive males tend to be poor parents (Wingfield et al., 1990; Forsgren 1997; Qvarnström & Forsgren 1998; Wong 2004).

Previous studies suggested that forced divorce may be important in populations that are at, or near, their carrying capacity and limited by high quality nesting sites (Heg et al. 2003, Jeschke et al. 2007). Our population does not fit this pattern because nesting

sites do not appear to be limited (Townsend et al. 2002) and nest site quality (microclimate, ease of flight initiation, substrate characteristics, etc.) has not explained any variation in the number of fledglings produced at a particular site (DJA, unpublished data). In addition, if high quality females (stayers) in our population selected preferred nest sites only, then they would have no reason to assist an incomer with the nest takeover. Instead, these females could settle for the winner of the male contest, but these stayer females were active participants in the takeover and we observed one female opt for an incomer that lost a fight with her former partner. So while site fidelity could contribute to mate fidelity in our system, it does not appear to be the driving force in mate rotation.

The mate rotation pattern of divorce appears to be due to a combination of two effects involving a female's abandonment of a previous partnership. In some cases, forced divorce included female collusion, and in other cases, the female chose another male at another nest site. Long-lived species with obligate bi-parental care, such as seabirds, are expected to divorce rarely because RS should improve as familiarity with a mate increases (Black 1996) and most seabirds form persistent pair-bonds (Ens et al. 1996). We found no evidence supporting a mate familiarity effect because mover females and incomer males had higher RS than faithful pairs. Recent studies that have disentangled age and breeding experience from pair-bond length have shown that pair-bond length correlated with an initial increase in RS, which is then followed by a decrease (Lewis et al. 2006; van de Pol et al. 2006; Naves et al. 2007). Experimental removal of either pair member reversed this downward reproductive trend once the "victim" of divorce paired with a new mate (van de Pol et al. 2006). If costs of

reproduction are ubiquitous in iteroparous organisms, as suggested by life-history theory (Stearns 1992), then divorce, after a period of time together, would seem advantageous for one or both members of a pair, particularly if non-breeding partners were available. A logical line of future inquiry then becomes “What constrains older pairs to stay together?”

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Table 1. Components and component loading scores from a principal components analysis of the 26 different behaviors (described in electronic supplementary material 1) performed by male Nazca boobies during the pre-egg-laying period in 2003. The behaviors reduced to five components and were named by the main behaviors that loaded onto them.

Component Loading Score									
Mate		Territorial		Prospecting		Aggression		Advertising	
Behavior	Score	Behavior	Score	Behavior	Score	Behavior	Score	Behavior	Score
gift	0.908	yes/no	0.864	roaming	0.675	squabble	0.841	skypoint	0.842
mutual jab	0.882	garden	0.699	roam flight	0.661	bill clash	0.732	garden	0.660
mutual gift	0.811	vocal	0.651	returned	0.633			parade	0.426
allopreen	0.748	wing flail	0.613	left	0.604				
bill touch	0.704	jab	0.477	flee	0.587				
copulation	0.619	circle flight	0.432						
cop. attempt	0.553	fight	0.296						
roam	0.496								
chased	0.478								
circle flight	0.457								
displaced	0.198								

Table 2. Logistic regression coefficients, standard error, statistical significance, odds ratio, and 95% confidence intervals of odds ratios of behavioral predictors of male mating success in Nazca boobies. A. All males, including those retained by their previous mates, were used in this analysis. B. Only non-breeding and males breeding with a new mate (retained males excluded) were used in this analysis.

A.

Predictor	β	standard error	p-value	Odds ratio	95% CI for OR	
					Lower	Upper
Constant	1.434	0.302	< 0.001			
Mate	2.159	0.403	< 0.001	8.67	3.94	19.10
Prospecting	-1.303	0.334	< 0.001	0.27	0.14	0.52
Territorial	0.615	0.290	0.034	1.85	1.05	3.26
Advertising	0.465	0.238	0.050	1.59	1.00	2.54
Aggression	0.149	0.234	0.525	1.16	0.73	1.84

B.

Predictor	β	standard error	p-value	Odds ratio	95% CI for OR	
					Lower	Upper
Constant	0.551	0.335	0.100			
Mate	1.635	0.437	< 0.001	5.13	2.18	12.08
Prospecting	-0.949	0.338	0.005	0.39	0.20	0.75
Territorial	0.775	0.321	0.016	2.17	1.16	4.07
Advertising	0.651	0.262	0.013	1.92	1.15	3.20
Aggression	0.252	0.241	0.297	1.29	0.80	2.06

Figure 1. Male reproductive success by partnership category over a three year period. Year X was the year prior to the divorce (or no divorce if retained), year X+1 was the year immediately after divorce, and year X+2 was the final year we followed the fate of pairs. Error bars represent 95% C.I. The flow diagram illustrates the partnership categories; the circles indicate nest sites and arrows represent yearly transitions. Individuals that stay at the same nest site between years are linked by horizontal arrows, while birds that change nest sites are linked by diagonal arrows. Solid arrows indicate transitions that must occur in a particular category, while dotted arrows represent transitions that may occur.

