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Rape and the Prevalence of Hybrids in Broadly Sympatric Species: a Case Study using Albatrosses

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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 present evidence that heterospecific rape may be the 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 heterospecific 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. The siring asymmetry found in our hybrids may have long persisted because an IM analysis suggests that long-term gene exchange between these species has been from Black-footed Albatrosses into Laysan Albatrosses. If hybrids are sired in heterospecific rapes, they 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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21 **Introduction**

22 Unidirectional hybridization is common in nature. A recent review showed that 50 of 80 cases
23 involving at least five hybrids, were predominantly unidirectional ([Wirtz 1999](#)). From a long list
24 of alternatives, a shortage of mates for females was the only general explanation supported for
25 unidirectional hybridization. In this paper we seek the beginnings of an answer to the question of
26 why hybrids vary so much in frequency between broadly sympatric species. For example, hybrids
27 between broadly sympatric species of waterfowl and grouse are far more common than they are in
28 other groups of birds (Grant and Grant 1992). Because hybridization usually arises as an
29 epiphenomenon of mating strategies within species ([Price 2008](#)), we think hybrids may be
30 disproportionately common in groups of birds characterized by forced copulations, as others have
31 suggested (Kabus 2002, McKee and Pyle 2002, Randler 2008). Forced copulations are used as a
32 supplemental reproductive tactic by males in many species of waterfowl (Brennan et al. 2009,
33 McKinney and Evarts 1998), but a comparative test by Randler (2008) found more support for
34 brood amalgamation than for forced copulations as alternative sources of hybrid waterfowl.

35 Here we suggest that predicting siring asymmetries offers a promising way to evaluate the
36 importance of heterospecific rape as a source of hybrids between broadly sympatric species. In
37 general, rape supplements male reproductive success when directed toward conspecifics ([Shields
38 & Shields 1983](#); [Thornhill 1980](#); [Thornhill & Palmer 2001](#); [Thornhill & Sauer 1991](#); [Thornhill &
39 Thornhill 1983](#)) but the urgent and aggressive nature of rape may result in males sometimes
40 forcing copulations on heterospecific females. While they may be uncommon, hybrids generated
41 by heterospecific rape should be found wherever the parental species breed sympatrically, rather
42 than being confined to zones where the ranges of parapatric species pairs meet and where hybrids
43 are often abundant.

44 We illustrate our predictions using hybrids between Laysan and Black-footed Albatrosses
45 (*Phoebastria immutabilis* and *P. nigripes*, respectively) because we had DNA samples for both
46 parental species and for hybrids between them that could be used to test for a siring bias in F1
47 hybrids and to evaluate long-term gene exchange between the parental species. Paired males of
48 both of these albatrosses are known to force copulations on conspecific females. If hybrids are
49 sired through heterospecific rape, differences between these albatrosses in behavior and the
50 timing of egg laying (detailed below) predict that Black-footed Albatrosses should sire most F1
51 hybrids. It is important that only F1 hybrids are used to evaluate siring biases predicted for

52 heterospecific rape because siring asymmetries will be lost if backcross hybrids are generated
53 through random mating with either parental species. Siring bias in F1 hybrids is easily assessed
54 using mitochondrial DNA (mtDNA) to identify the maternal species.

55 Forced and unforced extra pair copulations must be distinguished before the role of
56 heterospecific rape in the generation of hybrids can be assessed. Rape is not expected to be a
57 source of hybrids in species groups for which females control extrapair paternity (Dunn and
58 Cockburn 1999, Spottiswoode and Møller 2004, Stuchbury and Neudorf 1998). However, when
59 forced copulations are the result of extreme male aggression, sometimes carried out by groups of
60 males, rape can be a source of hybrids if males mistakenly attack heterospecific females. Of
61 course, male waterfowl have penises that can be used to forcibly inseminate resisting females
62 (Brennan et al. 2009), but even in species without penises, rapes may be so aggressive that
63 females must acquiesce to avoid being seriously injured or killed (Brekke et al. 2013, Fisher 1971,
64 McKinney and Everts 1998). Clear evidence of female coercion is required before heterospecific
65 rape appropriately can be considered a possible source of hybrids.

66 **Study System**

67 Laysan and Black-footed Albatrosses are closely related sister species ([Nunn et al. 1996](#)) that
68 breed sympatrically in the Northwestern Hawaiian Islands. Like other albatrosses, they are long-
69 lived, delay breeding until they are five to seven years old, form life-long pair bonds, lay single
70 eggs, and may breed for 20-50 years ([Fisher 1969](#); [Fisher 1971](#); [Fisher 1972](#); [Fisher 1975](#); [Fisher](#)
71 [1976](#); [Rice & Kenyon 1962](#)).

72 Our samples came from Midway Atoll where over 480,000 pairs of these albatrosses nest
73 and where the beach-nesting Black-footed Albatross comprises about five percent of all pairs (E.
74 Flint, personal communication). Although interbreeding between Black-footed and Laysan
75 Albatrosses is rare, putative hybrids have been noted for decades ([Fisher 1948](#); [Fisher 1971](#);
76 [McKee & Pyle 2002](#)) and up to 20 presumptive hybrids were observed at Midway Atoll between
77 1997 and 2000 ([McKee & Pyle 2002](#)).

