

Hybridization and cuckoldry between black-browed and grey-headed albatrosses

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Abstract: A vagrant black-browed albatross *Thalassarche melanophris* bred with a grey-headed albatross *T. chrysostoma* on Marion Island at least four times between 2000 and 2009 (and continued to return to the colony until at least 2019). The eggs failed to hatch in three breeding attempts, but the pair fledged a chick in the 2006/07 breeding season. Genetic sexing identified the black-browed albatross as female and she shared all eight sampled microsatellite alleles with the chick, whereas the grey-headed albatross social parent did not match the chick. The fledgling was banded and re-sighted in its natal breeding colony in 2016 and 2018, when it displayed an intermediate black-browed x grey-headed albatross phenotype, similar to a putative hybrid photographed at sea off Australia. These results suggest that the black-browed albatross cuckolded its social mate with another grey-headed albatross in 2006/07. The failures of the other three breeding attempts at the egg stage possibly indicate genetic incompatibility with the social partner.

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Introduction

Although inter-specific hybridization is fairly common among some birds (Grant & Grant 1992), it is rare in albatrosses, and the few hybrid pairs that have been recorded usually fail to produce chicks (Phillips *et al.* 2018). Some mixed pairs of black-footed (*Phoebastria nigripes*) and Laysan albatrosses (*P. immutabilis*) occasionally produce hybrid young, of which at least some are viable (Fisher 1948, 1972, McCarthy 2006, Rutt 2013). At Bird Island a black-browed (*Thalassarche melanophris*) and a white-capped albatross (*T. [cauta] steari*) pair produced two hybrid chicks, but both died before fledging (Phillips *et al.* 2018). Most other records of hybridization among albatrosses are confined to closely-related taxa formerly regarded as subspecies such as between southern royal (*D. [e.] epomophora*) and northern royal albatrosses (*Diomedea [e.] sanfordi*; Warham 1996, McCarthy 2006), and between black-browed and Campbell albatrosses (*T. [m.] impavida*; Moore *et al.* 2001). At least in the latter case there is evidence of high levels of genetic mixing among these taxa (Burg & Croxall 2001).

Genetic studies have found surprisingly high rates of extra-pair paternity among albatrosses, given their obligate social monogamy and strong mate fidelity between years (Huyvaert *et al.* 2000, Abbott *et al.* 2006, Burg & Croxall 2006, Huyvaert *et al.* 2006, Jouventin *et al.* 2007, Jones *et al.* 2012). These observations raise concerns about the validity of hybrid records based

solely on social pairings, particularly in cases where the male parent is a vagrant in the breeding colony. We report the first record of successful hybridization between black-browed and grey-headed albatrosses *Thalassarche chrysostoma*. Genetic paternity tests were conducted to confirm the putative hybrid chick's provenance. The chick was ringed and it returned to its natal colony 9 and 11 years later, so we also provide a description of the adult phenotype of this hybrid offspring.

Methods

Bird observations have been conducted at Marion Island, the larger of the two Prince Edward Islands, sporadically since the 1950s, and more regularly since the 1970s (Cooper & Brown 1990). The grey-headed albatross is the only *Thalassarche* albatross that breeds on the island. Annual censuses of grey-headed albatross colonies have been made since 1997, when a study colony in which all adults and chicks are ringed was established on Grey-headed Albatross Ridge (46°52'S, 37°41'E, Converse *et al.* 2009). In December 2000, a black-browed albatross was found incubating an egg in another part of the grey-headed albatross colony on Grey-headed Albatross Ridge (away from the study area). It was caught and banded, and its partner subsequently confirmed to be a grey-headed albatross. Observers have looked for the

presence of the black-browed albatross in most years since its initial discovery.

Blood was collected from both putative parents and their chick in 2007 and stored in lysis buffer. The sex of the parents was determined by amplification of the CHD gene, using primers 2550F and 2718R following the protocol of Fridolfsson & Ellegren (1999). Total genomic DNA was extracted using an extraction solution of 10% Chelex® 100 Resin (BioRad), 10 mM Tris, 0.2% SDS and 5 µl of Proteinase K (100 mg/mL). Approximately 20 µl of blood was added to 200 µl of the extraction solution and incubated at 65°C for at least 8 hours followed by boiling for 10 min. Eight microsatellite loci were used to genotype both parents and the chick: 6A3, 11F3, 12H8, 12C8, 10C5, 12E1, 11H1 and 7D8 (Dubois *et al.* 2005). Microsatellite loci were amplified by polymerase chain reaction (PCR). PCRs were multiplexed in 2 subsets of loci (6A3, 11F3, 12H8, 12C8, 10C5, 11H1 and 7D8; 12E1) in a 10 µl final volume, including 0.2 µM of each primer and 1 µl genomic DNA, using the Qiagen multiplex PCR kit (Qiagen). PCRs were conducted using an ABI GeneAmp® PCR System 2700 under the following conditions: 15 min activation of the HotStart *Taq* DNA polymerase at 95°C, 30 cycles of 30 sec of initial denaturation at 94°C, 90 sec annealing (58°C for 6A3, 11F3, 12H8, 12C8, 10C5, 11H1 and 7D8; 54°C for 12E1) and 60 sec extension at 72°C, and then a final extension of 30 min at 60°C. PCR products were combined and electrophoresed on an ABI3730xl using POP7 and a 50 cm capillary using Rox350 (Applied Biosystems) as the standard at the Central DNA Sequencing Facility of the University of Stellenbosch (<http://www.sun.ac.za/saf>). Profiles were analysed using GeneMapper Software version 3 (Applied Biosystems). Genotypes were confirmed by repeating PCRs and electrophoresis for each individual.

