Rape and the Prevalence of Hybrids in Broadly Sympatric Species: a Case Study using 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 source of hybrids between broadly sympatric species. We show heterospecific rape to be the probable 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.

2   Sievert Rohwer[1,2], Rebecca B. Harris[1,3] and Hollie E. Walsh[1,4],

5   [1]Department of Biology and Burke Museum of Natural History, University of Washington,

6   Seattle, WA, 98195-1800 USA

7   [2] *Corresponding author:* rohwer@uw.edu

8   [3]rbharris@uw.edu

9   [4]hwalsh@uw.edu

10   Keywords: *Phoebastria*, hybridization, gene flow, Isolation with Migration, heterospecific rape,

11   forced copulation

14   Running title: Rape and hybridization

15   Corresponding author:

16   Sievert Rohwer
17   Department of Biology
18   University of Washington
19   Seattle, WA 98195-1800 USA
20   Phione: 206-543-4066
21   Email: rohwer@uw.edu

Introduction

22 **Introduction**

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 suggest that predicting siring asymmetries

27 offers a strong way to evaluate the importance of heterospecific rape as a source of hybrids

28 between broadly sympatric species. Although this hypothesis seems likely to apply strongly to

29 waterfowl, where wild hybrids are more frequently reported than for any other group of birds

30 (Grant & Grant 1992; Randler 2008), we illustrate our predictions using hybrids between Laysan

31 and Black-footed albatrosses for which we had genetic samples.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

51 identify the maternal species.

Study System

Laysan and black-footed albatrosses are closely related sister species (Nunn et al. 1996) that breed sympatrically in the Northwestern Hawaiian Islands. Like other albatrosses, they are long-lived, delay breeding until they five to seven years old, form life-long pair bonds, lay single eggs, and may breed for 20-50 years (Fisher 1969; Fisher 1971; Fisher 1972; Fisher 1975; Fisher 1976; Rice & Kenyon 1962).

Our samples came from Midway Atoll where over 480,000 pairs of these albatrosses nest and where the beach-nesting black-footed albatross comprises about five percent of the total pairs (E. Flint, personal communication). Although interbreeding between black-footed and Laysan albatrosses is relatively rare, putative hybrids between them have been noted for decades (Fisher 1948; Fisher 1971; McKee & Pyle 2002) and up to 20 presumptive hybrids were observed at Midway Atoll between 1997 and 2000 (McKee & Pyle 2002).

Mature black-footed albatrosses are primarily dark brown, whereas Laysan albatrosses are largely white on the body and dark grey to black on the wings and back. Presumed hybrids are intermediate between the parental species in plumage and soft part coloration, ranging in plumage from very pale grey to fairly dark, with pale under wings (Fisher 1972; McKee & Pyle 2002). The lightest presumptive hybrids can resemble the darkest Laysan albatrosses in plumage color, but the darkest putative hybrids are not as dark as black-footed albatrosses. Because both Laysan albatrosses and hybrids are variable in coloration, identifying or excluding progeny that might result from backcrosses is not possible based on plumage characteristics alone (McKee & Pyle 2002). However, calculating the hybrid index for multiple nuclear loci allowed us to assess the existence of hybrids beyond the F1 generation.

Conspecific rapes are observed in both Laysan and black-footed albatrosses (Fisher 1971; Fisher 1972), and black-footed albatross males sometimes direct rape attempts at Laysan females, suggesting that hybrids could result from heterospecific rapes (McKee & Pyle 2002). In Laysan Albatrosses conspecific rape is very aggressive, often carried out by groups of males, and sometimes results in serious injury of the female (Fisher 1971). Multiple males regularly join these rape attempts, mounting other males until the pile topples over. Given that albatrosses lack the explosive penis that facilitates forced copulation by male waterfowl (Brennan et al. 2005), it seems likely that females may sometimes evert their cloaca to receive sperm just to prevent further harassment and injury by attacking males; however, we should note that Fisher 1971 found

83   no evidence of sperm transfer in albatrosses he examined closely following attacks by males.

