Rape and the Prevalence of Hybrids in Broadly Sympatric Species: a Test with Albatrosses

Conspecific rape often increases male reproductive success. However, the haste and aggression of forced copulations suggests that males may sometimes rape heterospecific females, thus making rape a likely, but undocumented, source of hybrids between broadly sympatric species. We show heterospecific rape to be the likely source of hybrids between black-footed and Laysan albatrosses (*Phoebastria nigripes*, and *P. immutabilis*, respectively). Extensive field studies have shown that paired (but not unpaired) males of both of these albatross species use rape as a supplemental reproductive strategy. Between species differences in size, timing of laying, and aggressiveness suggest that black-footed albatrosses should be more successful than Laysan albatrosses in heteropspecific rape attempts, and male black-footed albatrosses have been observed attempting to force copulations on female Laysan albatrosses. Nuclear markers showed that the six hybrids we studied were F1s and mitochondrial markers showed that male black-footed albatrosses sired all six hybrids. Long-term gene exchange between these species has been from black-footed albatrosses into Laysan albatrosses, suggesting that the siring asymmetry associated with heterospecific rape has long persisted. Hybrids sired through rape presumably would be raised and sexually imprinted on Laysan albatrosses, and two unmated hybrids in a previous study courted only Laysan albatrosses.

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Introduction

22

23   Unidirectional hybridization is common in nature. A recent review showed that 50 of 80

24 cases involving at least five hybrids, were predominantly unidirectional (Wirtz 1999). From a

25 long list of alternatives, a shortage of mates for females was the only general explanation

26 supported for unidirectional hybridization. Here we test an additional hypothesis for

27 unidirectional hybridization, that hybrids are generated by forced copulations occurring between

28 sympatric species pairs for which the males of at least one of the species commonly engage in

29 conspecific rape (Brown & Brown 1996; McKinney & Evarts 1998).

30   Hybridization usually arises as an epiphenomenon of mating strategies within species

31 (Price 2008). In general, rape supplements male reproductive success when directed toward

32 conspecifics (Shields & Shields 1983; Thornhill 1980; Thornhill & Palmer 2001; Thornhill &

33 Sauer 1991; Thornhill & Thornhill 1983) but the urgent and aggressive nature of rape may result

34 in males sometimes forcing copulations on heterospecific females. While they may be

35 uncommon, hybrids generated by heterospecific rape should be found wherever the parental

36 species breed sympatrically, rather than being confined to zones where the ranges of parapatric

37 species pairs meet and where hybrids are often abundant. While others have suggested

38 heterospecific rape as a source of hybrids (Kabus 2002; McKee & Pyle 2002; Randler 2005), the

39 idea remains controversial, poorly tested, and little supported.

40   We use Laysan and black-footed albatrosses (*Phoebastria immutabilis* and *P. nigripes,*

41 respectively) to test a predicted bias in the sire of F1 hybrids and to evaluate the effects of that

42 bias on long-term gene exchange between these species. Paired males of both species are known

43 to force copulations on conspecific females. If hybrids are sired through heterospecific rape,

44 differences between these albatrosses in behavior and the timing of egg laying (detailed below)

45 suggest that black-footed albatrosses should sire F1 hybrids. It is important that only F1 hybrids

46 are used to evaluate siring biases predicted for heterospecific rape because siring asymmetries

47 will be lost if backcross hybrids are generated through random mating with either parental

48 species. Siring bias in F1 hybrids is easily assessed using mitochondrial DNA (mtDNA) to

49 identify the maternal species.

50 **Study System**

51   Laysan and black-footed albatrosses are closely related sister species (Nunn et al. 1996)

52   that breed sympatrically in the Northwestern Hawaiian Islands. Like other albatrosses, they are

53   long-lived, delay breeding until they five to seven years old, form life-long pair bonds, lay single

54   eggs, and may breed for 20-50 years (Fisher 1969; Fisher 1971; Fisher 1972; Fisher 1975; Fisher

55   1976; Rice & Kenyon 1962).