CERVUS version 3.0.7 (Marshall *et al.* 1998, Kalinowski *et al.* 2007) was used to assess maternity and paternity. The programme uses a likelihood approach that allows for genotyping error and mutation to estimate the probability of maternity and paternity. Adult grey-headed albatrosses ($n = 200$) sampled from the same colony on Marion Island and screened for the same microsatellite loci (FitzPatrick Institute unpublished data), were used to calculate allele frequencies and to compute simulations. An LOD (logarithm of odds) score as well as a Delta score were calculated for the mother, putative father, and both parents and chick (trio). LOD scores are the sum of the log-likelihood ratios at each locus calculated for each candidate. Delta is a derivative of the LOD score and may be used as an additional measurement. When considering a set of candidates with a positive LOD score, Delta is defined as the difference in LOD scores between the most likely candidate parent

and the second most likely candidate parent. If no candidate has a positive LOD score, then Delta is undefined. Delta is especially useful when several candidates have positive LOD scores (Marshall *et al.* 1998). CERVUS compares the distribution of LOD or Delta scores of the simulation with the actual data. Consequently, confidence in assignment is defined as the proportion of candidate parents with LOD or Delta scores that exceed a given LOD or Delta score that are true parents (Marshall *et al.* 1998, Slate *et al.* 2000).

Results

The vagrant black-browed albatross ringed on Grey-headed Albatross Ridge in the 2000/01 breeding season has been resighted in 12 subsequent years from 2001/02 to 2018/19. However, breeding has only been recorded in four years: 2000/01, 2002/03, 2006/07 and 2008/09. It was only in 2006 that the partner was confirmed to be a grey-headed albatross, when it was also ringed. The egg apparently failed to hatch in three of these attempts; it was only in 2006/07 that a chick hatched (sometime between 26 December and 14 January). The chick was banded in March and fledged in May 2007. The black-browed albatross was seen in the 2007/08 season but did not breed (presumably because its partner was on sabbatical after raising a chick the preceding year). The same pair was found incubating in 2008, but the breeding attempt failed at the egg stage. Since then, the black-browed albatross has been observed loafing in the vicinity of its former breeding site in 2011/12 and each breeding season from 2013/14 to 2018/19. Its partner has not been re-sighted since 2008.

Genetic sexing indicated that the black-browed albatross was female. Unfortunately, despite running the sample from the grey-headed partner several times, we were unable to unequivocally confirm its sex, although one run showed faint amplification suggesting it was female. This raises the possibility of a female-female pairing (cf. Young *et al.* 2008). CERVUS version 3.0.7 analysis of maternity indicated the black-browed albatross to be the mother of the chick (no mismatches between genotypes, LOD of 2.16 and a Delta of 1.86, $P < 0.05$). A CERVUS analysis of paternity showed that although the grey-headed partner had an 80% confidence level of being the father when considered alone (three mismatches between genotypes), when considered with the known mother the trio LOD score was -7.7 (Delta 0.0; four mismatches between genotypes) and the Delta score for the father-chick pair was $< 80\%$ confidence level.

Juveniles of both parental species exhibit considerable variation in head pattern and can be difficult to differentiate (Marchant & Higgins 1990, Brooke 2004), but the fledgling had the grey head typical of a juvenile

grey-headed albatross, and lacked the white face and grey collar characteristic of juvenile black-browed albatrosses, suggesting the possibility that it may not be of hybrid origin. However, in 2018, 11 years later the putative hybrid was photographed in its natal breeding colony and its phenotype was intermediate between the two species (Fig. 1). Its nape was white, shading to pale grey towards the face, and the yellow culminicorn and lower ramicorn were broader than on an adult grey-headed albatross, with a smaller, mottled black region (on the upper ramicorn and sulcus) that petered out towards the gonyx. The peach-orange tip of the maxillary unguis was more extensive and paler than in a grey-headed albatross, resembling that of a black-browed albatross (Fig. 1). A second individual, resembling the hybrid, was seen in the colony in 2017. Although its history is unknown, its locality and hybrid phenotype suggest that the black-browed albatross may have fledged another chick.

