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1 **European colonisation, not Polynesian arrival, impacted population**
2 **size and genetic diversity in the critically endangered New Zealand**
3 **Kākāpō**

4

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18 Running header: Historical population genetics of kākāpō

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21

22 **Abstract**

23 Island endemic species are often vulnerable to decline and extinction
24 following human settlement, and the genetic study of historical museum
25 specimens can be useful in understanding these processes. The kākāpō
26 (*Strigops habroptilus*) is a critically endangered New Zealand parrot that was
27 formerly widespread and abundant. It is well established that both
28 Polynesian and European colonisation of New Zealand impacted the native
29 avifauna, but the timeframe and severity of impacts have differed depending
30 on species. Here we investigated the relative importance of the two waves of
31 human settlement on kākāpō decline, using microsatellites and mtDNA to
32 characterise recent kākāpō genetic and demographic history. We analysed
33 samples from 49 contemporary individuals and 54 museum specimens
34 dating from 1884 to 1985. Genetic diversity decreased significantly between
35 historical and contemporary kākāpō, with a decline in mean number of
36 microsatellite alleles from 6.15 to 3.08 and in number of mtDNA haplotypes
37 from 17 to 3. Modelling of demographic history indicated a recent population
38 bottleneck linked to the period of European colonisation (approximately 5
39 generations ago), but did not support a major decline linked to Polynesian
40 settlement. Effective population size estimates were also larger for historical
41 than contemporary kākāpō. Our findings inform contemporary kākāpō
42 management by indicating the timeframe and possible cause of the
43 bottleneck, which has implications for the management of extant genetic
44 diversity. We demonstrate the broader utility of a historical perspective in
45 understanding causes of decline and managing extinction risk in
46 contemporary endangered species.

47

48 **Keywords**

49 Endangered species; island endemic; museum DNA; population bottleneck;
50 parrot

51 **Subject areas**

52 Conservation genetics and biodiversity; population structure and
53 phylogeography

54

55

56 **Introduction**

57 The vulnerability of island endemic species to extinction is a well-known
58 phenomenon, with drivers of decline including anthropogenic factors such as
59 introduced predators (Blackburn *et al.*, 2004; Duncan & Blackburn, 2004)
60 and genetic factors such as inbreeding (Frankham, 1998; Jamieson, 2007).
61 However, disentangling causes of decline can be difficult, as islands have
62 often been colonised multiple times and drivers can act synergistically
63 (Brook, Sodhi & Bradshaw, 2008). Analysis of historical specimens using
64 ancient DNA techniques (Leonard, 2008; Ramakrishnan & Hadly, 2009) and
65 computational tools (Bruniche-Olsen *et al.*, 2014; Spurgin *et al.*, 2014) can
66 help in understanding the timing and magnitude of population bottlenecks,
67 as samples from small contemporary populations may be significantly
68 affected by genetic drift (Keller & Waller, 2002; Frankham, 2005; Allendorf &
69 Luikart, 2007), which can interfere with conclusions about historical
70 patterns and processes (Johnson, Dunn & Bouzat, 2007; Ramakrishnan &
71 Hadly, 2009; Spurgin *et al.*, 2014). Understanding the role of human activities
72 in the decline of endangered species can inform current management to
73 minimise risk of extinction (Leonard, 2008).

74 Multiple waves of human colonisation have led to dramatic declines of
75 endemic species on the islands of the Pacific Ocean (Steadman, 1995; Pimm
76 *et al.*, 2006). New Zealand has proven a particularly informative case study
77 for island endemic declines; it has a unique avifauna and is the most recently
78 colonised landmass, such that there is a relatively clear understanding of the