78 Mature Black-footed Albatrosses are primarily dark brown, whereas Laysan Albatrosses
79 are largely white on the body and dark grey to black on the wings and back. Presumed hybrids are
80 intermediate between the parental species in plumage and soft part coloration, ranging in plumage
81 from very pale grey to fairly dark, with pale under wings ([Fisher 1972](#); [McKee & Pyle 2002](#)). The

82 lightest presumptive hybrids can resemble the darkest Laysan Albatrosses in plumage color, but
83 the darkest putative hybrids are not as dark as Black-footed Albatrosses. Because Laysan
84 Albatrosses and hybrids are variable in coloration, identifying or excluding progeny that might
85 result from backcrosses is not possible based on plumage characteristics alone ([McKee & Pyle](#)
86 [2002](#)) and requires genetic assessment.

87 Conspecific rapes are observed in both Laysan and Black-footed Albatrosses ([Fisher 1971](#);
88 [Fisher 1972](#)), and Black-footed Albatross males sometimes direct rape attempts at Laysan females,
89 suggesting that hybrids could result from heterospecific rapes. In Laysan Albatrosses conspecific
90 rape is very aggressive, often carried out by groups of males, and sometimes results in serious
91 injury of the female ([Fisher 1971](#)). Multiple males regularly join these rape attempts, mounting
92 other males until the pile topples over. Given that albatrosses lack the explosive penis that
93 facilitates forced copulation by male waterfowl (Brennan et al. 2005), it is plausible that females
94 may sometimes evert their cloaca to receive sperm just to prevent further harassment and injury
95 by attacking males; however, we should note that Fisher (1971) found no evidence of sperm
96 transfer in albatrosses he examined closely following attacks by males. [Fisher \(1971\)](#) further
97 reports that he never observed an attempt by the female's mate to defend her from harassing
98 males, as does occur in waterfowl ([McKinney & Evarts 1998](#)). Although [Fisher \(1972\)](#) reports
99 failing to observe interspecific rape attempts, [McKee & Pyle \(2002\)](#) observed male Black-footed
100 Albatrosses attempting to rape female Laysan Albatrosses and believed these events to be the
101 source of hybrids. Neither [Fisher \(1972\)](#) nor [McKee & Pyle \(2002\)](#) observed mixed pairs
102 attending a nest.

103 Importantly, differences in the timing of breeding, body size, and aggressiveness all
104 suggest that F1 hybrids are sired when the larger and more aggressive male Black-footed
105 Albatrosses force copulations on female Laysan Albatrosses. Particularly important is that Black-
106 footed Albatrosses arrive at the breeding colonies and lay earlier than do Laysan Albatrosses
107 ([Fisher 1969](#); [Rice & Kenyon 1962](#)). Because females take the first incubation shift in these
108 albatrosses (Fisher 1971, Rice and Kenyon 1962), the species difference in breeding schedules
109 results in Laysan females being fertile and vulnerable to insemination through heterospecific rape
110 by Black-footed Albatross males paired to females that are already incubating.

111 We evaluated the F1 status of hybrids using fixed and near-fixed differences in their
112 nuclear genome, and we assessed siring bias using mtDNA from the hybrids. We also used an

113 isolation-migration (IM) model to test the hypothesis of asymmetric gene flow between these
114 species following their divergence approximately 1.03 million years ago ([Nunn et al. 1996](#)).

115 **Materials and Methods**

116 *Sampling*

117 Blood was sampled from 29 breeding Black-footed Albatrosses, 28 Laysan Albatrosses, and six
118 presumed hybrids (morphologically intermediate between the two species in plumage coloration)
119 at Midway Atoll National Wildlife Refuge (28°13' N, 177°22' W). Genomic DNA was extracted
120 from blood samples either by a standard phenol:chloroform procedure ([Sambrook et al. 1989](#)) or
121 using the Wizard SV Genomic DNA Purification System (Promega). All work was conducted in
122 accordance with policies of the University of Washington Institutional Animal Care and Use
123 Committee (protocol 2846-13).

124 *Molecular methods*

125 To assess gene flow between the parental species, we collected DNA sequence data for eight
126 anonymous nuclear loci, one coding nuclear locus (a fragment of a Major Histocompatibility
127 Complex (MHC) gene ([Walsh & Edwards 2005](#)), and the mtDNA *cytochrome-b* (*cyt-b*) locus.
128 Anonymous loci were derived from a fosmid library for Black-footed Albatross (Table 1). “FWD”
129 and “REV” designations indicate loci that were taken from opposite ends of a fosmid insert, and
130 therefore are separated by ~ 35 kb in the genome. Optimized PCR reactions for anonymous loci
131 contained 0.4 μM primer, 0.2 mM of an equimolar solution of dNTPs, 0.2 U of Taq DNA
132 polymerase (Roche, Indiana, USA), and approximately 20 ng of template DNA in 10 μl reaction
133 volumes. Thermal cycler reaction profiles consisted of initial denaturation at 94°C for 1 minute
134 30 seconds, followed by 30 cycles of 94°C for 30 seconds, 50-68°C for 30 seconds, 72°C for 45 to
135 60 seconds, and a final extension step of three minutes at 72°C.