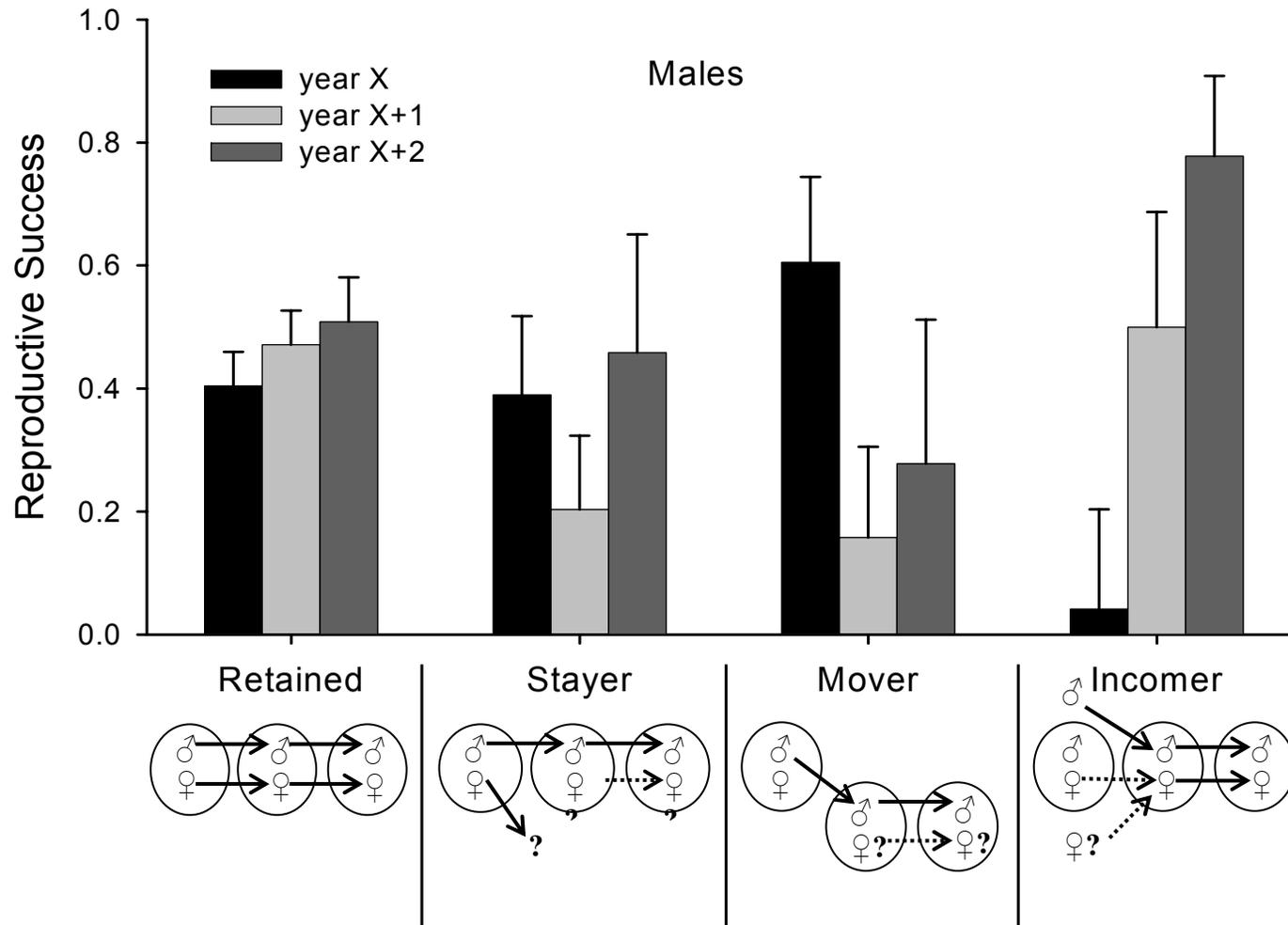
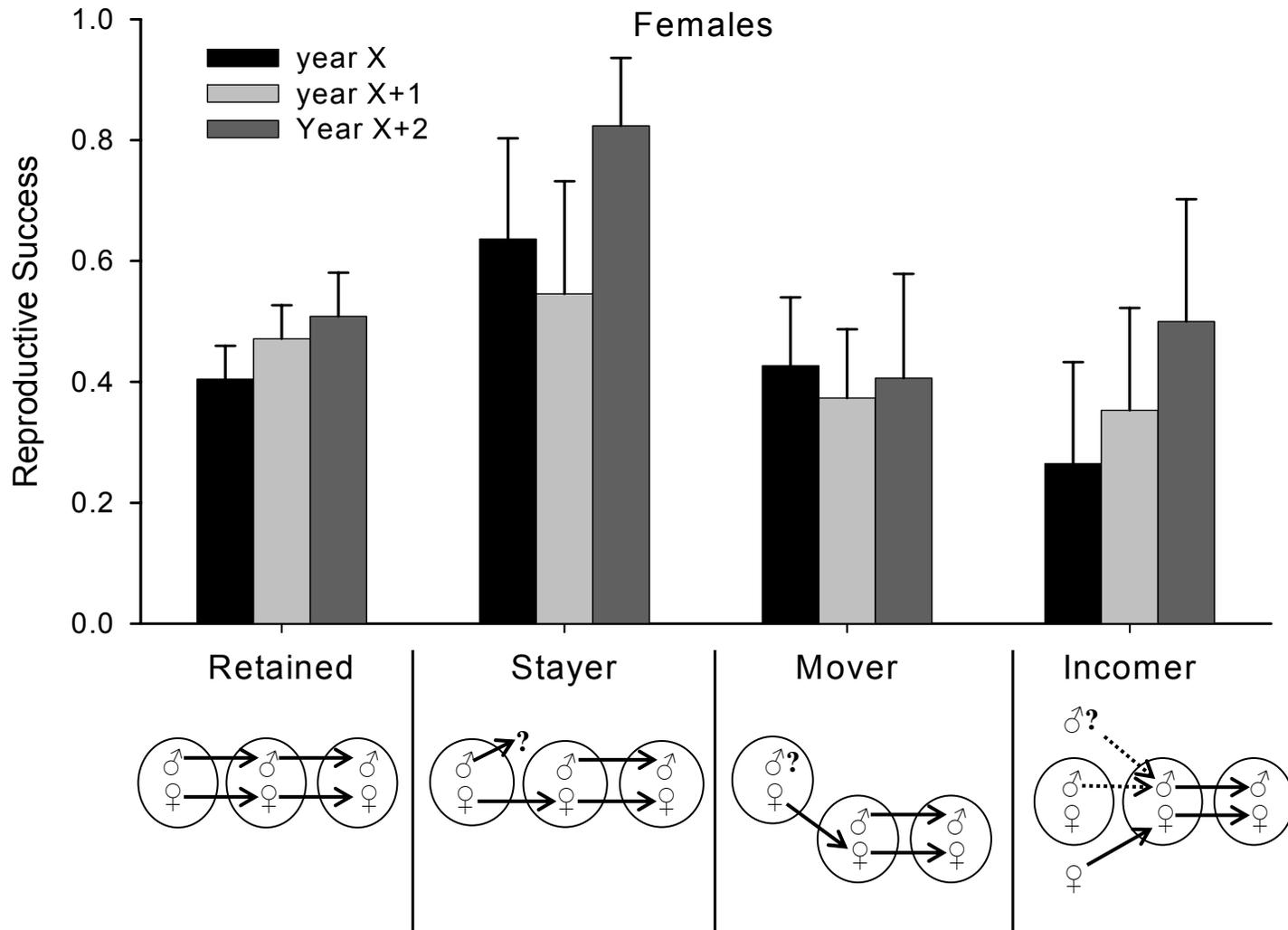


Figure 2. Female reproductive success by partnership category over a three year period. Year X was the year prior to the divorce (or no divorce if retained), year X+1 was the year immediately after divorce, and year X+2 was the final year we followed the fate of pairs. Error bars represent 95% C.I. The flow diagram illustrates the partnership categories; the circles indicate nest sites and arrows represent yearly transitions. Individuals that stay at the same nest site between years are linked by horizontal arrows, while birds that change nest sites are linked by diagonal arrows. Solid arrows indicate transitions that must occur in a particular category, while dotted arrows represent transitions that may occur.



APPENDIX

Electronic Supplementary Material:

Behavioral descriptions from Van Tets (1965) and Nelson (1978) unless otherwise noted:

Establishment and Maintenance of Territory:

Aerial Reconnaissance (Roam Flight; this study): A flight over the colony, presumably to search for vacant sites. Take off and landing is usually performed silently and does not occur at a particular site (see circle flight below).

Ground Reconnaissance (Roaming; this study): Wandering around the colony on foot, presumably to search for vacant sites, usually performed silently and without particular direction (see Roam below).

Circle Flight: A brief aerial excursion around the colony where a bird takes off from and returns to a particular site (at or near its nest site). Landing is performed with the wings held in a V-shape and is usually accompanied by a loud call.

Vocal (this study): A call, usually directed at an opponent.

Wing-flailing: A sudden opening and lifting of the wings which are then spread and brought down sharply. Wing-flailing can be a single swift movement or may be repeated several times.

Jabbing: A thrust of the bill toward (but not making contact with) an opponent usually delivered from a forward leaning posture.

Bill clash: A jab where the bills of two opponents come into contact often repeatedly and loudly.

Yes/No Headshake: A display that looks as if the bird were nodding a frenzied “yes” superimposed on a slow “no”. This display is often repeated and sometimes accompanied by vocalizations.

Squabbling (this study): A combination of Vocal, Wing-flailing, Jabbing, and Yes/No Headshaking behaviors.

Fight: Usually involves an interlocking of bills (and/or biting; this study) and pushing of opponents with an extended neck.

Chase (this study): Occurs when one bird runs toward another, presumably to chase away or remove an intruder.

Displace (this study): Usually occurs after a chase, where the focal bird displaces an individual from its current position (usually a nest site or conspicuous vantage point).

Flee (this study): Running away from an opponent, usually with the bill held close to the body (Pelican Posture).

Left (this study): Flying away from the colony or roaming out of the observation area.

Returned (this study): Returning to the colony or observation area after leaving.

Formation and Maintenance of Pairs

Sky-pointing: A display where an individual stretches its neck toward the sky, lifts its wings and whistles through a slightly opened bill.

Symbolic Nest Building (Gardening; this study): Nests of Nazca boobies have no

Structural significance, but birds will gather, present, arrange, and rearrange a variety of materials (pebbles, feathers, bones, dung, vegetation, crab shells, and other detritus) around the center of their nest.

Parading: An exaggerated walk where an individual lifts its feet higher than is necessary and displays them at an angle.

Roam (this study): Walking (without exaggerated steps; see Parading above) to and from the nest site, often to steal a neighbor's nest materials, to obtain gifts (see below) for a potential mate, or to sky-point from a conspicuous vantage point.

Mutual Jabbing: Performed by a pair, where the birds rush toward each other and thrust their open bill into the other's, sometimes rapidly shaking their heads and audibly clashing their bills together. Usually accompanied by vocalizations.