56   Our samples came from Midway Atoll where over 480,000 pairs of these albatrosses nest

57   and where the beach-nesting black-footed albatross comprises about five percent of the total pairs

58   (E. Flint, personal communication). Although interbreeding between black-footed and Laysan

59   albatrosses is relatively rare, putative hybrids between them have been noted for decades (Fisher

60   1948; Fisher 1971; McKee & Pyle 2002) and up to 20 presumptive hybrids were observed at

61   Midway Atoll between 1997 and 2000 (McKee & Pyle 2002).

62   Mature black-footed albatrosses are primarily dark brown, whereas Laysan albatrosses are

63   largely white on the body and dark grey to black on the wings and back. Presumed hybrids are

64   intermediate between the parental species in plumage and soft part coloration, ranging in from

65   very pale grey to fairly dark, with pale under wings (Fisher 1972; McKee & Pyle 2002). The

66   lightest presumptive hybrids can resemble the darkest Laysan albatrosses in plumage color, but

67   the darkest putative hybrids are not as dark as black-footed albatrosses. Because both Laysan

68   albatrosses and hybrids are variable in coloration, identifying or excluding progeny that might

69   result from backcrosses is not possible based on plumage characteristics alone (McKee & Pyle

70   2002). However, multilocus molecular methods allowed us to assess the existence of hybrids

71   beyond the F1 generation.

72   Conspecific rapes are observed in both Laysan and black-footed albatrosses (Fisher 1971;

73   Fisher 1972), and black-footed albatross males sometimes direct rape attempts at Laysan females,

74   suggesting that hybrids could result from heterospecific rapes (McKee & Pyle 2002). Importantly,

75   differences in the timing of breeding, body size, and aggressiveness all suggest that F1 hybrids

76   would be sired when the larger and more aggressive male black-footed albatrosses force

77   copulations on female Laysan albatrosses. Particularly important is that black-footed albatrosses

78   arrive at the breeding colonies and lay earlier than do Laysan albatrosses (Fisher 1969; Rice &

79   Kenyon 1962), with the result that Laysan females are fertile and vulnerable to insemination

80   through heterospecific rape by black-footed albatross males that are no longer occupied with mate

81   guarding.

82   We evaluated the F1 status of hybrids using fixed and near-fixed differences in their

83   nuclear genome, and we assessed siring bias using mtDNA from the hybrids. We also used an

84   isolation-migration (IM) model to assess asymmetries in long-term gene flow between these

85   species since their divergence approximately 1.03 million years ago (Nunn et al. 1996).

86   **Materials and Methods**

87   *Sampling*

88   Blood was sampled from 29 breeding black-footed albatrosses, 28 Laysan albatrosses, and

89   six presumed hybrids (morphologically intermediate between the two species in plumage

90   coloration) at Midway Atoll National Wildlife Refuge (28°13' N, 177°22' W). Genomic DNA

91   was extracted from blood samples either by a standard phenol:chloroform procedure (Sambrook

92   et al. 1989) or using the Wizard SV Genomic DNA Purification System (Promega). All work was

93   conducted in accordance with University of Washington Institutional Animal Care and Use

94   Committee (protocol 2846-13).

95   *Molecular methods*

96   To assess gene flow between the parental species, we collected DNA sequence data for

97   eight anonymous nuclear loci, one coding nuclear locus (a fragment of a Major

98   Histocompatibility Complex (MHC) gene (Walsh & Edwards 2005), and the mtDNA *cytochrome-*

99   *b* (*cyt-b*) locus . Anonymous loci were derived from a fosmid library for black-footed albatross

100   (Table 1). "FWD" and "REV" designations indicate loci that were taken from opposite ends of a

101   fosmid insert, and therefore are separated by ~ 35 kb in the genome. We followed standard

102   protocol to amplify and purify PCR products.

103   We assigned quality scores to base calls in sequence trace files using Phred (Ewing &

104   Green 1998; Ewing et al. 1998) and aligned homologous sequences using Phrap (Green 1994).

105   Polymorphic sites were identified using the program PolyPhred (Nickerson et al. 1997).

106   Assemblies were visualized in Consed (Gordon et al. 1998) and single nucleotide polymorphisms

107   (SNPs) and genotypes at each locus were confirmed by eye. Nuclear haplotypes were resolved

108   using PHASE v.2.1.1 (Stephens & Donnelly 2003; Stephens et al. 2001). All sequences have been

109   deposited in GenBank (accession numbers KF475302-KF475698).

110   Putative hybrids were sexed using primers 2550F and 2718R (Fridolfsson & Ellegren

111   1999); sex was scored by eye, with two bands indicating female and a single-band indicating

112   male. Sexing the hybrids enabled us to assess whether hybrid females (the heterogametic sex in

113   birds) were inviable, which could be expected under Haldane's rule (Haldane 1922)..