136 We assigned quality scores to base calls in sequence trace files using Phred ([Ewing &](#)
137 [Green 1998](#); [Ewing et al. 1998](#)) and aligned homologous sequences using Phrap ([Green 1994](#)).
138 Polymorphic sites were identified using the program PolyPhred ([Nickerson et al. 1997](#)).
139 Assemblies were visualized in Consed ([Gordon et al. 1998](#)) and single nucleotide polymorphisms
140 (SNPs) and genotypes at each locus were confirmed by eye. Nuclear haplotypes were resolved

141 using PHASE v.2.1.1 ([Stephens & Donnelly 2003](#); [Stephens et al. 2001](#)). All sequences have been
142 deposited in GenBank (accession numbers KF475302-KF475698).

143 Putative hybrids were sexed using primers 2550F and 2718R ([Fridolfsson & Ellegren](#)
144 [1999](#)); sex was scored by eye, with two bands indicating female and a single-band indicating
145 male. Sexing the hybrids enabled us to assess whether hybrid females (the heterogametic sex in
146 birds) were inviable, which could be expected under Haldane's rule ([Haldane 1922](#)).

147 *Hybrid Identification*

148 We computed two hybrid indices, both varying from 0 (pure Laysan Albatrosses) to 1 (pure
149 Black-footed Albatrosses). The first is most intuitive and includes only loci with fixed or near-
150 fixed sequence differences between black-footed and Laysan Albatrosses (Table 1). Using these
151 same loci, we also computed the probability that the six phenotypically intermediate specimens
152 were first generation (F1) hybrids or backcrosses (Table 2). For the second hybrid index we used
153 maximum likelihood in the introgress package implemented in R ([Gompert & Buerkle 2009](#)), and
154 included all of the nuclear loci.

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

156 *Migration estimation*

157 To assess the rate and direction of gene flow between Black-footed and Laysan Albatrosses, we
158 used the IM model implemented in IMA2 ([Hey & Nielsen 2004](#)). We applied the HKY mutation
159 model of nucleotide substitution and nuclear mutation rate scalars were free to vary in the model.
160 The nuclear and mitochondrial genes were assigned an inheritance scalar of 1.0 and 0.25,
161 respectively. To avoid violating the assumptions of no recombination and neutrality of markers,
162 we tested for within-locus recombination using the four-gamete test ([Hudson & Kaplan 1985](#)) for
163 each locus and within each species; we tested neutrality of markers using Tajima's D
164 implemented in R package PEGAS ([Paradis 2010](#)) (Table 1).

165 We ran 12 replicate IMA2 analyses, each using different starting seeds and 40-50
166 concurrent chains, for 10-50 million steps after an initial burn-in phase of 50,000-100,000
167 generations. To rescale estimates of population size and migration parameters into demographic
168 units, we used the geometric mean of previous mtDNA rate estimates for albatrosses of 3×10^{-5}
169 substitutions per locus per year for our fragment of *cyt-b*; ([Nunn & Stanley 1998](#)), and a

170 generation time of 25 years ([Cousins & Cooper 2000](#)). The results of these independent runs were
171 combined into a single L-mode analysis to evaluate the probabilities for all possible nested
172 models. For explanation of nested models, see the standard IMA2 documentation
173 (https://bio.cst.temple.edu/~hey/program_files/IMa2/Using_IMa2_8_24_2011.pdf). We
174 conducted model selection following Carstens et al. (2009).

175 **Results**

176 *Hybrid Indices and probability of hybrid genotypes*

177 All six putative hybrids were heterozygous at five diagnostic nuclear SNPs (Table 1). Using just
178 these diagnostic loci the hybrid index for a true F1 hybrid is expected to be 0.51 because Laysan
179 Albatrosses share in low frequency (10%) a single diagnostic SNP (dSNP2 in Tables 1 & 2) that
180 is fixed in Black-footed Albatrosses (Fig. 1).

181 In Table 2 we use the observed population allele frequencies to calculate the probability of
182 producing the genotype found in all six hybrids, under the assumption that they were either F1
183 hybrids or first generation backcrosses. The probability of producing the observed hybrid
184 genotype was 0.90 for a parental cross. The probability that the hybrid genotype resulted from a
185 backcross to either of the parental species varies by the sex of the hybrid and the sex and species
186 of the backcross parent (Table 2). Because all hybrids carried Laysan mtDNA haplotypes, the
187 probability of a backcross to a female Black-footed Albatross is 0. For the three other backcross
188 combinations, the probability of observing the hybrid genotype is either 0.028 or 0.034 (Table 2).
189 These calculations, based on the five diagnostic SNPs, show that the six hybrids are almost
190 certainly F1s and not backcross individuals.

191 We also evaluated the status of the six hybrids using a maximum likelihood estimator
192 ([Gompert & Buerkle 2010](#)), including in this analysis the four nuclear SNPs that were not
193 diagnostic (Table 1). All six hybrids received a score of 0.56, with a 95% confidence interval of
194 0.22 – 0.85.

195 *Siring bias and sex for the hybrids*

196 All six hybrids carried the Laysan Albatross mtDNA haplotype, indicating that F1 hybrids result
197 from male Black-footed Albatrosses inseminating female Laysan Albatrosses ($p = 0.031$). Three

198 of the hybrids were male and three were female, suggesting no inviability of the heterogametic
199 sex ([Haldane 1922](#)).