79 timing and impact of human activities (Hurles *et al.* 2003; Wilmshurst *et al.*
80 2008; Rawlence *et al.* 2012). Notable New Zealand endemics have been
81 driven extinct (Allentoft *et al.* 2014; Holdaway *et al.* 2014), while others have
82 experienced major declines (Taylor, Jamieson & Wallis, 2007; Tracy &
83 Jamieson, 2011; Shepherd *et al.*, 2012). Understanding the specific causes of
84 New Zealand avian declines requires differentiating between the impacts of
85 Polynesian (c. 1280 AD; Wilmshurst *et al.*, 2008) and European colonisation
86 (c. 1800s; Wilmshurst *et al.*, 2008), as their relative importance differs
87 depending on the species in question (Duncan & Blackburn, 2004; Bromham
88 *et al.*, 2012). For example, a small population of humans was responsible for
89 driving Moa to extinction (Allentoft *et al.*, 2014; Holdaway *et al.*, 2014),
90 however, many birds did not go extinct until after European colonisation and
91 still others remain extant today (Bromham *et al.*, 2012).

92 New Zealand's iconic and critically endangered (IUCN, 2014) kākāpō
93 (*Strigops habroptilus*) is an endemic parrot that was formerly widely
94 distributed and abundant (Fig. 1a) (Powlesland, Merton & Cockrem, 2006).
95 Observer and subfossil records suggest that kākāpō decline has been
96 occurring since human colonisation began with Polynesian arrival
97 (Department of Conservation, 1996; Powlesland *et al.*, 2006). After the 15th
98 century extirpation of larger fauna (Boessenkool *et al.*, 2008; Collins *et al.*,
99 2014; Holdaway *et al.*, 2014), kākāpō were one alternative food source for
100 Polynesians (Tipa, 2006). However, records suggest kākāpō remained locally
101 abundant in less intensively settled areas up to the 18th century, when
102 Europeans introduced new mammalian predators (Powlesland *et al.*, 2006).
103 While predators might have been the primary driver of kākāpō decline,
104 Polynesian hunting could have led to population fragmentation and made the
105 species vulnerable through the deleterious effects of inbreeding and genetic
106 drift. Consequently, the European introduction of mammalian predators may
107 have caused a rapid demographic decline in an already genetically
108 depauperate species (e.g. Palkopoulou *et al.* 2015). North Island kākāpō

109 likely went extinct in the early 20th century, and by the 1970s South Island
110 kākāpō were reduced to several males in Fiordland (southwest New Zealand)
111 (Lloyd & Powlesland, 1994; Powlesland *et al.*, 2006) and a small population
112 on Stewart Island, 30 km south of the South Island (Fig. 1a) (Powlesland *et*
113 *al.*, 1995).

114 There are 123 contemporary adult kākāpō (D. Eason, pers. comm.)
115 inhabiting three offshore islands (Fig. 1a); they are primarily descended from
116 Stewart Island individuals translocated in the 1990s, though Stewart Island
117 only represents a small fraction of their historical range (Powlesland *et al.*,
118 1995; 2006). At the time of these translocations, the kākāpō population
119 experienced a founder bottleneck of 51 individuals (Powlesland *et al.*, 2006;
120 Robertson 2006). An important aspect of current kākāpō conservation is
121 genetic management (Robertson, 2006; Neill, 2008), using tools such as
122 planned matings and artificial insemination to manage the risks of
123 inbreeding and genetic drift in a population descended from a small number
124 of founders (Robertson, 2006; Robertson *et al.*, 2011). Thus, a better
125 understanding of the timeframe and severity of kākāpō decline, and its
126 effects on genetic diversity, could inform contemporary management. In
127 addition, differentiating between the impacts of the two waves of human
128 settlement could identify important agents of decline in kākāpō.

129 In this study, we examine changes over time in kākāpō population size
130 and genetic diversity using nuclear and mitochondrial data from
131 contemporary and historical samples. We test for population bottlenecks
132 associated with Polynesian and European settlement; these hypothesised
133 bottlenecks are based on observational records of long-term, widespread
134 kākāpō decline after Polynesian settlement followed by rapid decline of
135 already isolated populations after European settlement. The well-
136 characterised nature of human influence in New Zealand allows us to
137 speculate as to which introduced threats (e.g. human settlement, introduced
138 predators) were most important in driving kākāpō decline. We use our

139 findings to guide current conservation of kākāpō, and discuss how a
140 historical approach is broadly useful in understanding population history
141 and managing genetic diversity in endangered species.