84   Fisher (1971) further reports no attempt by the female's mate to defend her from harassing males,

85   as does occur in waterfowl (McKinney & Evarts 1998). Although Fisher (1972) reports failing to

86   observe interspecific rape attempts, McKee & Pyle (2002) observed male black-footed albatrosses

87   attempting to rape female Laysan albatrosses and believed these events to be the source of

88   hybrids. Neither Fisher (1972) nor McKee & Pyle (2002) observed mixed pairs.

89   Importantly, differences in the timing of breeding, body size, and aggressiveness all

90   suggest that F1 hybrids are sired when the larger and more aggressive male black-footed

91   albatrosses force copulations on female Laysan albatrosses. Particularly important is that black-

92   footed albatrosses arrive at the breeding colonies and lay earlier than do Laysan albatrosses

93   (Fisher 1969; Rice & Kenyon 1962), with the result that Laysan females are fertile and vulnerable

94   to insemination through heterospecific rape by black-footed albatross males whose females are

95   incubating.

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

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

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

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

100   **Materials and Methods**

101   *Sampling*

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

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

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

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

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

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

108   Committee (protocol 2846-13).

109   *Molecular methods*

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

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

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

113 *b* (*cyt-b*) locus (Bretagnolle et al. 1998; Nunn et al. 1996). Anonymous loci were derived from a

114 fosmid library for black-footed albatross (Table 1). "FWD" and "REV" designations indicate loci

115 that were taken from opposite ends of a fosmid insert, and therefore are separated by ~ 35 kb in

116 the genome. We followed standard protocol to amplify and purify PCR products.

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

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

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

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

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

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

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

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

125 1999); sex was scored by eye, with two bands indicating female and a single-band indicating

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

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

128 *Hybrid Identification*

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

130 (pure black-footed albatrosses). The first is most intuitive and includes only loci with fixed or

131 near-fixed sequence differences between black-footed and Laysan albatrosses (Table 1). Using

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

133 specimens were first generation (F1) hybrids or backcrosses (Table 2). For the second hybrid

134 index we used maximum likelihood in the introgress package implemented in R (Gompert &

135 Buerkle 2009), and included all of the nuclear loci.

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

137 *Migration Estimation*

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

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

140 2004). We applied the HKY mutation model of nucleotide substitution and nuclear mutation rate

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

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

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

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

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

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

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

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

149 units, we used the geometric mean of previous mtDNA rate estimates for albatrosses of $3x10^{-5}$

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

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

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

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

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

155 **Results**

156 *Hybrid Indices and probability of hybrid genotypes*

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

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

159 because Laysan albatrosses share in low frequency (10%) a single diagnostic SNP (dSNP2 in

160 Tables 1) that is fixed in black-footed albatrosses (Fig. 1).

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

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

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

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

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

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

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

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

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

170 certainly F1s and not backcross individuals.

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

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

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

174   0.22 – 0.85.

175   *Siring bias and sex for the hybrids*

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

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

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

179   heterogametic sex (Haldane 1922).

180   *Gene flow*

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

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

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

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

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

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

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

188   55% of the variation in the weighted AIC models (Table 3).

189   **Discussion**

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

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

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

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

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

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

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

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

198   asymmetrical.

199   *Effects of Phenology and Behavior on Insemination Biases*

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

201   1969; Rice & Kenyon 1962), so most female black-footed albatross have begun incubating when

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

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

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

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

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

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

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

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

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

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

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

213   to sire hybrids through rapes, and all reported heterospecific rape attempts have involved male

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

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

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

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

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

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

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

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

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

223   1972). Thus, when plumage differences between these species were likely less dramatic, F1

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

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

226   *Alternative Explanations for Asymmetric Gene Flow*

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

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

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

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

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

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

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

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

235   untenably assumes that hybrids form life-long pair-bounds and breed the first year that they return

236   to their breeding islands. Instead, pre-breeding Laysan albatrosses typically spend one or two

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

238   bias the pattern of backcross matings toward Laysan albatrosses.

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

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

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

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

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

244   *Tests with other groups*

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

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

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

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

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

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

251   1998). Unfortunately, whether EPC are forced or accepted is rarely described in the literature

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

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

254   2002; Martin 1980). Improved behavioral descriptions of whether or not conspecific EPC are

255   forced are required to predict whether heterospecific rape can be a source of hybrids in most

256   groups.