200 *Gene flow*

201 Tajima's D values showed no significant deviation from neutrality for any of the loci examined
202 (Table 1) and no evidence of recombination within loci was found.

203 Under the IM model, the rate of gene flow was significantly higher from Black-footed
204 Albatrosses into Laysan Albatrosses ($p=0.028$). The mean rate of gene flow ($2Nm$) was 0.09 gene
205 copies per generation from Black-footed Albatross into Laysan Albatross (95% HPD 0.024 -
206 0.23), whereas this rate was zero in the reverse direction (95% HPD 0 - 0.10).

207 Asymmetrical gene flow from Laysan to Black-footed Albatross was constrained to zero
208 in the top four models, which, together, account for 55% of the variation in the weighted AIC
209 (Table 3). A commonly used standard for AIC model ranking is that models within two units of
210 the best model cannot be dismissed. The 5th ranked model does not support unidirectional gene
211 flow (Table 3) and is within two AIC units of the best model. However, this model differs from
212 the best model by one parameter ($k= 3$ vs. 4) and the maximized log-likelihood value of model 5
213 is similar to that of the best model. This suggests that model 5 is not competitive with the best
214 model and instead is "close" only because it adds one parameter, even though the fit is not
215 improved (Burnham & Anderson 2002).

216 **Discussion**

217 Using diagnostic nuclear loci, we show that all six presumed hybrids between Laysan and Black-
218 footed Albatross were F1 hybrids. All six carried Laysan mtDNA haplotypes, indicating that male
219 Black-footed Albatrosses were their sires. This contradicts the hypothesis that a scarcity of mates
220 for females of the rare species results in hybrid pairings ([Wirtz 1999](#)) because all six hybrids had
221 Laysan Albatross mothers, instead of mothers of the much less abundant Black-footed Albatross.
222 Finally, we found limited, but significant gene flow from Black-footed Albatrosses into Laysan
223 Albatrosses, suggesting that past F1 hybrids have backcrossed to Laysan Albatrosses. As we
224 discuss below, this is consistent with our hypothesis that forced copulations are asymmetrical.

225 *Effects of phenology and behavior on insemination biases*

226 Black-footed Albatrosses lay eggs 10 days to two weeks earlier than Laysan Albatrosses ([Fisher](#)
227 [1969](#); [Rice & Kenyon 1962](#)), so most female Black-footed Albatross have begun incubating when
228 Laysan females are fertile. This difference in breeding schedules undoubtedly contributes strongly
229 to the asymmetry in inseminations that generate hybrids because only paired males have been
230 reported to engage in rape attempts in these albatrosses ([Fisher 1971](#); [McKee & Pyle 2002](#)).
231 Unmated males spend their time at breeding colonies courting females and have not been
232 observed attempting rapes ([Fisher 1971](#)). Other factors may also contribute to the observed siring
233 asymmetry. Notably, female Laysan Albatrosses are 5-10% smaller than male Black-footed
234 Albatrosses ([Dunning 2007](#)), and male Black-footed Albatrosses are much more aggressive in
235 conspecific rape attempts than are male Laysan Albatrosses ([Fisher 1972](#)). Finally, because Black-
236 footed Albatrosses constitute only 5% of the population of these two species breeding at Midway
237 Atoll, they have far more opportunity to engage in forced heterospecific copulations than do
238 Laysan Albatrosses. These differences suggest that male Black-footed Albatrosses are more likely
239 to sire hybrids through rapes, and all reported heterospecific rape attempts have involved male
240 Black-footed Albatrosses and female Laysan Albatrosses ([McKee & Pyle 2002](#)).

241 The asymmetry in gene exchange suggested by the isolation-migration model implies a
242 long history of unidirectional gene flow from Black-footed Albatrosses into Laysan Albatrosses.
243 Although modern hybrids appear to have no success in attracting mates ([Fisher 1972](#); [McKee &](#)
244 [Pyle 2002](#); [Rice & Kenyon 1962](#)), two carefully observed hybrids (unsuccessfully) addressed all
245 courtship attempts at Laysan Albatrosses ([Fisher 1972](#)). If hybrids are sired by male Black-footed
246 Albatrosses raping female Laysan Albatrosses, they would be raised by and sexually imprinted on
247 Laysan Albatrosses ([Slagsvold et al. 2002](#); [ten Cate & Vos 1999](#)) and, thus, are expected to prefer
248 pairing with Laysans.

249 *Alternative explanations for asymmetric gene flow*

250 If we assume that hybrids have been able to pair and raise chicks in the past, then we can think of
251 two alternatives to our hypothesis of heterospecific rape and sexual imprinting as the cause of the
252 observed asymmetry in gene flow between Black-footed and Laysan Albatrosses. First, is the
253 possibility that F1 backcrosses into the Black-footed Albatross population have not been viable.
254 Definitely addressing this alternative would require breeding experiments, but Fisher's (1972)
255 observation that two closely observed hybrids courted only Laysan Albatrosses tends to preclude

256 it. Although Fisher closely observed just two hybrids, the number of Laysan Albatrosses they
257 attempted to court was large.