114   *Hybrid Identification*

115   We computed two hybrid indices, both varying from from 0 (pure Laysan albatrosses) to 1

116   (pure black-footed albatrosses). The first is most intuitive and includes only loci that

117   demonstrated diagnostic sequence differences between black-footed and Laysan albatrosses.

118   Using these same loci, we also computed the probability that the six phenotypically intermediate

119   specimens were first generation (F1) hybrids or backcrosses. For the second hybrid index we used

120   maximum likelihood in the introgress package implemented in R (Gompert & Buerkle 2009), and

121   included all of the nuclear loci.

122   Siring asymmetries for the hybrids were assessed using a binomial test on mtDNA data.

123   *Migration Estimation*

124   To assess effective population sizes and the rate and direction of gene flow between black-

125   footed and Laysan albatrosses, we used the IM model implemented in IMa2 (Hey & Nielsen

126   2004). We applied the HKY mutation model of nucleotide substitution and nuclear mutation rate

127   scalars were free to vary in the model. The nuclear and mitochondrial genes were assigned an

128   inheritance scalar of 1.0 and 0.25, respectively. To avoid violating the assumptions of no

129   recombination and neutrality of markers, we tested for within locus recombination using the four-

130   gamete test (Hudson & Kaplan 1985) for each locus and within each species; we tested neutrality

131   of markers using Tajima's D implemented in R package PEGAS (Paradis 2010) (Table 1).

132   We ran 12 replicate IMa2 analyses, each using different starting seeds and 40-50

133   concurrent chains, for 10-50 million steps after an initial burn-in phase of 50,000-100,000

134   generations. To rescale estimates of population size and migration parameter into demographic

135   units, we used the geometric mean of previous mtDNA rate estimates for albatrosses of 3×10^{-5}

136   substitutions per locus per year for our fragment of *cyt-b*; (Nunn & Stanley 1998), and a

137   generation time of 25 years (Cousins & Cooper 2000). We evaluated a total of 25 nested models

138   of the full migration model (all migration rates and all population sizes estimated as different

139   parameters). Models of population divergence were compared using likelihood ratio tests and

140   ranked based on the information-theoretic criterion (Carstens et al. 2009).

141   **Results**

142   *Hybrid Indices and probability of hybrid genotypes*

143   All six putative hybrids were heterozygous at five diagnostic nuclear SNPs. (Table 1).

144   Using just these diagnostic loci the hybrid index for a true F1 hybrid is expected to be 0.51

145   because Laysan albatrosses share in low frequency (10%) a single diagnostic SNP that is fixed in

146   black-footed albatrosses (Fig. 1).

147   In Table 2 we use the observed population allele frequencies to calculate the probability of

148   producing the genotype found in all six hybrids, under the assumption that they were either F1

149   hybrids or first generation backcrosses. The probability of producing the observed hybrid

150   genotype was 0.90 for a parental cross. The probability that the hybrid genotype resulted from a

151   backcross to either of the parental species varies by the sex of the hybrid and the sex and species

152   of the backcross parent. Because all hybrids carried Laysan mtDNA haplotypes, the probability

153   of a backcross to a female Black-footed albatross is 0. For the three other backcross

154   combinations, the probability of observing the hybrid genotype is either 0.028 or 0.034 (Table 2).

155   These calculations, based on the five diagnostic SNPs show that the six hybrids are almost

156   certainly F1s, and not backcross individuals.

157   We also evaluated the status of the six hybrids using a maximum likelihood estimator

158   (Gompert & Buerkle 2010), including in this analysis the four nuclear SNPs that were not

159   diagnostic (Table 1). All six hybrids received a score of 0.56, with a 95% confidence interval of

160    $0.22 - 0.85$.

161   *Siring bias and sex for the hybrids*

162   All six hybrids carried the Laysan albatross mtDNA haplotype, indicating that F1 hybrids

163   result from male black-footed albatrosses inseminating female Laysan albatrosses ($p = 0.031$).

164   Three of the hybrids were male and three were female, suggesting no inviability of the

165   heterogametic sex (Haldane 1922).

166   *Gene flow*

167   Tajima's D values showed no significant deviation from neutrality for any of the loci

168   examined (Table 1) and no evidence of recombination within loci was found.