257   Naturally occurring hybrids are abundant in waterfowl (Grant & Grant 1992; Randler
258   1998; Randler 2008) and male ducks are known to direct rape attempts at females of other species
259   (Muñez-Fuentes et al. 2007; Randler 2002; Seymour 1990). However, we could find no genetic
260   assessments of insemination biases in the generation of hybrids between naturally sympatric
261   waterfowl. An obvious test would be to compare insemination bias when one parental species is
262   characterized by forced copulations and the other is not. For example, hybrids between northern
263   shovelers (*Anas clypeata)* and both mallards (*A. platyrynchos)* and pintails (*A. acuta)* are reported
264   from North America and Eurasia (McCarthy 2006). Because shoveler males are territorial, and
265   seldom attempt conspecific rapes, F1 hybrids should be sired by mallards or pintails (McKinney
266   & Evarts 1998). Siring bias can also be predicted for the abundant hybrids between common
267   pochards and tufted ducks (*Aythya ferrina* x *A. fuligula,* respectively) (Randler 2008). Because
268   conspecific rape is unreported in common pochards but frequent in tufted ducks (McKinney &
269   Evarts 1998), F1 hybrids should be sired by tufted ducks if they were produced through
270   heterospecific rape. Heterospecific rape probably accounts for the frequent hybrids reported
271   between barn swallows and house martins in Europe and between barn swallows and cliff
272   swallows (*Petrochelidon pyrrhonota*) in North America. Barn swallows are characterized by
273   many EPCs, but females choose whether or not to accept these EPCs, and they are almost never
274   forced (Møller 1994). In contrast, conspecific rape is frequently observed in both cliff swallows
275   and house martins at communal mud-gathering sites (Brown & Brown 1996; Møller 1994). That
276   male cliff swallows and house martins are characterized by conspecific rape, presumably, renders
277   female barn swallows vulnerable to heterospecific rape when they gather mud at sites frequented
278   by males of these two species. When identified as nestlings, hybrids between barn swallows and
279   house martins were always found in barn swallow nests, had barn swallow siblings, and had two
280   barn swallow parents; similarly, nestling hybrids between barn swallows and cliff swallows or
281   cave swallows (*P. fulva*) were found, in all cases but one, in barn swallow nests, attended by two
282   barn swallow parents (Martin 1980). These are likely F1 hybrids sired through heterospecific
283   rapes.

284   *Broader implications*
285   Two comparative studies have addressed the role of EPC in the generation of avian
286   hybrids. In a survey of open nesting birds Randler (2006) found EPC to be uncorrelated with the

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

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

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

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

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

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

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

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

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

296 generate a stronger test of the heterospecific rape hypothesis.

297   In some cases evaluating siring asymmetries can generate tests of the hypothesis that

298 heterospecific brood parasitism results in ducks forming heterospecific pair bonds (Randler

299 2005). For example, redheads (*Aythys americana*) are facultative brood parasites of canvasbacks

300 (*Aythya valisineria*), whereas canvasbacks do not parasitize redhead nests (Sorenson et al. 2010).

301 Redhead ducklings raised by canvasback females in broods of mostly canvasback ducklings

302 should be sexually imprinted on canvasbacks and, therefore, be more willing to pair or at least

303 mate with canvasbacks (Sorenson et al. 2010). An excess of adult males in both species predicts

304 the siring bias: Female redheads imprinted on canvasbacks (Sorenson et al. 2010), should be able

305 to attract unmated male canvasbacks as mates. In contrast, male redheads imprinted on

306 canvasbacks would be unlikely to attract canvasback mates because canvasback females have

307 many unmated males to choose from. Thus canvasback males should sire F1 hybrids between

308 these species. In contrast, Barrows (*Bucephala islandica*) and common (*B. clangula*) goldeneyes

309 parasitize each other, so males of both species are expected to be sires of hybrids. Although rare,

310 hybrids between both of these species pairs are regularly reported (McCarthy 2006), and none of

311 the parentals is characterized by conspecific rape.

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

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

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

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

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

317 have a much larger effective population size (Kraus et al. 2012). Heterospecific rapes may be

318 responsible for generating F1 hybrids between these ducks and, unlike long-lived albatrosses, F1

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

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

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

322 breeding by hybrid females.