258 Second, if hybrids were intermediate in their breeding schedule relative to the parental
259 species, then hybrids may have had greater opportunity to mate with Laysan Albatrosses, which
260 return later to the breeding colonies than Black-footed Albatrosses. However, this explanation
261 untenably assumes that hybrids form life-long pair-bonds and breed the first year that they return
262 to the breeding islands. Instead, pre-breeding Laysan Albatrosses typically spend one or two
263 years choosing a mate ([Fisher 1972](#)), making the two-week difference in laying dates unlikely to
264 bias the hypothesized pattern of backcross matings toward Laysan Albatrosses.

265 It seems likely to us that the gene flow revealed by the IM analysis reflects gene exchange
266 that took place as the species were diverging in coloration. This is supported by the fact that
267 courting birds focus their attention on the breasts of their dance partners, where the two species
268 differ most in color ([Fisher 1972](#)), and by the failure of field workers to find any hybrids that were
269 paired ([Fisher 1972](#); [McKee & Pyle 2002](#)).

270 *Tests with other groups*

271 The contrast between species in which conspecific Extra-Pair Copulations (EPC) are forced, as
272 opposed to species in which females accept or solicit such copulations, is critical to our thesis that
273 hybrids between broadly sympatric species will be more common in groups where forced
274 copulations are frequent. Although EPC are common in many passerines, they are mostly
275 unforced and apparently controlled by females to increase the genetic quality of offspring ([Dunn
276 & Cockburn 1998](#); [Dunn & Cockburn 1999](#); [Spottiswoode & Møller 2004](#); [Stutchbury & Neudorf
277 1998](#)). Unfortunately, whether EPC are forced or accepted is rarely described in the literature
278 (although there are good descriptions of rape in albatrosses, waterfowl, bee-eaters, swallows and
279 the New Zealand Hihi (*Notiomystis cincta*) ([Brekke et al. 2013](#); [Emlen & Wrege 1986](#); [Kabus
280 2002](#); [Martin 1980](#)). Obviously, heterospecific rape should not be entertained as a source of
281 hybrids except in groups for which conspecific EPC are clearly forced.

282 Naturally occurring hybrids are abundant in waterfowl ([Grant & Grant 1992](#); [Randler
283 1998](#); [Randler 2008](#)) and male ducks are known to direct rape attempts at females of other species
284 ([Muñez-Fuentes et al. 2007](#); [Randler 2002](#); [Seymour 1990](#)). However, we could find no genetic
285 assessments of insemination biases in the generation of hybrids between naturally sympatric

286 waterfowl. An obvious test would be to compare insemination bias when one parental species is
287 characterized by forced copulations and the other is not. For example, hybrids between Northern
288 Shovelers (*Anas clypeata*) and both Mallards (*Anas platyrhynchos*) and Northern Pintails (*Anas*
289 *acuta*) are reported from North America and Eurasia ([McCarthy 2006](#)). Because Northern
290 Shoveler males are territorial, and seldom attempt conspecific rapes, the heterospecific rape
291 hypothesis predicts F1 hybrids will have Mallard or Northern Pintail sires ([McKinney & Evarts](#)
292 [1998](#)). Siring bias is also predicted for the abundant hybrids between Common Pochards and
293 Tufted Ducks (*Aythya ferrina* x *Aythya fuligula*, respectively) ([Randler 2008](#)) because conspecific
294 rape is unreported in Common Pochards but frequent in Tufted Ducks ([McKinney & Evarts](#)
295 [1998](#)); thus, F1 hybrids should be sired by tufted ducks if they are produced in heterospecific
296 rapes.

297 Heterospecific rape may also account for the frequent hybrids reported between Barn
298 Swallows (*Hirundo rustica*) and House Martins (*Delichon urbica*) in Europe and between Barn
299 Swallows and Cliff Swallows (*Petrochelidon pyrrhonota*) in North America. Barn Swallows are
300 characterized by many EPCs, but females choose whether or not to accept these EPCs, which are
301 almost never forced ([Møller 1994](#)). In contrast, aggressive conspecific rape is frequently observed
302 at communal mud-gathering sites in both Cliff Swallows and House Martins ([Brown & Brown](#)
303 [1996](#); [Møller 1994](#)). That male Cliff Swallows and House Martins are characterized by
304 conspecific rape may render female Barn Swallows vulnerable to heterospecific rape when they
305 gather mud at sites frequented by males of these species. Correspondingly, when identified as
306 nestlings, hybrids between Barn Swallows and House Martins were always found in Barn
307 Swallow nests, had Barn Swallow siblings, and had two Barn Swallow parents (Kabus 2002);
308 similarly, nestling hybrids between Barn Swallows and Cliff Swallows or Cave Swallows (*P.*
309 *fulva*) were found, in all cases but one, in Barn Swallow nests, attended by two Barn Swallow
310 parents ([Martin 1980](#)). Given that male Barn Swallows do not force copulations on females, but
311 that males of the three other parental species do force copulations on conspecific females, it
312 seems plausible that most of these were F1s sired through heterospecific rapes.