169   Under the IM model, the rate of gene flow was significantly higher from black-footed

170   albatrosses into Laysan albatrosses (p=0.028). The mean rate of gene flow ($2Nm$) was 0.09 gene

171   copies per generation from black-footed albatross into Laysan albatross (95% HPD 0.024 - 0.23),

172   whereas this rate was zero in the reverse direction (95% HPD 0 - 0.10). Migration from Laysan to

173   black-footed albatross was constrained to zero in the top four models, which, together, account for

174   55% of the variation in the weighted AIC models (Table 3).

175   **Discussion**

176   Using diagnostic nuclear loci, we show that all six presumed hybrids between Laysan and

177   black-footed albatross were F1 hybrids. All six carried Laysan mtDNA haplotypes, indicating that

178   male black-footed albatrosses were their sires. This contradicts the hypothesis that a scarcity of

179   mates for females of the rare species results in hybrid pairings (Wirtz 1999) because all six

180   hybrids had Laysan albatross mothers, instead of mothers of the much less abundant black-footed

181   albatross. Finally, we found limited, but significant gene flow from black-footed albatrosses into

182   Laysan albatrosses, suggesting that past F1 hybrids have backcrossed to Laysan albatrosses. As

183   we discuss below, this further supports to our hypothesis that forced copulations are

184   asymmetrical.

185   *Effects of Phenology and Behavior on Insemination Biases*

186   Black-footed albatrosses lay 10 days to two weeks earlier than Laysan albatrosses (Fisher

187   1969; Rice & Kenyon 1962), so most female black-footed albatross have begun incubating when

188   Laysan females are fertile. This difference in breeding schedules undoubtedly contributes strongly

189   to the asymmetry in inseminations that generate hybrids because only paired males have been

190   reported to engage in rape attempts in these albatrosses (Fisher 1971; McKee & Pyle 2002).

191   Unmated males spend their time at breeding colonies courting females and have not been

192   observed attempting rapes (Fisher 1971). Other factors may also contribute to the observed siring

193   asymmetry. Notably, female Laysan albatrosses are 5-10% smaller than male black-footed

194   albatrosses (Dunning 2007), and male black-footed albatrosses are much more aggressive in

195   conspecific rape attempts than are male Laysan albatrosses (Fisher 1972). Finally, because black-

196   footed albatrosses constitute only 5% of the population of these two species breeding at Midway

197   Atoll, they have far more opportunity to engage in forced heterospecific copulations than do

198   Laysan albatrosses. These differences suggest that male black-footed albatrosses are more likely

199   to sire hybrids through rapes, and all reported heterospecific rape attempts have been involved

200   male black-footed albatrosses and female Laysan albatrosses (McKee & Pyle 2002).

201   The asymmetry in gene exchange revealed by the isolation-migration model suggests a

202   long history of unidirectional gene flow from Black-footed Albatrosses into Laysan Albatrosses.

203   Although modern hybrids appear to have no success in attracting mates (Fisher 1972; McKee &

204   Pyle 2002; Rice & Kenyon 1962), two carefully observed hybrids (unsuccessfully) addressed all

205   courtship attempts at Laysan albatrosses (Fisher 1972). Hybrids sired by male black-footed

206   albatrosses raping female Laysan albatrosses would be raised by and sexually imprinted on

207   Laysan albatrosses (Slagsvold et al. 2002; ten Cate & Vos 1999); further, courting individuals of

208   both species spend a great deal of time focusing on the breast color of their dance partners (Fisher

209   1972). Thus, when plumage differences between these species were likely less dramatic, F1

210   hybrids, generated by heterospecific forced copulations, may have resembled the darkest Laysan

211   albatrosses well enough to pair and breed with them in the past.

212   *Alternative Explanations for Asymmetric Gene Flow*

213   We can think of two alternatives to our hypothesis of heterospecific rape as the cause of

214   the observed asymmetry in gene flow between black-footed and Laysan albatrosses. First, is the

215   possibility that F1 backcrosses into the black-footed albatross population have not been viable.

216   Definitively addressing this alternative this would require breeding experiments, but the

217   observation that hybrids court only Laysan albatrosses renders this alternative moot (Fisher 1971).