Bill Touching: Delicately touching another's bill with the tips of one's own closed bill.

Gift: Presenting a potential mate with symbolic nest materials (held in the bill).

Mutual Gifts (this study): Mutual presentation of gifts (see above) by a pair.

Allopreening: Preening another's feathers (non-reciprocal) or mutual preening (reciprocal) performed by a pair.

Copulation attempt (this study): An attempted but failed copulation, usually because the male fails to mount the female or is prevented (by female) from mounting.

Copulation: Successful meeting of cloacal openings.

Other behaviors not always noted when performed and used in this study only for determining time present, or performed after egg-laying began and subsequently not used in behavioral analyses:

Gazing: An intent stare performed with an out-stretched neck.

Sleeping (this study): Sitting still with eyes closed.

Awake (this study): Sitting still with eyes open.

Out-posting: Standing in a conspicuous vantage point near one's nest site.

Preen: Cleaning and arranging one's own feathers.

New arrival (this study): Arriving at the colony for the first time during an observation period.

Gone (this study): A bird that was present previously during an observation period but now not seen.

Bite (this study): Closing the bill on a body part (other than the bill; see fight above) of another bird.

Gift Attempt (this study): Presentation of a gift (see above) by an unpaired male usually to an incubating female.

Incubate: Sitting or standing with feet covering the egg(s).

Not Incubating (this study): Standing near the nest but not in contact with or shading the egg(s) or leaving the egg(s) unattended.

CHAPTER 4

Hematological and morphological parameters are associated with male mating success in Nazca boobies

Terri J. Maness, Victor Apanius, and David J. Anderson

Minor stylistic variations and the inclusion of multiple authors are due to this chapter being based on an article to be submitted for publication. T. Maness did IgG assays, performed all analyses, and prepared the manuscript. V. Apanius guided laboratory analyses of plasma metabolites and acted in an advisory capacity. D. Anderson acted in an advisory and editorial capacity.

ABSTRACT

Behavioral ecologists traditionally link the latent variable called “individual quality” with exaggerated secondary sexual characteristics or suites of phenotypically variable cues or behaviors. Life history theory suggests that high quality individuals are better able to afford investment in breeding than are low quality individuals, and so should express these relevant cues in a more attractive manner. Populations with biased operational sex ratios provide opportunities to investigate mate choice since the bias offers members of the limiting sex the opportunity to choose among competing potential mates. We investigated mate choice in a male-biased population of Nazca boobies and asked whether males that were chosen as mates differed in morphology or blood chemistry from those that were not chosen. Nazca booby males that were selected as mates in our study were in better body condition, had significantly lower immunoglobulin G levels, and, as a group, had lower variance in circulating albumin concentration than did males not selected as mates. Our results suggest that females may use several long-term, rather than short-term, indicators of nutritional health and condition to determine with whom to mate.

INTRODUCTION

Theories of mate choice are based on the premise that choosers exploit phenotypic cues that accurately predict the potential mate's fitness (Andersson 1994, Kokko et al. 2003). The reliability of sexually selected cues involves life-history trade-offs between allocation of time and energy to parental effort vs. other fitness-related functions, like self-maintenance. Under such a trade-off, higher-quality individuals are generally better able to afford investment in breeding than are lower-quality individuals (Stearns 1992). To optimize reproduction, members of monogamous taxa should select partners with superior reproductive performance (via parental care (Trivers 1972) and/or "good genes" (reviewed in Andersson 1994)), but how that is signaled at the proximate level is an open question.

A biased operational sex ratio (OSR; relative ratio of individuals ready to mate: males/(males + females); Enders 1993, Kvarnemo and Ahnesjö 1996, 2002) offers members of the limiting sex more opportunity to choose among competing potential mates. As a consequence, a biased OSR is expected to drive sexual selection on members of the over-represented sex (Emlen and Oring 1977, Andersson 1994, Shuster and Wade 2003). Thus, populations with biased OSRs provide opportunities to investigate mate choice in monogamous species.

Previously, we investigated pairing patterns in a population of Nazca boobies (*Sula granti*) with a significantly male-biased OSR (Townsend and Anderson 2007, Maness and Anderson 2007, 2008). Nazca boobies are long-lived seabirds (Anderson and Apanius 2003) with social and genetic monogamy (Anderson and Boag 2006) and obligate bi-parental care (Nelson 1978, Anderson 1993). As is typical of long-lived taxa

(Stearns 1992), Nazca boobies exhibit low annual fecundity (Humphries et al. 2006) and long developmental periods (Nelson 1978, Anderson 1993, Apanius et al. 2008). Parents produce at most one fledgling per breeding attempt (Nelson 1978; Humphries et al. 2006) and offspring require 160-180 days to develop from the egg to fledging (Nelson 1978, Anderson 1993, Apanius et al. 2008). Since parents invest considerable time and energy in reproduction, we would expect Nazca boobies to be choosy about their mates. The divorce rate in Nazca boobies is atypically high (28-38% per year; Maness and Anderson 2007, 2008) for a single-brooded, long-lived species (Ens et al. 1996, Jeschke and Kokko 2008). Females in our male-biased population frequently exchange their previous partners for new mates from the current pool of non-breeding males (Maness and Anderson 2007, 2008). Surprisingly, divorce occurred after successful, as well as unsuccessful breeding attempts, implicating costs of successful reproduction in mate retention (Maness and Anderson 2007, 2008). Thus, the male-biased OSR provided the opportunity for selection on female mating preferences (Maness and Anderson 2007, 2008), and we deduced that female choice is based at least in part on the current condition of potential mates. Here we ask whether males that were chosen as mates differed from rejected males in physiological condition, reflected in their morphology and blood chemistry.

Since provisioning ability is essential to breeding success in seabirds (Nur 1984; Schreiber and Burger 2002), it is commonly predicted that body mass or body condition index (BCI - usually mass corrected for body size; reviewed in Brown 1996) at the start of the reproductive cycle is an important determinant of parental performance. Individuals with greater mass for their body size are expected to be more proficient

foragers (Chastel et al. 1995). Furthermore, potential mates with greater body reserves (e.g., higher BCI scores) may have more endurance during non-feeding incubation bouts (Chaurand and Wiemerskirch 1994a, Olsson 1997, Gauthier-Clerc et al. 2001) and may guard young chicks for longer periods of time (Tveraa et al. 1998, Tveraa and Christensen 2002). The methods used to calculate BCIs have generated much recent discussion and theoretical models indicate that the relationship between body mass and condition indices may be difficult to interpret without additional sources of information (Brown 1996, Jakob et al. 1996, Green 2001, Schulte-Hostedde et al. 2005). Recognizing the controversy surrounding the use and method of calculating BCI, we used (in separate analyses) uncorrected body mass as well as mass corrected for structural size (BCI) as predictors of mating success. We predicted that males with greater body mass and/or higher BCI scores would be more likely to be selected as mates.

Veterinarians use blood chemistry to assess pathological changes due to dehydration, malnutrition, trauma, or disease (Fudge 1997, Harr 2002). The ability of blood parameters to resolve variation in physiological status within wild populations of breeding (i.e., healthy) birds is receiving increasing scrutiny. Generally, decreased body condition, when assessed by mass loss, is reflected in diverse blood parameters (Table 1). Whether these hematological parameters provide more sensitive measures of mate quality, compared to morphological traits, has not been previously addressed. We focused on concentrations of three hematological parameters with sufficient background information for unambiguous interpretation of the outcomes; uric acid (UA), albumin (ALB), and immunoglobulin G (IgG). We know of no other study that has compared these blood parameters in a mate choice study.