142 **Methods**

143 *Sampling*

144 Blood samples from 49 individuals (Table S1) considered founders of the
145 contemporary kākāpō population were collected between 1985 and 2005; one
146 individual was from Fiordland and 48 were from Stewart Island (Robertson, 2006).
147 We refer to all founders of the contemporary population (aside from the Fiordland
148 individual) as Stewart Island individuals although all kākāpō originating from
149 Stewart Island have since been translocated to smaller offshore islands (Fig. 1). In
150 this study, we focus only on founders, as genetic composition of individuals on
151 current offshore islands has likely been highly influenced by the bottleneck of 51
152 individuals and current kākāpō genetic management. We also examined one
153 historical blood sample and 54 museum skins from six regions across the former
154 range of kākāpō (Fig. 1b, Table S2).

155 *DNA extractions, amplification and sequencing*

156 For contemporary samples, genomic DNA was extracted from whole blood using a
157 5% Chelex protocol (Walsh, Metzger & Higuchi 1991). Genomic DNA was extracted
158 from museum skins using a DNeasy Blood & Tissue Kit (Qiagen); appropriate
159 precautions were taken to minimise the risk of contamination in historical samples
160 (Gilbert *et al.*, 2003; Sefc, Payne & Sorenson, 2007; Allentoft *et al.*, 2011; Knapp *et*
161 *al.*, 2012; Dussex, Rawlence & Robertson, 2015).

162 We designed four primers based on a published kākāpō mitochondrial
163 genome (Harrison *et al.*, 2004) to amplify and sequence a c. 1200 bp fragment of the
164 hypervariable mitochondrial DNA (mtDNA) control region in contemporary kākāpō
165 (Table S3). For museum samples, primers were designed to amplify and sequence
166 four overlapping fragments of approximately 200 bp, yielding a 420 bp subset of the
167 control region (Supplementary Methods; Table S4). All variable sites that defined

168 less common museum sample haplotypes (haplotypes occurring in less than 10
169 samples) were confirmed by repeating the PCR and sequencing at least twice.

170 Contemporary and historical kākāpō were genotyped at 13 microsatellite loci
171 (Supplementary Methods; Table S5) using methods described previously
172 (Robertson *et al.*, 2009; Bergner, Jamieson & Robertson, 2014; White *et al.*, 2014a).
173 In historical kākāpō, all samples were independently amplified at least five times in
174 order to decrease the chances of allelic dropout (Allentoft *et al.*, 2011).

175

176 *Genetic diversity*

177 Mitochondrial genetic diversity indices (number of haplotypes, nucleotide diversity,
178 number of segregating sites, haplotypic diversity) were calculated using the *pegas*
179 package (Paradis, 2010) in R (R Core Team, 2014). Many of the variable sites in the
180 kākāpō control region were defined by insertions/deletions (indels), including
181 multiple base-pair indels. Many sequence analysis programs consider indels by
182 coding gaps as a fifth character, thus a five base-pair deletion would be considered
183 to be five mutational events even though it could also be one five base-pair
184 mutational event. Consequently, an R function was written to code multiple base-
185 pair indels as one mutational event, and this was used to calculate diversity indices
186 for contemporary and historic samples (D. Winter, pers. comm.).

187 We also examined temporal haplotype variation by creating one haplotype network
188 per sampling period (historical and contemporary) using the statistical parsimony
189 method implemented in TCS (Clement, Posada & Crandall, 2000). We also analysed
190 all samples together to infer relationships between haplotypes not present during
191 the same time period, and combined the networks to visualise the changes in
192 haplotypes through time.