218   Second, if hybrids were intermediate in their breeding schedule relative to the parental

219   species, then hybrids may have had greater opportunity to mate with Laysan albatrosses, which

220   return later to the breeding colonies than black-footed albatrosses. However, this explanation

221   assumes that hybrids form their life-long pair-bounds and breed the first year that they return to

222   their breeding islands. Instead, pre-breeding Laysan albatrosses typically spend one or two years

223   choosing mates (Fisher 1972), making the two-week difference in laying dates unlikely to bias the

224   pattern of backcross matings toward Laysan albatrosses.

225   It seems most likely to us that the gene flow revealed by the IM analysis reflects gene

226   exchange that took place as the species were diverging in coloration. This is supported by the fact

227   that courting birds focus their attention of the breasts of their dance partners, where the two

228   species differ most in color (Fisher 1972), and by the failure of field workers to find any hybrids

229   that were paired (Fisher 1972; McKee & Pyle 2002).

230   *Tests with other groups*

231   The contrast between species in which conspecific Extra-Pair Copulations (EPC) are

232   forced, as opposed to species in which females accept or solicit such copulations, is critical to our

233   thesis that hybrids between broadly sympatric species will be more common in groups where

234   forced copulations are frequent. Although EPC are common in many passerines, they are mostly

235   unforced and apparently controlled by females to increase the genetic quality of offspring (Dunn

236   & Cockburn 1998; Dunn & Cockburn 1999; Spottiswoode & Møller 2004; Stutchbury & Neudorf

237   1998). Unfortunately, whether EPC are forced or accepted is rarely described in the literature

238   (although there are good descriptions of rape in albatrosses, waterfowl, bee-eaters, swallows and

239   the New Zealand hihi, *Notiomystis cincta* (Brekke et al. 2013; Emlen & Wrege 1986; Kabus 2002;

240   Martin 1980). Improved behavioral descriptions of whether or not conspecific EPC are forced are

241   required to predict whether heterospecific rape can be a source of hybrids in most groups.

242   Naturally occurring hybrids are abundant in waterfowl (Randler 1998; Randler 2008) and

243   male ducks are known to direct rape attempts at females of other species (Muñez-Fuentes et al.

244   2007; Randler 2002; Seymour 1990). However, we could find no genetic assessments of

245   insemination biases in the generation of hybrids between naturally sympatric waterfowl. An

246   obvious test would be to compare insemination bias when one parental species is characterized by

247   forced copulations and the other is not. For example, hybrids between northern shovelers (*Anas*

248   *clypeata)* and both mallards (*A. platyrynchos)* and pintails (*A. acuta)* are reported from North

249   America and Eurasia (McCarthy 2006). Because shoveler males are territorial, and seldom

250   attempt conspecific rapes, F1 hybrids should be sired by mallards or pintails (McKinney & Evarts

251   1998). Siring bias can also be predicted for the abundant hybrids between common pochards and

252   tufted ducks (*Aythya ferrina* x *A. fuligula,* respectively) (Randler 2008). Because conspecific rape

253   is unreported in common pochards but frequent in tufted ducks (McKinney & Evarts 1998), F1

254   hybrids should be sired by tufted ducks if they were produced through heterospecific rape.

255   Heterospecific rape probably accounts for the frequent hybrids reported between barn

256   swallows and house martins in Europe and between barn swallows and cliff swallows

257   (*Petrochelidon pyrrhonota*) in North America. Barn swallows are characterized by many EPCs,

258   but females choose whether or not to accept these EPCs, and they are almost never forced (Møller

259   1994). In contrast, conspecific rape is frequently observed in both cliff swallows and house

260   martins at communal mud-gathering sites (Brown & Brown 1996; Møller 1994). That male cliff

261   swallows and house martins are characterized by conspecific rape, presumably, renders female

262   barn swallows vulnerable to heterospecific rape when they gather mud at sites frequented by

263   males of these two species. When identified as nestlings, hybrids between barn swallows and

264   house martins were always found in barn swallow nests, had barn swallow siblings, and had two

265   barn swallow parents; similarly, nestling hybrids between barn swallows and cliff swallows or

266   cave swallows (*P. fulva*) were found, in all cases but one, in barn swallow nests, attended by two

267   barn swallow parents (Martin 1980). These are likely F1 hybrids sired through heterospecific

268   rapes.