UA is the principal waste product of dietary protein catabolism in birds. Carnivorous raptors and piscivorous seabirds show a pronounced post-prandial increase in UA concentration, especially when prey is ingested in a single, large meal (Kolmstetter and Ramsay 2000, Harr 2002, Lumeij and Remple 1991). Circulating UA also rises in conjunction with mass loss during starvation and during extreme non-feeding incubation bouts (Cherel et al. 1988, Jenni-Eiermann and Jenni 1991, 1994, and 1998, Alonso-Alvarez and Ferrer 2001; Alonso-Alvarez et al. 2002, 2003). Catabolism of body proteins is only expected when fat reserves are depleted (Cherel et al. 1988) because of their role in body structure and muscle function (Castellini and Rea 1992); therefore, UA concentration is only expected to increase appreciably during phase III of fasting or shortly before death by starvation (Le Maho et al. 1981, Boismenu et al. 1992, Alonso-Alvarez and Ferrer 2001). In our study, we assumed that elevated UA concentration indicates recent foraging success, as it signals the digestion of a large meal. If elevated UA concentration was due to depletion of body reserves, we would expect a negative correlation between UA concentration and body mass. If recent foraging success reflects the likelihood of continued foraging success, then males selected as mates should have an elevated UA level.

ALB is the most abundant serum protein. Circulating levels are under tight homeostatic regulation, reflecting its critical role in maintaining colloidal osmotic pressure in the vasculature (Griminger 1976, Sturkie 1976). It also has a central role in the circulatory transport of hydrophobic fatty acids, vitamins, and hormones and can be taken up by peripheral cells as a source of amino acids for protein synthesis (Griminger 1976). An elevated ALB level indicates hemoconcentration due to dehydration, while a

low level indicates protein malnutrition (Griminger 1976, Harr 2002). In our study, we assumed that ALB concentration is maintained at a homeostatic set-point and that deviations from that point represent lax regulation, which may be associated with erratic physiological performance. Accordingly, we expected males selected as mates to have a lower variance in ALB level, as a group, compared to the higher variance expected in the cohort of rejected males.

IgG, also referred to as IgY, is the second most abundant plasma protein. It provides a critical self-maintenance function by binding to and eliminating toxins, viruses, bacteria, and parasites that infiltrate body surface barriers (Warr 1995, Hanson 1979, Lemke et al. 2004). IgGs are produced by splenic B-lymphocytes and have the ability to confer rapid responses to previous antigenic exposure (“immunological memory”; Hanson 1979, Warr 1995). Extensive studies in laboratory animals and humans show that the circulating IgG level is maintained around homeostatic set-points by independent control of synthesis and degradation rates (Waldmann et al. 1970). Repeated measurements of IgG concentration in adult Nazca boobies during the chick-rearing period showed that it fluctuated around individual homeostatic set-points and that both parents maintained constant levels, in contrast to the decline across the reproductive cycle consistently shown for short-lived taxa (Apanius et al. 2008). An increased IgG level could be the result of antigen exposure (McFarlane 1973) or increased allocation to this self-maintenance component (Sarker et al. 2000) while a low IgG level may be induced by stress (Barnard et al. 1994, de Vries et al. 1997). As with ALB, we assumed that the cohort of selected males, as a group, will have a lower variance in IgG

concentration, compared to rejected males that are unable to maintain consistent regulation of this self-maintenance trait.

Summary of predictions tested:

1. If mass and/or BCI reflects long-term foraging success, then males with higher mass and/or BCI are expected to be selected as mates.
2. If recent foraging success reflects the likelihood of continued foraging success, then males with high UA levels are expected to be selected as mates.
3. If the variance of ALB concentration represents the degree of homeostatic regulation of this physiological trait, then the cohort of selected mates is expected to have a lower variance.
4. If the variance of IgG concentration indicates the ability to regulate this self-maintenance trait homeostatically, then the cohort of selected mates is expected to have a lower variance.

METHODS

Study site and blood sampling:

Our group has been conducting long-term demographic studies on a population of Nazca boobies at Punta Cevallos, Isla Española, Galápagos Islands (1°23'S, 89°37'W), Ecuador since 1984. Although extra pair copulations are common in this population, the extra pair fertilization rate is estimated to be zero (Anderson and Boag 2006); so parentage can be assigned reliably through behavior. Beginning on 17 August 2003 and continuing through 28 September 2003, 2-3mL blood samples were collected by brachial

venipuncture from resident male boobies (sexes are dimorphic by voice; Nelson 1978) in a subsection of the “study area” known as the “mini-area” (see fig. 2 of Apanius et al. 2008 for detailed description and map). Blood sampling was standardized to the same circadian phase (2000 to 2400h). Sampling was done at this time because foragers (successful and unsuccessful) would have returned to the colony by this time (Anderson and Ricklefs 1992) and mass of ingested food would be highest (contrasting with time body mass was determined; see below). Most samples (83.4%) were collected as males began courtship display (Van Tets 1965, Nelson 1978, Maness and Anderson 2008) in late August and early September of 2003 and all sampling was completed at least two weeks before egg-laying began in mid-October of 2003. Blood was placed in 1.5 mL polypropylene microcentrifuge tubes and allowed to clot at ambient temperature for 2-4 hrs, then centrifuged at 10,000 rpm for 10 min. Serum was then transferred to a clean 1.5 mL cryovial and frozen in the field in a propane powered freezer. Samples were transported from the field on ice, and then stored at -80°C until laboratory analysis.

Morphometric measurements:

We measured male body mass with a Pesola spring scale ($2500\text{g} \pm 20\text{g}$), culmen and tarsus length were determined with vernier calipers ($\pm 0.1\text{ mm}$), and flattened wing length and ulna length were measured with a wing rule ($\pm 1.0\text{ mm}$). Measurements were taken during the same circadian phase (0200 – 0600 h) when mass of ingested food was lowest to best estimate body reserves. Dates measurements were taken were from 23 August to 30 September 2003. Mass did not correlate with measurement date (Spearman $R = -0.12$, $P = 0.14$). Structural size was determined by principal component analysis

(PCA) of the four skeletal/feather measurements. A BCI was calculated as the residuals from an ordinary least squares regression of mass and the first principle component (PC1) of body size (see Schulte-Hostedde et al. 2005).

We obtained morphological measurements from 146 males and blood and morphological measurements from 136 males. All structural measurements significantly correlated with each other ($p < 0.04$, Table 2) and PC1 (table 3) explained 75.7% of the variation in structural size. The regression of structural size and mass was significant ($F_{(1, 144)} = 7.88$, $P < 0.006$), and PC1 explained 5.2% of the variation in mass.

Uric Acid measurement:

Serum concentration of UA was measured through enzymatic rate reactions following manufacturer's instructions (Thermo Electron, TR24321). Assays were performed in triplicate in 96-well micro-plate and absorbance at 520nm was measured with a micro-plate reader (Victor3, Perkin-Elmer). Normal human serum (Data-Trol N, Thermo Electron, TR40001/1902-050) was used as a reference in every plate.

UA level correlated with sampling date (Spearman's $R = -0.328$, $P < 0.001$). An analysis of the main effects of UA and sampling date, and their interaction (UA*Date) on male breeding success revealed that sampling date and its interaction with UA concentration had no significant effects on male breeding success (data not shown), and so sampling date was not a confounding variable in our analyses.

Albumin measurement:

Serum concentration of ALB was measured through dye binding of Bromocresol Green following manufacturer's instructions (Thermo Electron, TR36021/1105-250). Assays were performed in triplicate in 96-well micro-plate and absorbance at 625nm was measured with a micro-plate reader (Victor3, Perkin-Elmer). Normal human serum (Data-Trol N, Thermo Electron, TR40001/1902-050) was used as a reference in every plate.

ALB level correlated with sampling date (Spearman $R = 0.225$, $P < 0.007$). An analysis of the main effects of ALB and sampling date and their interaction (ALB*Date) on male breeding success revealed that sampling date and its interaction with ALB concentration had no significant effects on male breeding success (data not shown), and so sampling date was not a confounding variable in our analyses.

Immunoglobulin G measurement:

IgG was identified based on the molecular weight of the native protein and of the subunits following reductive dissociation in two-dimensional electrophoresis after Apanius et al. (1983). IgG concentration was measured by electrophoretic separation from other serum proteins in 7.5% polyacrylamide gels followed by quantitative staining and densitometry (Apanius and Nisbet 2003; Fig. 3). Purified Chicken IgG (I4881, Sigma-Aldrich) was used to construct a standard curve (2, 4, 6, 8, 10 mg/mL) in each gel. This concentration range produced a linear standard curve with $r^2 > 0.95$ for each gel. Reference chicken serum (Sigma-Aldrich, C5405) was used in each gel.