193 For microsatellite data, we checked for scoring error using MICRO-CHECKER
194 2.2.3 (Van Oosterhout *et al.*, 2004). Tests for Hardy-Weinberg proportions and
195 genotypic disequilibrium were conducted using exact probability tests with 1000
196 iterations in GENEPOP 4.1 (Rousset, 2008). An unbiased estimate of the exact P-

197 value was determined using a Markov chain method following the permutation
198 algorithm of Guo & Thompson (1992). The significance threshold values for multiple
199 statistical tests was adjusted using a sequential Bonferroni correction (adjusted
200 $\alpha=0.003$) (Rice, 1989). Indices of genetic diversity were calculated for both
201 contemporary and historical samples. To assess the level of genetic variation in
202 microsatellites, FSTAT (Goudet, 1995) was used to calculate mean allelic richness,
203 which corrects for unequal sample sizes among sampled populations (El Mousadik
204 & Petit, 1996), and GENALEX (Peakall & Smouse, 2006) was used to estimate
205 observed and expected heterozygosity. Statistical comparison between measures of
206 microsatellite diversity in contemporary and historical samples were matched by
207 loci and tested using a pairwise Wilcoxon signed-ranked test (adjusted $\alpha=0.003$) in
208 R (R Core Team, 2014).

209

210 *Population structure*

211 Pairwise F_{ST} values were calculated between contemporary and historical groups, as
212 well as between the six historical regions, using ARLEQUIN 3.5 (Excoffier & Lischer,
213 2010) for mtDNA data and FSTAT for microsatellite data. A Mantel test (10,000
214 permutations) in GENEPOP and linear regression in R were used to test for isolation
215 by distance using both mtDNA and microsatellite genetic distances; these
216 relationships were examined between historical individuals and regions.

217 For microsatellite data, genetic structure was assessed with Bayesian
218 clustering methodology implemented in STRUCTURE 2.3.3 (Pritchard, Stephens &
219 Donnelly, 2000; Falush, Stephens & Pritchard, 2003) using an admixture model with
220 correlated allele frequencies. Twenty iterations (chain length 500,000 steps, burn-in
221 200,000 steps) were performed for each K (1 to 7 populations). The number of
222 distinct genetic clusters was inferred using the ΔK (Evanno, Regnaut & Goudet,
223 2005) method implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012).
224 Multiple runs of the selected K were averaged using CLUMPP (Jakobsson &
225 Rosenberg, 2007) and the output was visualised using DISTRUCT (Rosenberg,

226 2003). This analysis was performed for both contemporary and historical samples,
227 and for historical samples only.

228 Because STRUCTURE attempts to maximise HWE for each value of K, results
229 can be biased in non-equilibrium populations (Jombart, Devillard & Balloux, 2010).
230 We also applied Discriminant Analysis of Principal Components (DAPC, Jombart et
231 al. 2010) using the *adegenet* package (Jombart & Ahmed, 2011) in R (R Core Team
232 2014). This analysis was performed with and without prior information on
233 individual populations.

234

235 *Characterisation of bottlenecks*

236 We used an approximate Bayesian computation (ABC) approach (Beaumont,
237 Zhang & Balding, 2002) implemented in DIYABC 1.0.4 (Cornuet, Ravignie & Estoup,
238 2010) to test for population bottlenecks and estimate values of key demographic
239 parameters. Specifically, our models were designed so that we could distinguish the
240 potential impacts of Polynesian and European arrivals on kākāpō demography by
241 varying the timing of bottlenecks. We assumed a generation time of 25 years for
242 kākāpō (G. Elliott, pers. comm.). Four models were tested: a (i) model of ‘Polynesian
243 bottleneck’ described a bottleneck occurring 15 to 50 generations ago, a period
244 encompassing the arrival of Polynesians to New Zealand; a (ii) model of ‘European
245 bottleneck’ described a more recent bottleneck occurring 1 to 15 generations ago; a
246 (iii) model of ‘Polynesian and European bottlenecks’ combined the previous two
247 models; finally, a (iv) null model of ‘constant population size’ since the last
248 glaciation was also tested with N_e uniformly distributed through time (for 0 to 500
249 generations). We tested these models using both microsatellite and mtDNA data
250 from mainland (historical only, n=45) samples and Stewart Island (n=48
251 contemporary, n=2 historical) samples, resulting in a total of 8 models tested.