269   *Broader implications*

270   Two comparative studies have addressed the role of EPC in the generation of avian

271   hybrids. In a survey of open nesting birds Randler (2006) found EPC to be uncorrelated with the

272   production of hybrids; however, this study failed to distinguish forced and unforced extra pair

273   copulations and failed to consider whether hybrids were rare or common. In another study

274   Randler (2005) assessed the roles of forced EPC and brood amalgamation on the production of

275   hybrid waterfowl, and found a significant effect only of brood amalgamation when both factors

276   were included in the model. However, both causal variables were treated as binary characters,

277   which masks their importance in species pairs where either factor causes the production of many

278   hybrids. For example, over 800 common pochard x tufted duck hybrids have been reported from

279   Europe (Randler 2008), yet these were treated as equivalent to a single report of a natural hybrid

280   between other species pairs. Testing for siring asymmetries in F1 hybrid waterfowl would

281   generate a stronger test of the heterospecific rape hypothesis.

282   Among *Anas* ducks gene sharing through hybridization apparently has strongly affected

283   effective population sizes. For pintails and green-winged teal, census population sizes are too

284   small for certain shared alleles to have persisted for more than 2 and 2.6 million years. But these

285 alleles, which are shared with mallards, are estimated to have persisted for 6.2 and 7.9 million

286 years, respectively, suggesting a long history of horizontal gene exchange with mallards, which

287 have a much larger effective population size (Kraus et al. 2012). Heterospecific rapes are likely

288 responsible for generating F1 hybrids between these ducks and, unlike the situation in albatrosses,

289 F1 hybrid females in these short-lived ducks probably do form pair-bonds and breed. Hybrid

290 female ducks should be sexually imprinted on the species that raised them (ten Cate & Vos 1999)

291 and the strong male bias in the breeding sex ratios of north temperate ducks should facilitate

292 pairing and breeding by hybrid females.

293 **Conclusion**

294   Although unidirectional hybridization often predominates in nature, only a shortage of

295 mates for females previously had emerged from a long list of alternative hypotheses as a general

296 explanation for asymmetric hybridization (Wirtz 1999). This case study of hybrid albatrosses

297 makes the general point that, when hybrids result from heterospecific rape, differences in

298 behavior and life history of the parental species can be used to predict the direction of crosses.

299 Predicting the mother and father species more rigorously tests the suggestion that heterospecific

300 rape may be an important source of hybrids (McKee & Pyle 2002; Møller 1994; Randler 2005).

301 Although heterospecific rape is unlikely to be adaptive, it has the potential to explain differences

302 in the prevalence of F1 hybrids between broadly sympatric species according to whether or not

303 they are characterized by conspecific forced copulation.

304   Several authors have suggested that heterospecific rape may be a source of avian hybrids

305 (McKee & Pyle 2002; Møller 1994; Randler 2005), but this study of albatrosses apparently

306 represents the first critical evaluation of the idea for any bird. Forced copulations have been

307 reported for various insects (Arnqvist 1989; Thornhill 1980; Thornhill & Sauer 1991), fish

308 (Valero et al. 2008), lizards (Cooper 1985; Olsson 1995; Rodda 1992) and mammals (Harris et al.

309 2010), but whether or not forced copulations generate hybrids in these groups has not yet been

310 addressed.

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Figure 1

Hybrid scores based on the five diagnostic SNPS (Table 1), with pure black-footed albatrosses scored as 0 and pure Laysan albatrosses scored as 1.