IgG level did not correlate with sampling date (Spearman $R = -0.06$, $P = 0.47$).

Statistical Analyses:

Levene's test was used to compare homogeneity of variances of ALB and IgG concentrations between breeding and non-breeding males. Logistic regressions were used to assess directional predictors (mass and UA) of male mating success and Akaike's Information Criterion (corrected for small sample sizes (AICc)) was used for logistic regression model selection and ranking (Burnham and Anderson 2002). Since AICc converges to AIC with large sample sizes, AICc should be employed regardless of sample size (Burnham and Anderson, 2002). All predictors were entered using a block method. We also report Bayesian Information Criterion (BIC) values as an alternative model selection tool. The BIC penalizes free parameters more strongly than does AIC (Schwarz 1978).

All statistical tests were performed in SPSS (ver.15.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

Variance tests

The variance in ALB concentration was significantly higher in non-breeding males (Table 5, Figure 1). The variance in IgG concentration (log transformed, see below) was not different between groups (Table 5), contrary to our original prediction. None the less, females might select mates with a particular IgG concentration and we added IgG to the logistic regression analysis.

Logistic regressions

The behaviors of mass and BCI in logistic regressions were nearly identical, and we report only the results for BCI here. The first logistic model tested included BCI, UA, and IgG levels as predictors of male mating success. UA and IgG levels were log-transformed prior to input because their distributions were positively skewed (Sokal and Rohlf 1995). Diagnostics of logistic regression residuals revealed one extreme outlier (standardized residual > 3.0), and this individual was omitted from further analyses (Jennings 1986). The omnibus test of model coefficients was significant ($\chi^2_3 = 14.478$, $P = 0.002$) and the model was a good fit (-2LL = 142.10; Hosmer and Lemeshow $\chi^2_8 = 8.88$, $P = 0.35$; AICc = 150.41, BIC = 161.72) and classified 74.1% of males correctly. BCI was a significant positive predictor and IgG level was a significant negative predictor of male mating success, while UA level was not significant (Table 6). Subsequent testing of nested and reduced models resulted in a final model that included IgG and BCI as predictors. This model's omnibus test of model coefficients was significant ($\chi^2_2 = 14.01$, $P = 0.001$) and the model was a good fit (-2LL = 142.57; Hosmer and Lemeshow $\chi^2_8 = 13.99$, $P = 0.082$, AICc = 148.75, BIC = 157.28) and classified 74.1% of males correctly; IgG level was a significant negative predictor and BCI was a significant positive predictor of mating success (Table 6). The difference in AICc rankings of the two models ($\Delta\text{AICc} = 1.66$) suggested that the two models have equal predictive value (Burnham & Anderson 2002), while the difference between BIC rankings ($\Delta\text{BIC} = 4.44$) provided "positive" evidence (Raftery 1995) that the more complex model should be rejected. Thus, because both models correctly classified the same percentage of subjects, we selected the more parsimonious model as the best model

to evaluate the relationship between physiological parameters and mate choice. IgG concentration was a negative predictor of mating success, indicating that males with high IgG level were less likely to be selected as mates (Table 6). BCI was a positive predictor of mating success, indicating that males with more mass for their body size were more likely to be selected as mates (Table 6).

A female who retains her mate may use different criteria than a naïve female when deciding with whom to mate due to her prior experience with the male (Dubois et al. 2004); therefore, a second logistic regression was performed with retained males excluded from the model. The final model was similar to the logistic regression which included all males, except that BCI was only marginally significant (Table 6). Additionally, variance in ALB concentration was significantly higher for the group of non-breeding males ($F_{1,76} = 4.07, P < 0.001$).

Predictions

Consistent with prediction 1, males with higher BCI scores were more likely to be selected as mates. The effect size for BCI was relatively small (Table 6), but suggests that a male carrying 200 extra grams for his structural size ($BCI = 200$) would be twice as likely to breed as a male with a $BCI = 0$. The range of BCI values was -224.85 to 358.06, so the male with the highest BCI would be almost four times more likely to breed than the male with the lowest BCI, all else being equal.

Prediction 2 was not supported because UA concentration was not a significant predictor of male mating success (Table 6).

Consistent with prediction 3, the group of males selected as mates had significantly lower variance in ALB level than did the non-selected group of males (Table 5, Figure 1).

Prediction 4 was not supported because variance in IgG level was not different between breeder and non-breeder males (Table 5). Instead, IgG level was a significant negative predictor of male mating success. The effect size of the log-transformed IgG level was large (Table 6) and, after reverting back to the original scale, suggested that a 1.0 g/L increase in circulating IgG level would make a male 20% less likely to be selected as a mate, all else being equal. The range in IgG concentration was 2.16 to 26.04 g/L; thus, the male with the highest IgG level should be nearly five times less likely to breed than the male with the lowest level, all else being equal.

DISCUSSION

Nazca booby males that were selected as mates in our study had significantly higher BCI scores, significantly lower IgG levels, and, as a group, had lower variance in ALB concentration than did males not selected as mates. Recent foraging success, as indicated UA, was not a significant predictor of mating success. While many studies have used morphological and/or hematological parameters to examine trade-offs between reproduction and self-maintenance (e.g., see Table 1), we know of no other study that has used these variables to examine individuals during the process of mate selection in a wild population.

Individuals with greater mass for their structural size are expected to have more body reserves (reviewed in Brown 1996) and could be more successful foragers over

time. Heavier males are known to achieve greater reproductive success (Aldrich and Raveling 1983, Gibbons 1989) and available body reserves may affect the decision to breed in intermittent breeders (Weimerskirch 1992, Chastel et al. 1995) and influence chick provisioning (Weimerskirch et al. 1995, Chaurand and Weimerskirch 1994b). In Nazca boobies, both fathers and mothers lose mass during the chick rearing period (Apanius et al. 2008), which could represent a physiological cost, such as loss of energy reserves or muscle mass, or an adaptive shift in wing-loading favored by long-foraging flights (Jones 1994, Rands et al. 2006). Males in our study with larger BCI scores at the start of the breeding season may be less likely to abandon the reproductive attempt later if this mass loss is a physiological cost and would therefore be more desirable as mates. Alternatively, an initially heavier male may be better able to optimally adjust his mass loss/wing loading to the increasing demands of a growing chick than an initially lighter male (Rands et al. 2006).

The higher IgG level in rejected males could indicate that these males were responding to antigen exposure (McFarlane 1973, Lemke et al. 2004); however, no males in this study had the physical appearance of illness (lethargy, avoidance of others, reduced feather maintenance, etc.) or injury. Thus, overt illness or injury does not provide an explanation for their higher IgG level and rejection as mates. If the high IgG indicates an immune response to a fitness-reducing viral, bacterial, and/or parasitic infection, then females, who can detect the infection, the IgG response, or a correlate, should discriminate against infected males as mates.

We predicted that the group of selected males would have lower variance in circulating IgG concentration because laboratory studies have shown that serum IgG

level is maintained around a homeostatic set-point (Waldmann et al. 1970) and Apanius et al. (2008) found that breeding Nazca boobies show low variance in IgG level across the chick rearing period. However, males in the Apanius et al. (2008) study represent a non-random subset of the population in that they were already successful in obtaining a mate and hatching a chick when they entered the study. The immunocompetence handicap hypothesis (Folstad and Karter 1992) posits a negative relationship between testosterone and immune function, which could indicate that males with a high IgG level were rejected because they were not ready to breed and so had not increased testosterone level. However, this explanation seems unlikely in our population because IgG concentration did not correlate with sampling date and all males subsequently attempted to obtain a mate by performing mate attraction behaviors (Maness and Anderson 2007, 2008) and evidence that testosterone is immunosuppressive is equivocal at best (Roberts et al. 2004), especially in birds (Hasselquist et al. 1999).