323 **Conclusion**

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

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

326 explanation for asymmetric hybridization (Wirtz 1999). In this case study of hybrid albatrosses,

327 we attempt to make the general point that, when hybrids result from heterospecific rape,

328 differences in behavior and life history of the parental species can be used to predict the direction

329 of crosses. Predicting the mother and father species of F1 hybrids from different parental

330 combinations has the potential to considerably refine our understanding of the importance of

331 heterospecific forced copulation (and brood parasitism), in the generation of hybrids (McKee &

332 Pyle 2002; Møller 1994; Randler 2005). Although heterospecific rape is unlikely to be adaptive, it

333 has the potential to explain differences in the prevalence of F1 hybrids between broadly sympatric

334 species pairs according to whether or not they are characterized by conspecific forced

335 copulations.

336   Several authors have suggested that heterospecific rape may be an important source of

337 avian hybrids (McKee & Pyle 2002; Møller 1994; Randler 2005), but a comparative study of

338 waterfowl found only weak support for this hypothesis (Randler 2005). We believe that testing for

339 siring asymmetries will provide a stronger assessment of this hypothesis in waterfowl and game

340 birds, groups for which wild hybrids between a many pairs of broadly sympatric species (Grant &

341 Grant 1992). Forced copulations have been reported for various insects (Arnqvist 1989; Thornhill

342 1980; Thornhill & Sauer 1991), fish (Valero et al. 2008), lizards (Cooper 1985; Olsson 1995;

343 Rodda 1992) and mammals (Harris et al. 2010), but whether or not forced copulations generate

344 hybrids in these groups has not yet been addressed.

345 **Acknowledgements**

346   Nancy Hoffman and Peter Pyle collected and provided hybrid blood samples for this

347 study, and S. Edwards provided blood samples for black-footed and Laysan albatrosses. E. Flint

348 and N. Hoffman engaged in helpful discussions. A. Møller provided information on swallows and

349 helped with references. W. Swanson provided workspace, and J. Felsenstein, S. Edwards, and

350 members of the Rohwer, Leaché and Klicka lab groups commented on the manuscript. Thanks to

351 all for their help.

352 **References**

353 Arnqvist G. 1989. Multiple mating in a water strider: mutual benefits or intersexual conflict?
354   *Animal behaviour* 38:749-756.

355 Brekke P, Cassey P, Ariani C, and Ewen JG. 2013. Evolution of extreme-mating behaviour:
356   patterns of extrapair paternity in a species with forced extrapair copulation. *Behavioral*
357   *Ecology and Sociobiology* DOI 10.1007/s00265-013-1522-9.

358 Bretagnolle V, Attié C, and Pasquet E. 1998. Cytochrome-B evidence for validity and
359   phylogenetic relationships of Pseudobulweria and Bulweria (Procellariidae). *Auk* 115:188-
360   195.

361 Brown CR, and Brown MB. 1996. *Coloniality in the Cliff Swallow*. Chicago: University of
362   Chicago Press.

363 Carstens BC, Stoute HN, and Reid NM. 2009. An information-theoretical approach to
364   phylogeography. *Molecular ecology* 18:4270-4282.

365 Cooper WE. 1985. Female residency and courtship intensity in a territorial lizard, Holbrookia
366   propinqua. *Amphibia-Reptilia* 6:63-69.

367 Cousins K, and Cooper J. 2000. The Population Biology of the Black-Footed Albatross in
368   Relation to Mortality Caused by Longline Fishing. Western Pacific Regional Fishery
369   Management Council. Honolulu, HI.

370 Dunn PO, and Cockburn A. 1998. Costs and benefits of extra-group matings in superb fairy-
371   wrens. In: Parker P, and Burley N, editors. Avian reproductive tactics: female and male
372   perspectives. Washington, D.C.: American Ornithologists' Union.

373 Dunn PO, and Cockburn A. 1999. Extrapair mate choice and honest signaling in cooperatively
374   breeding superb fairy-wrens. *Evolution* 53:938-946.

375 Dunning JB, Jr. 2007. *CRC Handbook of Avian Body Masses*. London: CRC Press.

376 Emlen ST, and Wrege PH. 1986. Forced copulations and intra-specific parasitism: Two costs of
377   social living in the white-fronted bee-eater. *Ethology* 71:2-29.