252 For each model, 1 million datasets were simulated with the defined
253 demographic and marker parameters (Supplementary Methods; Table 2). For the
254 Stewart Island population, we considered one contemporary sample collected at
255 time t=0 generations and one historical sample collected at time t=4 generations in
256 each model. For the mainland population, we only considered historical samples

257 (we excluded Richard Henry, the contemporary Fiordland individual), such that all
258 samples were taken either before or during the presumed European bottleneck.

259 As summary statistics for microsatellite data, we used the mean number of
260 alleles across loci, mean gene diversity (Nei, 1987), Garza and Williamson's $M's$
261 within each population, and the overall number of alleles across loci and mean gene
262 diversity (Nei 1987). For mtDNA, summary statistics used were number of
263 haplotypes, number of segregating sites, mean of pairwise differences and Tajima's
264 D within each population (these statistics did not include indels).

265 Normalised Euclidian distances were calculated between the observed
266 dataset and each of the simulated datasets using the local linear regression method
267 of Beaumont et al. (2002). The posterior probabilities of each scenario were
268 estimated using both the direct and logistic regression approaches (Fagundes *et al.*,
269 2007; Cornuet *et al.*, 2010). The ten thousand datasets (1%) with the smallest
270 Euclidian distances were then retained to build posterior parameter distribution,
271 which were smooth-weighted using the *Locfit* function within R (Loader 2007).
272 Further details on parameter priors, mutation models and model checking can be
273 found in the Supplementary Methods.

274

275 *Population size estimates*

276 We estimated N_e in contemporary kākāpō using the bias-corrected Linkage
277 Disequilibrium (LD) method (Waples, 2006) implemented in NeEstimator 2.01 (Do
278 *et al.*, 2014). Because contemporary kākāpō are intensively monitored by the
279 Department of Conservation (Eason *et al.*, 2006), we compared our contemporary
280 genetic estimates of N_e with observed number of adults (N_a) and number of
281 breeding adults (N_b) in recent breeding years (Supplementary Methods; Table S7,
282 S8).

283 We then estimated historical variation in N_e before European arrival using the
284 coalescent approach implemented in MSVAR 1.3 (Storz & Beaumont, 2002) and 14
285 historical samples from Fiordland collected between 1884 and 1898
286 (Supplementary Methods). This reduced sample set was used to avoid the detection

287 of a false bottleneck associated with overlapping generations or potential
288 population differentiation (Chikhi *et al.*, 2010).

289 *Data Availability*

290 In accordance with data archiving guidelines (Baker 2013), we have deposited the
291 mtDNA sequences and microsatellite genotypes in Genbank and Dryad (pending MS
292 number).

293 **Results**

294 *Genetic diversity*

295 We analysed 420 bp of the mtDNA control region for contemporary (n=45) and
296 historical (n=51) kākāpō. Contemporary (n=49) and historical (n=47) kākāpō were
297 genotyped at 13 microsatellite loci. Loci Strhab13, Strhab33, and Strhab41 showed
298 significant departure from Hardy-Weinberg equilibrium after Bonferroni correction,
299 but only among historical Fiordland samples. No tests for linkage disequilibrium
300 were significant. Genetic diversity was greater among historical samples for both
301 mtDNA and microsatellite data (Table 1, S5). For microsatellite data, there were
302 significant differences between contemporary and historical samples for all
303 measures of diversity aside from H_o (Table S5). The haplotype network indicated a
304 loss of genetic diversity between historical and contemporary kākāpō (Fig. 2). There
305 was only one historical haplotype present in contemporary kākāpō, while two of the
306 three contemporary haplotypes were not represented in historical samples.
307 Between historical and contemporary samples, there was a decrease in h , S , Hd , and
308 indel π , though not in π (Table 1).

309 *Population structure*

310 There was no apparent geographic pattern of mtDNA population structure in either
311 historical or contemporary kākāpō (Fig. 1b,c). F_{ST} estimates between contemporary
312 and historical samples were significant for both mtDNA ($F_{ST} = 0.395$) and
313 microsatellites ($F_{ST} = 0.164$) (Table S9a). F_{ST} values were low and non-significant for
314 microsatellites and mtDNA between Fiordland and Westland, the only historical

315 regions with sufficiently large sample sizes for comparison (Table S9b). We also did
316 not observe evidence for isolation by distance between historical regions or
317 individuals (Fig. S1).