Alternatively, a male with a high IgG concentration may have a high genetically determined homeostatic set-point (Rees and Nordskog 1981, Sarker et al. 1999, 2000). Long-lived taxa are expected to bias energy allocation to self-maintenance, such as immune function, compared to short-lived taxa (Kirkwood and Holliday 1979, Apanius and Nisbet 2003, 2006, Apanius et al. 2008). However, increased allocation to immune function has important implications regarding immunopathology and autoimmunity (Råberg et al. 1998, 2000), and may necessitate a down-regulation of other physiological functions such as growth, tissue repair, or reproduction (Sheldon and Verhulst 1996, Wikelski and Ricklefs 2001). As such, an individual directing too much personal resource to circulating IgG may be less desirable as a mate. Further studies with repeated

measures of IgG level in selected and unselected males are needed before these two possibilities (antigen response vs. increased homeostatic levels) can be teased apart.

The group of rejected males had significantly higher variance in ALB concentration than did the group of males subsequently selected as mates, suggesting that rejected males had difficulty maintaining ALB homeostasis. Reduction of ALB level indicates several pathological states, mainly malnutrition (Griminger 1976, Harr 2002) and infection (Kuklina and Kuklin 2007), while an increase in ALB level indicates dehydration (Harr 2002). Nazca boobies do not drink fresh water when available (Anderson 1993), so input of water likely comes from their main diet of fish and squid (Anderson 1993). Another possible explanation involves the circulating stress hormone corticosterone, which binds to ALB, decreasing the metabolic clearance rate of the hormone (Wingfield et al. 1984, Silverin 1986, Klukowski et al. 1997). An increased level of corticosterone could result in an increase in ALB concentration, as has been found in rats (Pung et al. 2003) or in a decreased clearance rate of ALB. Individual Nazca boobies with abnormal ALB levels could be experiencing short-term foraging failures (which would increase the probability of dehydration), sustained low levels of foraging success (malnutrition), stress, and/or exposure to environmental antigens.

Finally, UA was not a significant predictor of male mating success; this was the only short-term indicator of condition we examined. Females were not more likely to select mates that were recently successful foragers. Instead, the more long-term indicators of condition that we examined had more influence on mating decisions of females.

The biased OSR in our study population allows the limiting sex, in this case females, the opportunity to choose the best available partner among many potential mates (Maness and Anderson 2007, 2008). Our results suggest that females may use several long-term, rather than short-term, indicators of nutritional health and condition (BCI, ALB, and IgG) to determine with whom to mate. These parameters permitted discrimination between males subsequently selected or rejected as mates. How females are able to distinguish between these males remains an open question. This is the first study to examine these parameters in a group of presumably healthy males actively attempting to attract mates. Follow up studies are now needed to examine how these parameters are related to visual appearance, behavior, or other sexually selected traits of individual males.

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Table 1. Relationships between inferred body condition in relation to observed body mass and plasma metabolite concentrations in several species of birds. BC = body condition, BM = body mass, CK = creatine kinase, CRE = creatinine, GLU = glucose, TG = triglycerides, FFA = free fatty acids, GLY = glycerol, BHB = β -hydroxybutyrate, CHL = cholesterol, TPP = total plasma protein, ALB = albumin, BUN = blood urea nitrogen, UA = uric acid. Upward and downward arrows indicate direction of statistically significant changes; horizontal line indicates that the parameter was measured but no significant changes were observed. Empty cells indicate that the parameter was not measured in that particular study.

Species/source of variation	BC	BM	CK	CRE	AST	LDH	GLU	TG	FFA	GLY	BHB	CHL	TPP	ALB	BUN	UA	Study
Cory's Shearwater <i>Calonectris diomedea</i> Breeding	↓							-			-		-			-	Forero et al. 2006
Cory's Shearwater <i>Calonectris diomedea</i> Pre-breeding vs. egg-laying		↑						↓				↓	↓		↑	-	Navarro et al. 2007
Cory's Shearwater <i>Calonectris diomedea</i> Hatching vs. chick rearing		↓						↑				↑	-		↓	↑	Navarro et al. 2007
Adelie Penguin <i>Pygoscelis adeliae</i> Early fasting		↓					-				-					↓	Vleck & Vleck 2002
Adelie Penguin <i>Pygoscelis adeliae</i> Late fasting		↓					-				↑					↑	Vleck & Vleck 2002

Macaroni Penguin <i>Eudyptes chrsolophus</i> Pre vs. late molt	↓			↓	↓							↑	↓	↓	-	↓	Ghebremeskel et al. 1992
Gentoo Penguin <i>Pygoscelis papua</i> Early vs. late molt	↓			-	-							↑	↓	↓	↑	-	Ghebremeskel et al. 1992
Chinstrap Penguin <i>Pygoscelis antarctica</i> Fast (11 day)	↓	↓					↓	-				-			↑	↑	Alonso-Alvarez et al. 2003
White Pelican <i>Pelecanus otocrotalus</i> Fast (2 day)	↓		-	-		-	-	↑				-	-	-	↓	-	Shmueli et al. 2000
Yellow-legged Gull <i>Larus cachinnans</i> Food restriction	↓	↓		↓			↓					↓			↑	↑	Alonso-Alvarez et al. 2002
Yellow-legged Gull <i>Larus cachinnans</i> Fast (8-12 day)	↓	↓		-			↓	-				↓	-		↑	↑	Alonso-Alvarez & Ferrer 2001
Yellow-legged Gull <i>Larus cachinnans</i> Food restriction (10-18 day)	↓	↓		↓			↓	↓				↓	↓		↑	↑	Alonso-Alvarez & Ferrer 2001
Herring Gull <i>Larus argentatus</i> Fast (6 day)	↓	↓	-		-	-	-	-	↑		↑	-	↓		-	-	Totzke et al. 1999
Lesser Scaup <i>Aythya affinis</i> 1day mass change	↓						↓			-	↑						Anteau & Afton 2008
Black-necked Swan <i>Cygnus melanocoryphus</i> Chronic malnutrition	↓		↑		↑		↓				↑		↓	↓	↑	↓	Artacho et al. 2006

Bald Ibis <i>Geronticus eremita</i> Fast (12-15hr)	↓				-	↑	↓	↓				-	-		↑	-	Villegas et al. 2004
Western Sandpiper <i>Calidris mauri</i> Fast (1 day)	↓	↓						↓		↑	↑						Williams et al. 1999
Western Sandpiper <i>Calidris mauri</i> Food restriction	↓	↓						-		↑	↑					↓	Seaman et al. 2005
Western Sandpiper <i>Calidris mauri</i> Re-feeding after restiction	↑	↑						-		↓	↓					↑	Seaman et al. 2005
Northern Goshawk <i>Accipiter gentilis</i> Breeding	↓		-		-	-						-	-	-		-	Hanauska- Brown et al. 2003
Swainson's Hawk <i>Buteo swainsoni</i> Wintering	↓							↓				-			-	-	Sarasola et al. 2004
Spanish Imperial Eagle <i>Aquila adaberti</i> Re-feeding after emaciation	↑							-	-			↑			↓	↓	Ferrer & Dabado- Berrios 1998
Common Buzzard <i>Buteo buteo</i> Fast (13 day)	↓ ↑	↓ ↑						↑ -				↑ ↓	- ↑	- -	↑ ↓	↑ ↓	Garcia- Rodriguez et al. 1987
Common Buzzard <i>Buteo buteo</i> Re-feeding after fast	↑	↑						-				↓	↑	-	↓	↓	Garcia- Rodriguez et al. 1987

Table 2. Correlations between morphological measurements in Nazca booby males. All correlations were significant (N = 146, $p < 0.04$).

	Culmen	Wing length	Ulna	tarsus	mass
Culmen	1.00	0.25	0.44	0.33	0.23
Wing length		1.00	0.49	0.33	0.17
Ulna			1.00	0.57	0.36
Tarsus				1.00	0.29
Mass					1.00

Table 3. Component loading scores for four morphological measures in male Nazca boobies. The first principal component (PC1) explained 75.7% of the variation in structural size.

Trait	PC1 loading
Culmen	0.343
Wing Length	0.982
Ulna	0.642
Tarsus	0.427

Table 4. Spearman correlations between morphological measures and concentrations of blood parameters. Bolded values are significant ($P < 0.05$).