378 Ewing B, and Green P. 1998. Base-calling of automated sequencer traces usingPhred. II. error
379   probabilities. *Genome research* 8:186-194.

380 Ewing B, Hillier L, Wendl MC, and Green P. 1998. Base-calling of automated sequencer traces
381   usingPhred. I. Accuracy assessment. *Genome research* 8:175-185.

382 Fisher HI. 1948. Notes. Interbreeding of Laysan and Black-footed Albatrosses.

383 Fisher HI. 1969. Eggs and Egg-Laying in the Laysan Albatross, *Diomedea immutabilis*. *The*
384   *Condor* 71:102-112.

385 Fisher HI. 1971. Incubation, hatching and related behavior in the Laysan Albatross. *Living Bird*.

386 Fisher HI. 1972. Sympatry of Laysan and black-footed albatrosses. *The Auk* 89:381-402.

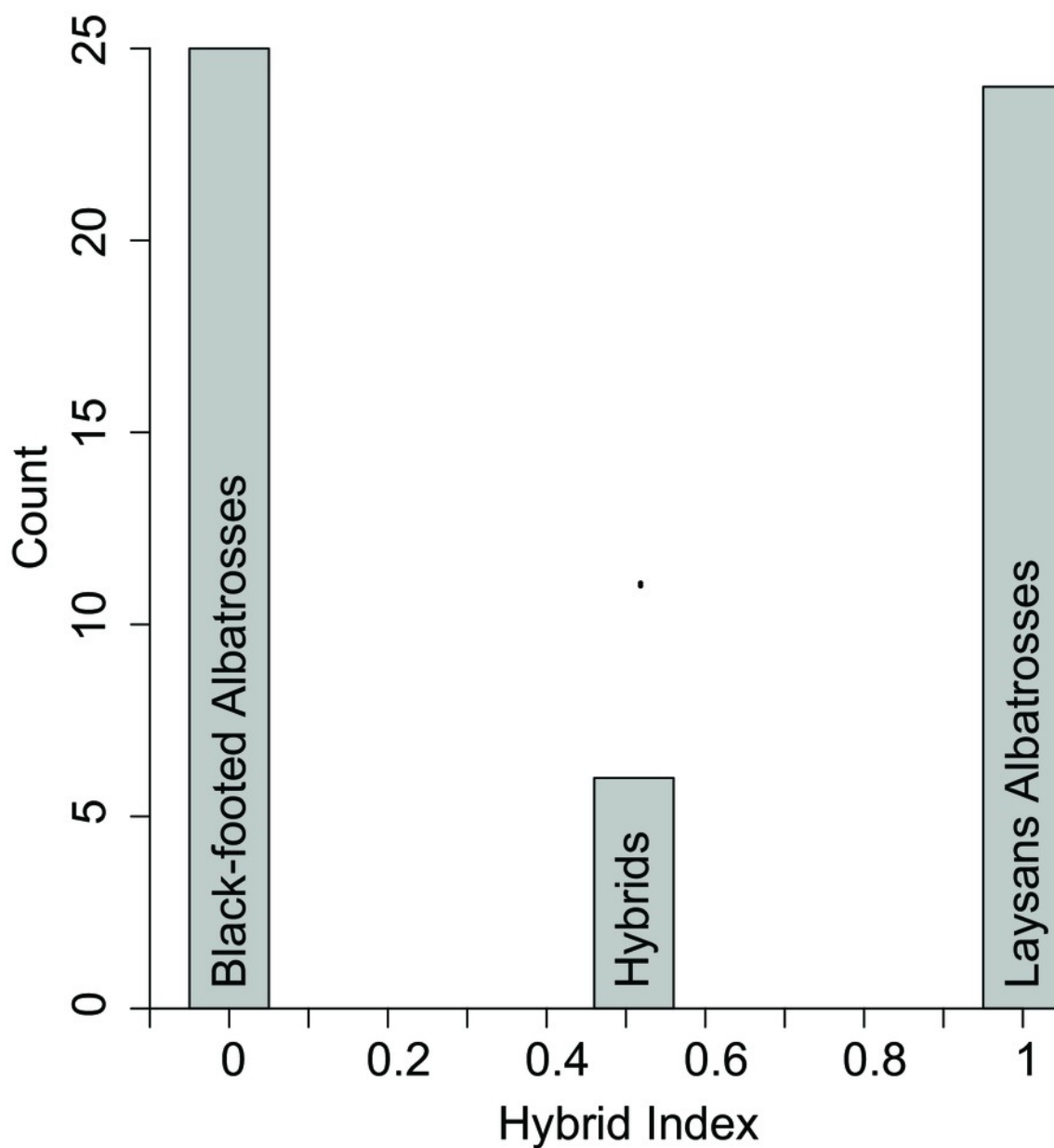
387   Fisher HI. 1975. The relationship between deferred breeding and mortality in the Laysan
388   Albatross. *The Auk* 92:433-441.
389   Fisher HI. 1976. Some dynamics of a breeding colony of Laysan Albatrosses. *Wilson Bulletin*
390   88:121-142.
391   Fridolfsson A-K, and Ellegren H. 1999. A simple and universal method for molecular sexing of
392   non-ratite birds. *Journal of Avian Biology* 30:116-121.
393   Gompert Z, and Buerkle C. 2009. A powerful regression-based method for admixture mapping of
394   isolation across the genome of hybrids. *Molecular ecology* 18:1207-1224.
395   Gompert Z, and Buerkle CA. 2010. Introgress: a software package for mapping components of
396   isolation in hybrids. *Molecular Ecology Resources* 10:378-384.
397   Gordon D, Abajian C, and Green P. 1998. Consed: a graphical tool for sequence finishing.
398   *Genome research* 8:195-202.
399   Grant PR, and Grant BR. 1992. Hybridization of bird species. *Science* 256:193-197.
400   Green P. 1994. PHRAP documentation. *Algorithms*.
401   Haldane JBS. 1922. Sex ratio and unisexual sterility in hybrid animals. *Journal of genetics*
402   12:101-109.
403   Harris HS, Oates SC, Staedler MM, Tinker MT, Jessup DA, Harvey JT, and Miller MA. 2010.