The six putative hybrids all scored as 0.51, rather than 0.50, because Laysan albatrosses share a rare allele with Black-footed albatrosses at one of our diagnostic loci.
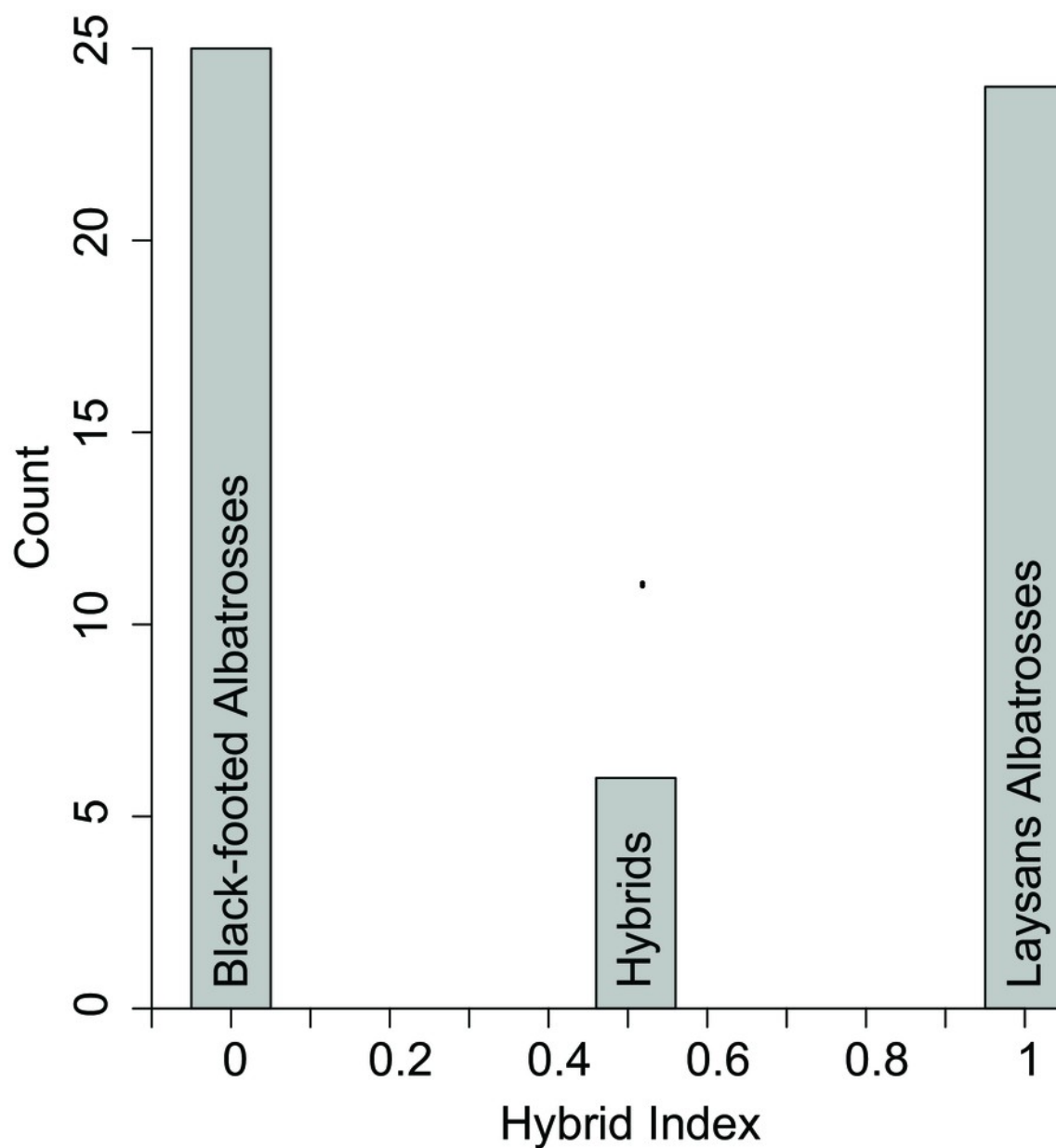
Table 1

Probabilities of F1 and backcross hybrids carrying the observed hybrid genotype.

All six hybrids carried genotype (LA)(A/G)(A/C)(CAG/TGC)(C/T)(A/C); observed allele frequencies are listed in parentheses for the mitochondrial gene and the diagnostic SNPs (dSNP). The fixed mitochondrial differences render some parental combinations impossible. The shared polymorphism at dSNP 2 makes it possible that the observed hybrid genotype derives from backcrossing, albeit at very low probabilities (<0.05). Abbreviations: LA, Laysan albatross; BF black-footed albatross; f, female; m, male.

F1 genotype

F1 combinations	mtDNA	dSNP 1	dSNP 2	dSNP 3,4,5	dSNP 6	dSNP 7	Probability
LA f x BF m	LA (1.0)	A/G (1.00)	A/C (0.90) C/C (0.10)[1]	CAG/TGC (1.0)	C/T (1.0)	A/C (1.0)	0.90
LA m x BF f	BF (1.0)	A/G (1.00)	A/C (0.90) C/C (0.10)[1]	CAG/TGC (1.0)	C/T (1.0)	A/C (1.0)	0.00[2]