	Mass	PC1 size	BCI	IgG	ALB	UA
Mass	1.000	0.236	0.963	0.115	0.027	-0.009
PC1 size		1.000	-0.006	0.029	-0.012	-0.110
BCI			1.000	0.125	0.032	0.009
IgG				1.000	0.039	0.134
ALB					1.000	0.034
UA						1.000

Table 5. Mean and standard deviation (SD) of morphological and physiological measures in male Nazca boobies by group.

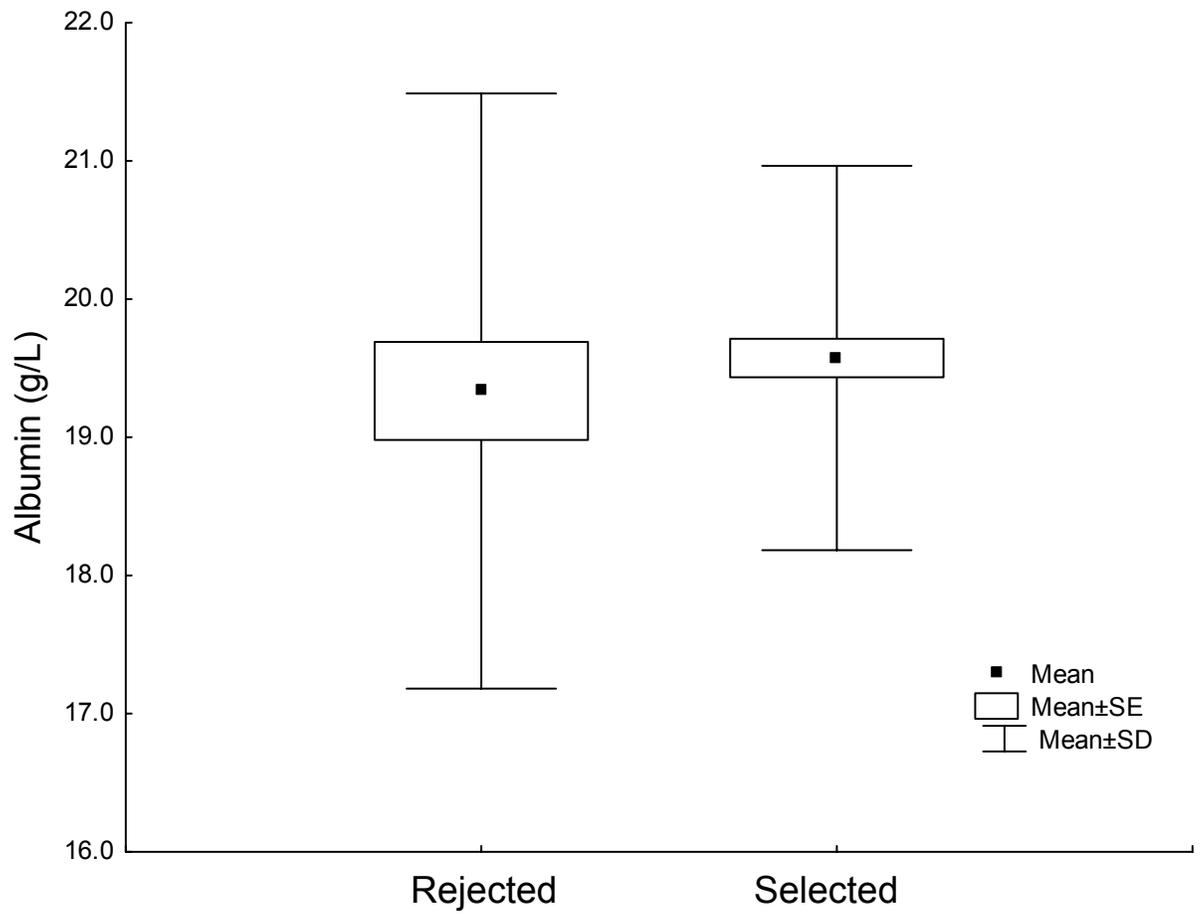
Homogeneity of variance and Welch’s ANOVA tests are also given for each parameter. “N” is the sample size and “ α -crit” is the critical alpha value adjusted for multiple comparisons using the false discovery method (Benjamini and Hochberg 1995, Curran-Everett 2000). The bolded text indicates a significant P value. PC1 size = first principal component score for structural size, BCI = body condition index (residuals of OLS regression of mass and PC1), ALB = albumin, UA = uric acid, IgG = immunoglobulin G.

Trait	All Males		Breeder		Non-breeder		Homogeneity of Variance			Welch's ANOVA		
	Mean	SD	Mean	SD	Mean	SD	F ratio	P value	α -crit	t value	P value	α -crit
Morphological	N = 146		N = 107		N = 39							
PC1 Size	0.00	1.00	0.01	1.06	-0.02	0.82	1.66	0.08	0.010	0.17	0.862	0.050
Mass (g)	1670.44	111.38	1679.30	115.51	1646.15	96.41	1.44	0.21	0.013	1.74	0.086	0.010
BCI	0.00	108.46	8.66	110.80	-23.75	99.23	1.25	0.44	0.017	1.69	0.095	0.013
Physiological	N = 136		N = 99		N = 37							
ALB (g/L)	19.51	1.63	19.57	1.39	19.34	2.15	2.40	<0.001	0.008	0.62	0.534	0.025
UA (g/L)	0.72	0.29	0.71	0.29	0.76	0.30	1.06	0.807	0.025	-0.94	0.352	0.017
IgG (g/L)	6.07	2.66	5.78	2.17	6.86	3.60	1.03	0.954	0.050	-2.27	0.026	0.008

Table 6. Logistic regression coefficients, standard errors, statistical significance, odds ratios, and 95% confidence intervals of odds ratios of predictors of male mating success in Nazca boobies. BCI = body condition index, UA = uric acid, IgG = immunoglobulin G.

Predictor	β	standard error	p-value	Odds ratio	95% CI for odds ratio
Model 1					
BCI	0.005	0.002	0.017	1.005	1.001 - 1.009
UA	-0.873	1.272	0.493	0.418	0.034 - 5.059
IgG	-3.844	1.411	0.006	0.021	0.001 - 0.340
Constant	3.894	1.156	0.001		
Model 2					
BCI	0.005	0.002	0.018	1.005	1.001 - 1.009
IgG	-3.893	1.408	0.006	0.020	0.001 - 0.322
Constant	4.076	1.128	<0.001		
Retained males excluded (breeder N = 42, non-breeder N = 35)					
BCI	0.005	0.003	0.081	1.005	0.999 - 1.010
IgG	-4.162	1.764	0.018	0.016	0.0005 - 0.495
Constant	3.517	1.404	0.012		

Figure 1. Comparison of variance in circulating levels of albumin in Nazca booby males subsequently selected or rejected as mates.



CONCLUSIONS

Causes of the sex ratio bias

Nazca booby parents might adaptively alter the sex of their offspring according to current environmental or demographic conditions, or sex-specific mortality may occur in poor food years; however, these processes could not account for the male-biased adult sex ratio in our population. The hatching and fledging sex ratios were not biased. Instead, the sex ratio bias arose after the period of parental care ended, during the juvenile/subadult stage between fledging and return to the colony. Dispersal seldom moves members of either sex from their natal colony; consequently, the bias evidently arises from greater post-fledging mortality of females. Nazca booby fledglings leave the colony for several years before they return to breed; females could travel farther or to more hazardous areas than males while at sea, or incur different costs related to body size, during this sub-adult period. Alternatively, members of the larger sex (females, in this case) may fledge at a disadvantage if parents meet their requirements less well than those of the smaller sex. The post-fledging exodus makes Nazca boobies unavailable for study for several years after fledging, so tests of some hypotheses invoking sex-specific mortality are not possible at present.