404   Lesions and behavior associated with forced copulation of juvenile Pacific harbor seals
405   (*Phoca vitulina richardsi*) by southern sea otters (*Enhydra lutris nereis*). *Aquatic*
406   *Mammals* 36:331-341.
407   Hey J, and Nielsen R. 2004. Multilocus methods for estimating population sizes, migration rates
408   and divergence time, with applications to the divergence of *Drosophila pseudoobscura*
409   and *D. persimilis*. *Genetics* 167:747-760.
410   Hudson RR, and Kaplan NL. 1985. Statistical properties of the number of recombination events
411   in the history of a sample of DNA sequences. *Genetics* 111:147-164.
412   Kabus A. 2002. Hybriden zwischen Rauchschwalbe *Hirundo rustica* und Mehlschwalbe
413   *Delichon urbica*. *Limicola* 16:276-285.
414   Kraus RH, Kerstens HH, van Hooft P, Megens H-J, Elmberg J, Tsvey A, Sartakov D, Soloviev
415   SA, Crooijmans RP, and Groenen MA. 2012. Widespread horizontal genomic exchange
416   does not erode species barriers among sympatric ducks. *BMC evolutionary biology* 12:45.
417   Martin RF. 1980. Analysis of hybridization between the hirundinid genera *Hirundo* and
418   *Petrochelidon* in Texas. *The Auk*:148-159.
419   McCarthy EM. 2006. *Handbook of avian hybrids of the world*: Oxford University Press New
420   York.
421   McKee T, and Pyle P. 2002. Plumage variation and hybridization in Black-footed and Laysan
422   Albatrosses *North American Birds* 56:131-138.
423   McKinney F, and Evarts S. 1998. Sexual coercion in waterfowl and other birds. *Ornithological*
424   *Monographs*:163-195.
425   Møller AP. 1994. *Sexual Selection in the Barn Swallow*. Oxford: Oxford University Press.
426   Muñez-Fuentes V, Vilà C, Green AJ, Negro JJ, and Sorenson MD. 2007. Hybridization between
427   white-headed ducks and introduced ruddy ducks in Spain. *Molecular ecology* 16:629-638.
428   Nickerson DA, Tobe VO, and Taylor SL. 1997. PolyPhred: automating the detection and
429   genotyping of single nucleotide substitutions using fluorescence-based resequencing.
430   *Nucleic acids research* 25:2745-2751.