318 STRUCTURE did not identify population differentiation among historical
319 samples, but identified contemporary samples as a distinct cluster (Fig. 3a, c, S2).
320 One contemporary individual (Richard Henry, originating from Fiordland) grouped
321 with the historical cluster with a high membership score ($q = 0.83$; Table S10). DAPC
322 results supported STRUCTURE clustering with a strong distinction between
323 historical and contemporary individuals, with Richard Henry (black square)
324 clustering with historical individuals (Fig. 3b, d). The two historical Stewart Island
325 individuals (blue) grouped distinctly from contemporary Stewart Island individuals
326 (red) (Fig. 3d), indicating the presence of a mainland lineage on the island when
327 historical samples were collected around 1847 (Dawson 1962).

328 *Characterisation of bottlenecks*

329 When testing for population bottlenecks one scenario was strongly supported
330 (posterior probabilities of 99%) for each of the mainland and Stewart Island; a
331 scenario of ‘European bottleneck’ for Stewart Island, and a scenario of ‘constant
332 population size’ for the mainland. All other alternative scenarios for both locations
333 obtained posterior probabilities of <1%.

334 For the ‘European bottleneck’ scenario on Stewart Island, population size estimate
335 for historical $N_{e-historical-St}$ was 1,623 (100 – 143,468) and 2.3 (1.5 – 49.3) for
336 contemporary N_{e-St} . The estimated time since the population bottleneck was 5.07
337 generations (4.56 – 17.66), or approximately 125 years (Table 2). Type I and Type II
338 error rates were 0.84 and 0.06 respectively.

339 For the ‘constant population size’ scenario on the mainland, population size
340 estimate for $N_{e-main-pre-EU}$ was 46,764 (4915 – 163,789). Type I and Type II error rates
341 were both 0.02.

342 *Population size estimates*

343 Contemporary genetic N_e estimates were 25.9 (16 - 45.8) for LD and 2.3 (1.5 - 49.3)
344 for ABC, while our empirical estimates based on breeding records were 18 (7.75 -
345 28.25) for N_b and 56 (40.59 - 71.41) for N_a (Table 3). The MSVAR analysis did not
346 support a population decline prior to European arrival, with overlapping posterior
347 distributions for pre-Polynesian N_{HP} and pre-European N_{HE} , and modes of 1,683 (22
348 - 9,316) and of 2,528 (20 - 1,038,159) respectively. The ratio of the posterior
349 distributions of contemporary and historical population sizes ($r = N_c / N_H$) was 1.5.
350 Posterior distributions overlapped (Table S11, Fig. S3) and the Bayes factor value
351 was 0.5, giving no support to a pre-European population decline.

352 **Discussion**

353 Kākāpō have experienced a sharp population decline and striking loss of genetic
354 diversity most likely in association with European colonisation. The lack of support
355 for a decline associated with Polynesian settlement, the estimated timeframe of
356 decline on Stewart Island, and the non-detection of a bottleneck prior to European
357 arrival on the mainland further support a scenario of a stable kākāpō population at
358 European arrival. Our findings suggest that mammalian predators introduced by
359 Europeans may have been an important agent of decline in kākāpō. The rapid
360 decline resulted in a small contemporary population exhibiting low levels of genetic
361 diversity that are not reflective of historical patterns.

362 The loss of microsatellite and mtDNA genetic diversity in kākāpō shows the
363 stochastic nature by which genetic variation is lost through population bottlenecks
364 (Miller & Hedrick, 2001; Bouzat, 2010). Only one haplotype, which was relatively
365 uncommon historically, was shared between contemporary and historical kākāpō.
366 This could be due to biased sampling in different areas at different time periods, but
367 similarly striking shifts in haplotype frequency have been observed in other species
368 that have experienced a population bottleneck (e.g. Glenn, Stephan & Braun, 1999;
369 Weber, Stewart & Lehman, 2004; Xenikoudakis *et al.*, 2015). The rapid decline of
370 South Island kākāpō and the prolonged period of small population size and
371 inbreeding on Stewart Island (Jamieson, Wallis & Briskie, 2006; Robertson, 2006)

372 both likely contributed to the low contemporary diversity.