Consequences of the sex ratio bias for the mating system

Behavioral observations revealed that all males present in the colony attempted to attract mates during the pre-breeding season and nearly all (98.5%) females present bred; therefore, the operational sex ratio was the same as the population sex ratio and was significantly male biased. Divorce was more common in our population than is typically

expected for a long-lived species with obligate bi-parental care. Costs of reproduction provided a basis for females to adaptively switch mates, replacing a temporarily depleted male for a current non-breeder in better condition. This was supported by the observation that males entered and exited the breeding pool more often than females. Females, but not males, would have the opportunity to up-grade mates in a male-biased population.

To maximize reproduction, members of monogamous taxa with bi-parental care are expected to select partners that provide superior parental care as well as superior genetics. A biased sex ratio offers members of the limiting sex more opportunity to select among potential mates. Females in our population appeared to use several long-term indicators of health and nutritional status to distinguish among potential mates. Males selected as mates were in better body condition, had lower circulating immunoglobulin G levels, and, as a group, had lower variance in serum albumin concentration than did unselected males. In addition, selected males performed more mate, advertising, and territorial behaviors, and less prospecting behaviors, than did rejected males. Behavioral observations also revealed that mate choice and divorce were driven primarily by female choice. In some cases, a divorcing female chose another male at another nest site, and in other cases the divorcing female colluded with an incoming male to drive her former partner away forcibly. Females that followed this forced-divorce mate rotation strategy had the highest reproductive success of all females. As a whole, these results suggest that offspring provisioning ability, rather than persistent genetic quality (or experience), may be more important to potential mates in some instances, particularly in altricial species with prolonged parental care periods. If costs of reproduction are ubiquitous in iteroparous organisms, as suggested by life-history theory,

then divorce, after a period of time together, would seem advantageous for one or both members of a pair, particularly if non-breeding partners were available.

This body of work included the most complete documentation of the ontogeny of the sex ratio across the lifespan for any wild bird species, provided evidence for a previously undescribed mating system, uniquely investigated the behavior of mated pairs and non-breeding adults prior to divorce, and was the first study to examine the morphological and hematological parameters of individuals during the process of mate selection in a wild population.

SCHOLASTIC VITA

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EDUCATION

PhD Candidate, Biology. Wake Forest University, Winston-Salem, NC.

MS, Biology. University of North Carolina at Wilmington.

BS, Biology. University of North Carolina at Chapel Hill.

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Study Abroad Program. University of Wollongong, New South Wales, Australia.

PUBLICATIONS

Maness, TJ and DJ Anderson (2008) Mate rotation by female choice and coercive divorce in Nazca boobies (*Sula granti*). *Animal Behaviour* 76: 1267-1277.

Maness, TJ and DJ Anderson (2007) Serial monogamy and sex ratio bias in Nazca boobies. *Proceedings of the Royal Society B, Biology* 274: 2047–2054.

Maness, TJ, MA Westbrook, and DJ Anderson (2007) Ontogenic sex ratio variation in Nazca boobies ends in male-biased adult sex ratio. *Waterbirds* 30(1): 10-16.

Townsend, HM, TJ Maness, and DJ Anderson. (2007) Offspring growth and parental care in sexually dimorphic Nazca boobies (*Sula granti*). *Canadian Journal of Zoology* 85: 686-694.

Maness, TJ and SD Emslie. (2001) An analysis of possible genotoxic exposure in adult and juvenile Royal Terns in North Carolina, USA. *Waterbirds* 24(3): 352-360.

Maness, TJ (2000) An analysis of possible genotoxic exposure in adult and juvenile Royal Terns (*Sterna maxima*) in North Carolina. MS Thesis. UNC at Wilmington.

TEACHING EXPERIENCE

- 2004-2008 **Graduate Teaching Assistant.** Department of Biology, WFU, Courses: Biological Principles; Evolutionary and Ecological Biology; Avian Biology
- 2008 **Invited Speaker.** Forsyth County Audubon Society.
- 2007 **Guest Lecturer.** Animal Behavior, Department of Biology, Wake Forest University.
- Guest Lecturer.** Avian Biology, Department of Biology, Wake Forest University.
- Graduate Student Instructor.** University of Michigan Biological Station, Pellston, MI. Course: Behavioral Ecology
- Guest Lecturer.** Behavioral Ecology, University of Michigan Biological Station, Pellston, MI.
- 2006 **Guest Lecturer.** Avian Biology, Department of Biology, WFU.
- 1997-2000 **Graduate Teaching Assistant.** Department of Biology, UNC at Wilmington. Courses: Introductory Biology; Genetics; Microbiology

MENTORING

Undergraduate Thesis:

- Diego García, 2006. Influence of foraging efficiency and body condition on reproductive characteristics of the Nazca Booby (*Sula granti*).
- Diana Astorga, 2005. Growth and competition in an obligately siblicidal bird: the Nazca Booby (*Sula granti*).

Undergraduate Research:

- Kristen Bretz, 2008. Effects of silt run-off on the foraging behavior of the Queen snake (*Regina septemvittata*). Wake Forest University.

Molecular Techniques:

- WFU Graduate students: Jill Awkerman, Martina Müller, Mark Westbrook
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Work Study Students:

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RESEARCH EXPERIENCE

- 2000-2002 **Research Associate.** Department of Biology, UNC at Wilmington.
Project: Effects of genotoxic exposure on Royal Terns (*Sterna maxima*) in North Carolina
- 2001 **Research Assistant.** Department of Zoology, North Carolina State University, Raleigh, NC.
Project: Behavioral Ecology and Population Status of Wood Thrush and Ovenbird in Great Smoky Mountains National Park.
- 1999-2001 **Seabird Survey Monitor and Scientific Crew Member of R/V Cape Fear.** Department of Biology, UNC at Wilmington.
Project: Coastal Ocean Monitoring
- 1991-1997 **Laboratory Technician III.** Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.
Project: DNA binding kinetics and molecular activation of endonucleases and topoisomerases.
- 1987-1990 **Research Aide.** Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, RTP, NC.
Project: Molecular control and genetic organization of transposon-mediated genes in *Drosophila melanogaster*.

PRESENTATIONS

- Maness, TJ and DJ Anderson. (2008) Mate rotation by female choice and coercive divorce in Nazca Boobies. 45th Annual Meeting of the Animal Behavior Society, Snow Bird, Utah.
- Maness, TJ and DJ Anderson. (2007) Serial monogamy and sex ratio bias in Nazca Boobies. 44th Annual Meeting of the Animal Behavior Society, Burlington, VT.
- Maness, TJ and DJ Anderson. (2006) Mate-Switching in the Long-Lived Nazca Booby: Out with the Old, in with the New. 4th North American Ornithological Conference, Veracruz, Mexico.
- Maness, TJ, MA Westbrook, and DJ Anderson. (2005) Ontogenic sex ratio variation, male-biased adult sex ratio, and higher female variance in reproductive success in Nazca Boobies. 123rd Annual Meeting of the American Ornithological Union, Santa Barbara, CA.
- Maness, TJ, MA Westbrook, and DJ Anderson. (2005) Ontogeny of the sex ratio in Nazca Boobies. 28th Annual Meeting of the Waterbird Society, Portland Oregon.

Maness, TJ (2000) An analysis of possible genotoxic exposure in adult and juvenile Royal Terns in North Carolina. 24th Annual Meeting of the Waterbird Society. Manomet, Massachusetts.

GRANTS AND AWARDS

Cocke Travel Award, Wake Forest University, 2005, 2006, 2007, 2008.

Alumni Travel Award, Wake Forest University, 2007, 2008.

Sigma Xi Grants-in-aid of Research Award, 1999, 2006.

Marcia Brady Tucker Award, American Ornithological Union, 2005.

Student Travel Award, Waterbird Society, 2005.

Summer Stipend Award for Graduate Research, Center for Marine Science Research, UNC at Wilmington, 1998, 1999.

Escheats Scholarship. UNC at Chapel Hill, 1984-1988.

PROFESSIONAL AFFILIATIONS

Animal Behavior Society, American Ornithological Union, Waterbird Society, Sigma Xi, Phi Kappa Phi Honor Society.

PROFESSIONAL SERVICE

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2007-2008 **Graduate Representative to Biology Faculty,** Wake Forest University.

1998-1999 **President of Biology Graduate Student Association,** UNC at Wilmington.