431   Nunn GB, Cooper J, Jouventin P, Robertson CJR, and Robertson GG. 1996. Evolutionary
432   relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from
433   complete cytochrome-B gene sequences. *Auk* 113:784-801.
434   Nunn GB, and Stanley SE. 1998. Body size effects and rates of cytochrome b evolution in tube-
435   nosed seabirds. *Molecular Biology and Evolution* 15:1360-1371.
436   Olsson M. 1995. Forced copulation and costly female resistance behavior in the Lake Eyre
437   dragon, Ctenophorus maculosus. *Herpetologica*:19-24.
438   Paradis E. 2010. PEGAS: an R package for population genetics with an integrated-modular
439   approach. . *Bioinformatics* 26:419-420.
440   Price T. 2008. *Speciation in Birds*. Greenwood Village, Colorado: Roberts and Company.
441   Randler C. 1998. Hybrid waterfowl in western Central Europe. *British Birds* 91:573-573.
442   Randler C. 2002. Avian hybridization, mixed pairing and female choice. *Animal behaviour*
443   63:103-119.
444   Randler C. 2005. Do forced extrapair copulations and interspecific brood amalgamation facilitate
445   natural hybridisation in wildfowl? *Behaviour*:477-488.
446   Randler C. 2006. Extrapair paternity and hybridization in birds. *Journal of Avian Biology* 37:1-5.
447   Randler C. 2008. Hybrid wildfowl in Central Europe-an overview. *Waterbirds* 31:143-146.
448   Rice DW, and Kenyon KW. 1962. Breeding cycles and behavior of Laysan and Black-footed
449   Albatrosses. *Auk* 79:517-567.
450   Rodda GH. 1992. *The mating behavior of Iguana iguana*: Smithsonian Institution Press
451   Washington, DC.
452   Sambrook J, Fritsch EF, and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. New
453   York: Cold Spring Harbor Laboratory Press.
454   Seymour N. 1990. Forced copulation in sympatric American black ducks and mallards in Nova
455   Scotia. *Canadian Journal of Zoology* 68:1691-1696.
456   Shields WM, and Shields LM. 1983. Forcible rape: An evolutionary perspective. *Ethology and*
457   *Sociobiology* 4:115-136.
458   Slagsvold T, Hansen BT, Johannessen LE, and Lifjeld JT. 2002. Mate choice and imprinting in
459   birds studied by cross-fostering in the wild. *Proceedings of the Royal Society B-Biological*
460   *Sciences* 269:1449-1455.
461   Sorenson MD, Hauber ME, and Derrickson SR. 2010. Sexual imprinting misguides species
462   recognition in a facultative interspecific brood parasite. *Proceedings of the Royal Society*
463   *B: Biological Sciences* 277:3079-3085.
464   Spottiswoode C, and Møller AP. 2004. Extrapair paternity, migration, and breeding synchrony in
465   birds. *Behavioral Ecology* 15:41-57.
466   Stephens M, and Donnelly P. 2003. A comparison of bayesian methods for haplotype
467   reconstruction from population genotype data. *The American Journal of Human Genetics*
468   73:1162-1169.
469   Stephens M, Smith NJ, and Donnelly P. 2001. A new statistical method for haplotype
470   reconstruction from population data. *The American Journal of Human Genetics* 68:978-
471   989.
472   Stutchbury BJM, and Neudorf DL. 1998. Female control, breeding synchrony, and the evolution
473   of extra-pair mating systems. *Ornithological Monographs* 49:103-121.
474   ten Cate C, and Vos DR. 1999. Sexual imprinting and evolutionary processes in birds: a
475   reassessment. *Advances in the Study of Behaviour* 28:1-31.