313 *Broader implications*

314 Two comparative studies have addressed the role of EPC in the generation of avian hybrids. In a
315 survey of open nesting birds, [Randler \(2006\)](#) found EPC to be uncorrelated with the production of

316 hybrids; however, this study failed to distinguish forced and unforced extra pair copulations and
317 failed to consider whether hybrids were rare or common. In another study [Randler \(2005\)](#)
318 assessed the roles of forced EPC and brood amalgamation on the production of hybrid waterfowl,
319 and found a significant effect only of brood amalgamation when both factors were included in the
320 model. However, both causal variables were treated as binary characters, which masks their
321 relative importance in species pairs where both factors occur but one generates far more hybrids
322 than the other. Over 800 Common Pochard x Tufted Duck hybrids were reported from Europe
323 ([Randler 2008](#)), yet these were treated as equivalent to a single report of a natural hybrid between
324 other species pairs. If most of these 800 hybrids were caused by either factor, then the importance
325 of that factor will be greatly underestimated by failing to account for hybrid frequency.

326 In some cases evaluating siring asymmetries can generate strong tests of the hypothesis
327 that heterospecific brood parasitism results in ducks forming heterospecific pair bonds ([Randler](#)
328 [2005](#)). For example, Redheads (*Aythya americana*) are facultative brood parasites of
329 Canvasbacks (*Aythya valisineria*), whereas canvasbacks do not parasitize redhead nests ([Sorenson](#)
330 [et al. 2010](#)). Redhead ducklings raised by Canvasback females in broods of mostly Canvasback
331 ducklings should be sexually imprinted on Canvasbacks and, therefore, be more willing to pair or
332 at least mate with Canvasbacks. Indeed, males of both species cross-fostered into broods
333 predominated by the other species (without hens) preferentially courted heterospecific females
334 ([Sorenson et al. 2010](#)). An excess of adult males in both species predicts the siring bias: Female
335 Redheads imprinted on Canvasbacks ([Sorenson et al. 2010](#)), should be able to attract unmated
336 male Canvasbacks as mates. In contrast, male Redheads imprinted on Canvasbacks would be
337 unlikely to attract Canvasback mates because Canvasback females have many unmated males to
338 choose from. Thus Canvasback males should sire F1 hybrids between these species, if hybrids are
339 generated by brood parasitism and sexual imprinting. In contrast, Barrow's (*Bucephala islandica*)
340 and Common (*B. clangula*) Goldeneyes parasitize each other, so males of both species are
341 expected to be sires of hybrids. Although rare, hybrids between both the *Bucephala* and the
342 *Aythya* species pairs are regularly reported ([McCarthy 2006](#)), and none of the parentals is
343 characterized by conspecific rape.

344 Among *Anas* ducks gene sharing through hybridization apparently has strongly affected
345 effective population sizes. For Northern Pintails and Green-winged Teal (*Anas crecca*), census
346 population sizes are too small for certain shared alleles to have persisted for more than 2 and 2.6

347 million years. However, these alleles, which are shared with Mallards, are estimated to have
348 persisted for 6.2 and 7.9 million years, respectively, suggesting a long history of horizontal gene
349 exchange with Mallards, which have a much larger effective population size ([Kraus et al. 2012](#)).
350 Heterospecific rapes may be responsible for generating F1 hybrids between these ducks and,
351 unlike the situation in albatrosses, F1 hybrid females in these short-lived ducks may form pair-
352 bonds and breed. Hybrid female ducks should be sexually imprinted on the species that raised
353 them ([ten Cate & Vos 1999](#)) and the strong male bias in the breeding sex ratios of north temperate
354 ducks should facilitate pairing and breeding by hybrid females.

355 **Conclusion**

356 Although unidirectional hybridization often predominates in nature, only a shortage of mates for
357 females previously emerged from a long list of alternative hypotheses as a general explanation for
358 asymmetric hybridization ([Wirtz 1999](#)). Here we attempt to make the general point that, if hybrids
359 result from heterospecific rape, differences in behavior and life history of the parental species can
360 be used to predict the direction of crosses. Predicting the mother and father species of F1 hybrids
361 from different parental combinations has the potential to considerably refine our understanding of
362 the importance of heterospecific forced copulation (and brood parasitism), in the generation of
363 hybrids ([McKee & Pyle 2002](#); [Møller 1994](#); [Randler 2005](#)). Although heterospecific rape is
364 unlikely to be adaptive, it may explain differences in the prevalence of F1 hybrids between
365 broadly sympatric species pairs according to whether or not they are characterized by conspecific
366 forced copulations.

367 Several authors have suggested that heterospecific rape may be an important source of
368 avian hybrids ([McKee & Pyle 2002](#); [Møller 1994](#); [Randler 2005](#)), but Randler's (2005)
369 comparative study of waterfowl found only weak support for this hypothesis. We believe that
370 testing for siring asymmetries will provide a stronger assessment of this hypothesis in waterfowl,
371 a group for which wild hybrids have been reported between many pairs of broadly sympatric
372 species ([Grant & Grant 1992](#)). Forced copulations have been reported for various insects
373 ([Arnqvist 1989](#); [Thornhill 1980](#); [Thornhill & Sauer 1991](#)), fish ([Valero et al. 2008](#)), lizards
374 ([Cooper 1985](#); [Olsson 1995](#); [Rodda 1992](#)) and mammals ([Harris et al. 2010](#)), but whether or not
375 forced copulations generate hybrids in these groups has not yet been addressed.