Discussion

Although some hybrids among *Phoebastria* albatrosses apparently result from forced copulations (Rohwer *et al.* 2014), our observations suggest that the black-browed albatross formed a mixed species pair with a grey-headed albatross. We can only confirm it was the same partner in two breeding attempts, but it is possible that the same birds were involved in all four breeding attempts. The fact that only one egg hatched might reflect some level of genetic incompatibility between these two individuals; similar apparent incompatibility has been reported among within-species pairs of other albatrosses (e.g. Jones *et al.* 2012). The intermediate adult phenotype of the sole chick raised by this pair confirms its hybrid origin, and together with the microsatellite results, suggests that the



Fig. 1. Black-browed × grey-headed albatross hybrid photographed at its natal colony on Grey-headed Albatross Ridge, Marion Island, 11 years after fledging (photograph by C.W. Jones)

chick resulted from an extra-pair fertilization by another grey-headed albatross.

This is the third record of a mixed-species pairing between black-browed albatrosses and another *Thalassarche* albatross. Pairings between the genetically close (Chambers *et al.* 2009) Campbell and black-browed albatrosses at Campbell Island have resulted in viable F1 hybrids (Moore *et al.* 2001). However, at South Georgia both chicks hatched by a female black-browed albatross and sired by a more distantly related white-capped albatross (Chambers *et al.* 2009) died before fledging (Phillips *et al.* 2018). The grey-headed albatross is more closely related to black-browed/Campbell albatrosses than is the white-capped albatross (Chambers *et al.* 2009), which might explain the production of a hybrid that was able to both fledge and survive to return to the breeding colony a decade later.

Black-browed and grey-headed albatrosses share breeding colonies at South Georgia (Prince & Ricketts 1981), the Crozets (Weimerskirch *et al.* 1986), Campbell Island (Waugh *et al.* 2000) and Macquarie Island (Jaimie Cleeland, personal communication 2018). Their breeding phenology and behaviours, including courtship and aggressive repertoires, are similar (Tickell 1984). Courtship displays have been observed between the two species at South Georgia (Warham 1996), and experimental cross fostering of eggs between the species has shown that each can successfully rear chicks of the other species (Prince & Ricketts 1981). Ours is the first confirmed record of a mixed species pair between the two species (Marchant & Higgins 1990, Tickell 2000). However, at least one suspected black-browed × grey-headed albatross hybrid has been photographed off Australia (http://www.pbase.com/wildlifeimages/port_fairy_albatross). The appearance of the Australian bird(s), photographed in 2008, 2012 and 2017 (https://birdlifephotography.org.au/index.php?option=com_blphtotos&view=show_image&id=25679) is similar to (but not the same as) the hybrids seen on Marion in 2016 and 2018. Hybridization among birds often occurs when a species is out of its normal range and has few if any opportunities to breed with conspecifics (Grant & Grant 1992), as is the case in our study.

Strong mate fidelity is to be expected in albatrosses given their long pair bond formation and low reproductive rates (Moller 2000). However, at Bird Island, South Georgia, rates of extra-pair paternity were 0–9% among black-browed albatrosses and 3–10% among grey-headed albatrosses (Burg & Croxall 2006). Extra-pair copulations insure against male infertility or partner incompatibility and may enable females to produce young while benefiting from the assistance of her social mate (Wink & Dyrce 1999). Further, black-browed albatross typically breed annually whereas grey-headed albatrosses biennially (Prince *et al.* 1994,

Ryan *et al.* 2007) which may hinder the development of a stable pair bond and render such a mixed species pair more prone to extra-pair behaviour. Genetic compatibility in a black-browed and grey-headed albatross pairing may be limited and might explain the failure of the pair's other breeding attempts. Extra-pair fertilisation was possibly the only option for the female black-browed albatross (Gowaty 1996) but she was still constrained to mate with a grey-headed albatross since they are the only *Thalassarche* albatross present at her breeding site on Marion Island.

Albatrosses generally are philopatric to their natal colonies, so the appearance of a black-browed albatross at Marion Island is remarkable. However, occasional long-range dispersal movements do occur (e.g. Inchausti & Weimerskirch 2002), and are relatively common among black-browed albatrosses, as indicated by their recent colonisation at Campbell, Macquarie, Antipodes islands and The Snares (Moore *et al.* 2001) and the famous individual that was observed in gannet colonies in Scotland (Agreement on the Conservation of Albatrosses and Petrels 2010). It is unclear whether this reflects a greater dispersal tendency in this species, or is merely a result of their large population size and wide geographic range (Phillips *et al.* 2016).

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Author contributions

MGWJ, NMST and PGR wrote the manuscript and MMR and CWJ added to its preparation. NMST conducted the genetic analyses. MGWJ, MMR, CWJ, QAH and FT conducted the field work. PGR supervised the research.

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