476   Thornhill R. 1980. Pape in *Panorpa* scorpionflies and a general rape hypothesis. *Animal*
477   *behaviour* 28:52-59.
478   Thornhill R, and Palmer C. 2001. *A natural history of rape: Biological bases of sexual coercion*:
479   The MIT Press.
480   Thornhill R, and Sauer KP. 1991. The notal organ of the scorpionfly (Panorpa vulgaris): an
481   adaptation to coerce mating duration. *Behavioral Ecology* 2:156-164.
482   Thornhill R, and Thornhill NW. 1983. Human rape: An evolutionary analysis. *Ethology and*
483   *Sociobiology* 4:137-173.
484   Valero A, Garcia CM, and Magurran AE. 2008. Heterospecific harassment of native endangered
485   fishes by invasive guppies in Mexico. *Biology letters* 4:149-152.
486   Walsh H, and Edwards S. 2005. Conservation genetics and Pacific fisheries bycatch:
487   mitochondrial differentiation and population assignment in black-footed albatrosses
488   (Phoebastria nigripes). *Conservation Genetics* 6:289-295.
489   Wirtz P. 1999. Mother species–father species: unidirectional hybridization in animals with female
490   choice. *Animal behaviour* 58:1-12.

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.

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	Freq. of dSNP in LA	Forward Primer (5'-3')	Reverse Primer (5'-3')	Length (bp)	%GC	% Identical Sites	D BF	D LA
cyt-b	−	--	TTTGCCCTATCTATCCT	GATCCTGTTTCGTGGAGGAAGGT	609	48	97.7	-1.51	NA
MHC*	1	1.0	CCGGCAGCAGTACGTGCACTTCGNACAGCGA	GATGGGCTGCTGCAGGCTGGTGTGCT	571	63.5	99.1	-0.22	-1.28
1FWD*	2	0.90	GTGCCACCCATGTAACACCT	TGTGCTTTGGATGAACAGTTG	429	55	99.5	NA	-0.26
1REV*	3,4,5	1.0	ACTGTGTCACCCCATGCTC	CTGAGTCATTTCCATTCCTGG	407	58.7	99.0	-0.87	NA
4FWD*	6	1.0	TGGGCCAGGTTGTTAGGTAG	TATTGGTGGAATGGGCTTGT	464	34.3	99.4	-1.16	NA
4REV*	7	1.0	GGCTGGGGGTTTGGAATTA	CTTTCTACAGAGAAATAAACAAAGACC	443	36.9	99.5	-0.24	NA
6FWD	−	--	AGGGGTCTCTCAAACAGCAA	CTGGCCCTTTAGATAATAGCC	418	35.8	99.8	1.53	NA
6REV	−	--	GAACTGTAGTGAAGTATAACATCGTG	ATGCTGAGGGTGCCATCTTA	458	39.5	98.9	0.47	-1.76
10FWD	−	--	GGCAAAGGCTAAAGGCAAAG	TCAGAATTATTATAGCTTCAGGTGAG	548	43.4	99.6	NA	0.06
10REV	−	--	GGTGGTAGAACAGAAAGTCT	TTACCACCTTCCACCACACA	495	36.2	99.6	0.87	NA

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.

F1 genotype		
F1 combinations	**mtDNA**	**Probability**
LA f x BF m	LA (1.0)	0.90
LA m x BF f	BF (1.0)	0.00[1]

Backcross genotype		
Backcross combinations	**mtDNA**	**Probability**
F1 f x BF m	LA (1.0)	0.028
F1 f x LA m	LA (1.0)	0.034
F1 m x LA f	LA (1.0)	0.034
F1 m x BF f	BF (1.0)	0.00[1]

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

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

Model 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	ΔAIC	ω	q0	q1	q2	m0>1	m1>0
Pop. size BF = LA; Mig. from LA to BF = 0	2.48	3	1.04	0	0.16	0.24	[q0]	0.01	[0]	0.22
Mig. from LA to BF = 0	3.39	4	1.22	0.18	0.15	0.22	0.07	0	[0]	2.56
Ancestral pop. size = Bf pop. size; Mig. from LA to BF = 0	2.16	3	1.68	0.64	0.12	0.3	0.11	[q0]	[0]	1.82
Ancestral pop. size = LA pop. size; Mig. from LA to BF = 0	2.16	3	1.69	0.65	0.12	0.3	0.11	[q1]	[0]	1.76
Mig. from BF to LA = mig. from LA to BF	2.99	4	2.03	0.99	0.1	0.25	0.13	0	0.2	[m0>1]
Pop. size LA = BF	2.48	4	3.04	2	0.06	0.24	[q0]	0.01	0	0.22