376 **Postscript**

377 Coincident with our revision of this manuscript Hope Ronco and Pete Leary (US Fish & Wildlife
378 Service) informed us of a hybrid albatross (Fig. 2) at Midway Atoll that is paired with a Laysan
379 Albatross and that has successfully raised chicks several times since 2006. Its sex is unknown
380 because they have not observed it mating. As far as we know this is the first record of a Black-
381 footed x Laysan Albatross hybrid successfully breeding. Of course, it is only a single bird, but
382 that it is mated to a Laysan Albatross is consistent with the hypothesis that its sire would have
383 been a Black-footed Albatross and that it would have been raised and imprinted on Laysan
384 parents. Its apparent success at raising backcross chicks with a Laysan is also consistent with the
385 asymmetry in gene flow suggested by our IM analyses. Blood samples to confirm that it is an F1
386 hybrid, and blood samples from its chicks would add valuable additional information to this
387 remarkable observation.

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395 Service, provided information on the breeding hybrid, and Hope provided the striking photograph
396 of this bird. Thanks to all for their help.

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540 choice. *Animal Behaviour* 58:1-12.

Table 1 (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	Freq. of		Forward Primer (5'-3')	Reverse Primer (5'-3')	Length (bp)	%GC	% Identical		
		dSNP in	LA					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	CTGAGTCATTTCCATTCTGG	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		GGCTGGGGGTTTGAATTA	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	-	--		GGCAAAGGCTAAAGGCAAAG	TCAGAATTATTATAGCTTCAGGTGAG	548	43.4	99.6	NA	0.06
10REV	-	--		GGTGGTAGAACAGAAAGTCT	TTACCACCTTCCACCACACA	495	36.2	99.6	0.87	NA

Table 2 (on next page)

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); frequencies of the diagnostic SNPs are given Table 1. 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.

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

Backcross genotype		
Backcross	mtDNA	Probability
combinations		
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

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

Table 3(on next page)

AIC ranking of 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	Delta (AIC)	w	q0	q1	q2	M0>1	M1>0
Pop. size BF = LA; Mig. from LA to BF = 0	2.48	3	1.04	0.00	0.1 6	0.238 8	0.2388]	0.0085	[0]	0.2236
Mig. from LA to BF = 0	3.39	4	1.22	0.18	0.1 5	0.224 4	0.07	0.0008 7	[0]	2.5594
Anc. pop. size = BF; Mig. from LA to BF = 0	2.16	3	1.68	0.64	0.1 2	0.300 8	0.1094	[0.3008]	[0]	1.8228
Anc pop. size = LA; Mig. from LA to BF = 0	2.16	3	1.69	0.65	0.1 2	0.304 3	0.1101	[0.1101]	[0]	1.7566
Mig. from BF to LA = mig. from LA to BF	2.99	4	2.03	0.99	0.1 0	0.246 5	0.1291	0.0026	0.1998	[0.1998]
Pop. size LA = BF	2.48	4	3.04	2.00	0.0 6	0.238 8	[0.2388]	0.0085	0	0.2236
Mig. from LA to BF = 0; Pop. size LA & BF = anc	0.30	2	3.40	2.36	0.0 5	0.176 1	[0.1761]	[0.1761]	[0]	1.4231
Anc pop. Size = BF	2.16	4	3.68	2.64	0.0 4	0.300 8	0.1094	[0.3008]	0	1.8228
LA pop. size = anc	2.16	4	3.69	2.65	0.0 4	0.304 3	0.1101	[0.1101]	0	1.7566
BF pop. size = LA; Mig. from LA to BF = BF to LA	0.93	3	4.15	3.11	0.0 3	0.238 8	[0.2388]	0.0085	0.1034	[0.1034]
Full model	2.60	5	4.80	3.76	0.0 2	0.339 4	0.1015	0.0171	0	1.6769
BF pop. size = LA & anc	0.30	3	5.40	4.36	0.0 2	0.176 1	[0.1761]	[0.1761]	0	1.4231
BF pop. size = LA; Both mig. = 0	-0.83	2	5.66	4.62	0.0 2	0.259 5	[0.2595]	0.5181	[0]	[0]
Both mig. = 0	-0.19	3	6.38	5.34	0.0	0.250	0.2732	0.5181	[0]	[0]