Backcross genotype

Backcross combinations	mtDNA	dSNP 1	dSNP 2	dSNP 3,4,5	dSNP 6	dSNP 7	Probability
F1 f x BF m	LA (1.0)	A/G (0.50) G/G (0.50)	A/C (0.45) C/C (0.55)	CAG/TGC (0.5) TGC/TGC (0.5)	C/T (0.5) T/T (0.5)	A/C (0.5) C/C (0.5)	0.028
F1 f x LA m	LA (1.0)	A/A (0.50) G/A (0.50)	A/A (0.405) C/A (0.540) C/C (0.055)	CAG/CAG (0.5) TGC/CAG (0.5)	C/C (0.5) T/C (0.5)	A/A (0.50) C/A (0.50)	0.034
F1 m x LA f	LA (1.0)	A/A (0.50) G/A (0.50)	A/A (0.405) C/A (0.540) C/C (0.055)	CAG/CAG (0.5) TGC/CAG (0.5)	C/C (0.5) T/C (0.5)	A/A (0.50) C/A (0.50)	0.034
F1 m x BF f	BF (1.0)	A/G (0.50) G/G (0.50)	A/C (0.45) C/C (0.55)	CAG/TGC (0.5) TGC/TGC	C/T (0.5) T/T (0.5)	A/C (0.5) C/C (0.5)	0.00[2]

(0.5)

[1]None of the six hybrids showed this genotype.

[2]Probability is 0 due to the absence of BF mitochondrial haplotype in the observed hybrid genotype.

Table 2(on next page)

AIC ranking of the six best models using IMa2 based on ~ 300,000 sampled genealogies.

Models, subscripts of population size (q), and migration (m) parameters identify populations used in the analysis; 0, 1, and 2 represent the estimated population sizes for black-footed albatrosses, Laysan albatrosses, and the ancestral population, respectively. In each model brackets denote fixed parameters; other parameters were estimated.

Model	log(P)	k	AIC	∆AIC	ω	q0	q1	q2	m0>1	m1>0
8	2.48	3	1.04	0.00	0.16	0.24	[q0]	0.01	[0]	0.22
3	3.39	4	1.22	0.18	0.15	0.22	0.07	0.00	[0]	2.56
13	2.16	3	1.68	0.64	0.12	0.30	0.11	[q0]	[0]	1.82
18	2.16	3	1.69	0.65	0.12	0.30	0.11	[q1]	[0]	1.76
2	2.99	4	2.03	0.99	0.10	0.25	0.13	0.00	0.20	[m0>1]
6	2.48	4	3.04	2.00	0.06	0.24	[q0]	0.01	0	0.22

Table 3(on next page)

Primer and locus information. Diagnostic nuclear loci (dSNP) that provided at least a 90% probability of distinguishing between the parental species are starred.

Tajima's D of NA indicates no variation occurring at that locus; BF = black-footed albatross, LA = Laysan albatross.

Locus	dSNP	Forward Primer (5'-3')	Reverse Primer (5'-3')	Length (bp)	%GC	% Identical Sites	D BF	D LA
cyt-b	–	–	–	609	48	97.7	-1.51	NA
MHC*	1	–	–	571	63.5	99.1	-0.22	-1.28
1FWD*	2	GTGCCACCCATGTAACACCT	TGTGCTTTGGATGAACAGTTG	429	55	99.5	NA	-0.26
1REV*	3,4,5	ACTGTGTCACCCCATGCTC	CTGAGTCATTTCCATTCCTGG	407	58.7	99.0	-0.87	NA
4FWD*	6	TGGGCCAGGTTGTTAGGTAG	TATTGGTGGAATGGGCTTGT	464	34.3	99.4	-1.16	NA
4REV*	7	GGCTGGGGGTTTGGAATTA	CTTTCTACAGAGAAATAAACAAAGACC	443	36.9	99.5	-0.24	NA
6FWD	–	AGGGGTCTCTCAAACAGCAA	CTGGCCCTTTAGATAATAGCC	418	35.8	99.8	1.53	NA
6REV	–	GAAGCGTAGTGAAGTATAACATCGTG	ATGCTGAGGGTGCCATCTTA	458	39.5	98.9	0.47	-1.76
10FWD	–	GGCATAGGCTAAAGGCAAAG	TCAGAATTATTATAGCTTCAGGTGAG	548	43.4	99.6	NA	0.06
10REV	–	GGTGTTAGAACAGAAAGTCT	TTACCACCTTCCACCACACA	495	36.2	99.6	0.87	NA