BF pop. size = LA & anc; Mig. From LA to BF = BF to LA	-1.21	2	6.42	5.38	0.0	0.290	[0.2907]	[0.2907]	0.1121	[0.1121]
LA pop. size = anc; Mig. from LA to BF = BF to LA	-0.69	3	7.38	6.34	0.0	0.205	0.1214	[0.1214]	0.6553	[0.6553]
BF pop. size = anc; Both mig. = 0	-1.78	2	7.55	6.51	0.0	0.448	0.3151	[0.4489]	[0]	[0]
BF pop. size = LA; Mig. from BF to LA = 0	-0.83	3	7.66	6.62	0.0	0.259	[0.2595]	0.5181	0	[0]
BF pop. size = anc; Mig. from LA to BF = BF to LA	-0.85	3	7.71	6.67	0.0	0.256	0.1392	[0.2568]	0.2261	[0.2261]
LA pop. size = anc; Mig. from BF to LA = 0	-0.86	3	7.73	6.69	0.0	0.403	0.1815	[0.1815]	0.1434	[0]
BF pop. size = LA & anc; Mig. from BF to LA = 0	-2.06	2	8.13	7.09	0.0	0.271	[0.2717]	[0.2717]	0.4307	[0]
Mig. from BF to LA = 0	-0.19	4	8.38	7.34	0.0	0.250	0.2732	0.5181	0	[0]
BF pop. size = anc; Mig. from BF to LA = 0	-1.60	3	9.21	8.17	0.0	0.265	0.0985	[0.2659]	1.0792	[0]
LA pop. size = anc; Both mig. = 0	-3.53	2	11.0	10.01	0.0	0.250	0.2846	[0.2846]	[0]	[0]
BF pop. size = LA & anc.; Both mig. = 0	-4.97	1	11.9	10.90	0.0	0.264	[0.2644]	[0.2644]	[0]	[0]

Figure 1

Hybrid scores based on the five diagnostic SNPS (Table 1).

Pure Black-footed Albatrosses are scored as 0 and pure Laysan Albatrosses are 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 the diagnostic loci.

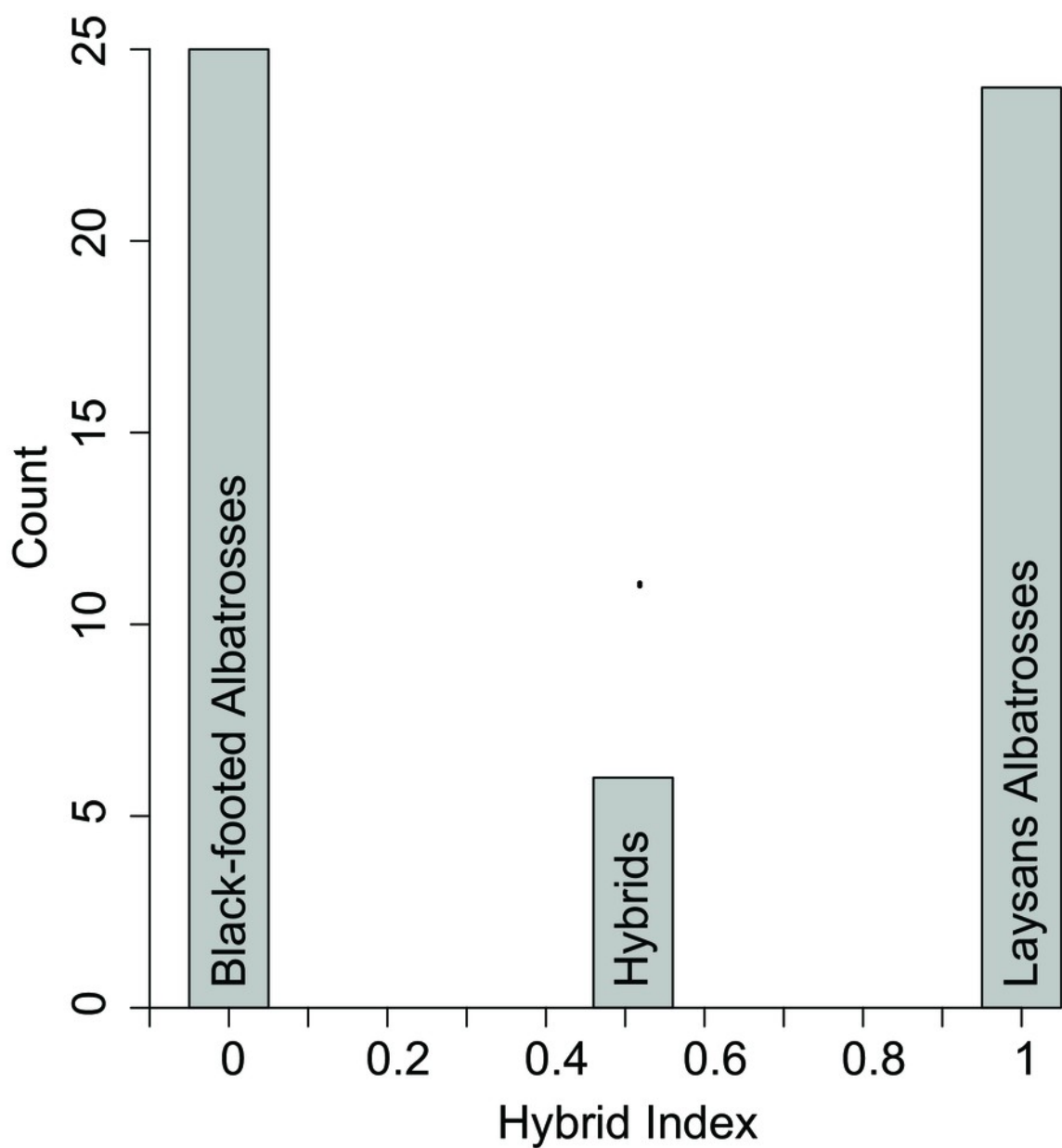


Figure 2

A recently documented hybrid that is mated to a Laysan Albatross and has raised chicks.

H. Ronco of the USFWS provided the photo.