373 The ABC analysis did not support the hypothesis of a pre-European
374 population bottleneck in kākāpō on the mainland, and the MSVAR analysis further
375 rejected this hypothesis based on our analysis of mainland historical samples only
376 (1884 to 1898). However, we did observe support for a recent reduction in kākāpō
377 population size in line with the timing of European colonisation. Estimates from
378 both ABC and MSVAR indicated a larger historical N_e than contemporary N_e , which is
379 consistent with the remaining population size of 123 adult birds and observational
380 records. Indeed, Buller (1888) stated that “According to native tradition, the Kakapo
381 was formerly abundant all over the North and South Islands; but at the present day
382 its range is confined to circumscribed limits, which are becoming narrower every
383 year.” All of the contemporary estimates, both genetic and empirical, indicated
384 smaller population sizes with overlapping confidence intervals. Empirical estimates
385 of contemporary N_b are much smaller than N_a , which is likely exacerbated by a
386 polygynous mating system (Nunney, 1996; Hedrick, 2005) found in kākāpō (Merton,
387 Morris & Atkinson, 1984; Clout, Elliott & Robertson, 2002; Eason *et al.*, 2006)

388 It is important to mention that despite the large Type I error for the scenario of
389 ‘European bottleneck’ for Stewart Island, this scenario is still the most likely. Indeed,
390 widening prior range (from 15-50 to 1-50 generations ago) for the timing of
391 bottleneck in the alternative scenario of ‘Polynesian bottleneck’ produced a similar
392 mode of ~ 5 generations (Results not shown). Also, estimating parameters for the
393 alternative scenario of ‘Polynesian and European bottlenecks’ produced a flat
394 distribution for the timing of the Polynesian bottleneck (meaning an inability to date
395 a decline) and a mode of ~ 5 generations for the European bottleneck. The high
396 Type I error might be explained by the low historical sample size for the Stewart
397 Island population ($n=2$). In addition, the small sample sizes from most historical
398 regions also make conclusions about historical population structure tentative; there
399 may have been substructure between regions, as suggested by heterozygote
400 deficiencies at several loci. However, historical population structure was not

401 detected based on either STRUCTURE results from only historical individuals or
402 DAPC, the latter of which is free of assumptions about HWE. Our ability to better
403 sample across regions is limited by the availability of pre-European kākāpō museum
404 samples for this project, but future studies including older samples, such as those
405 from Māori middens or pitfalls (e.g. Trewick, 1997), could shed more light on
406 historical kākāpō demography and the impacts of Polynesian colonisation on the
407 species.

408 Although several lines of evidence in our analyses support a European
409 bottleneck (e.g. lack of support for Polynesian decline, loss of genetic diversity
410 between contemporary and historical samples), we cannot completely rule out an
411 older bottleneck associated with Polynesian settlement that was over-ridden by
412 strong genetic drift from a secondary European bottleneck. Given the HPD intervals
413 of the bottleneck timing from the ABC analysis for Stewart Island (maximum 17.66
414 generations which equates to 441.5 years), we cannot exclude a Polynesian
415 bottleneck, given that this encompasses a time period before European colonisation.
416 Our MSVAR results indicate that prior to European settlement, kākāpō on the
417 mainland had a constant population size, suggesting there was no Polynesian-
418 associated bottleneck. However, it remains a possibility that there were two
419 bottleneck events in kākāpō associated with human settlement, with the more
420 recent European bottleneck eclipsing the effects of a previous Polynesian
421 bottleneck. It is also worth noting that our mainland samples only represent the
422 South Island, so North Island kākāpō may well have a different demographic history
423 given that the island was settled earlier and more intensively than the South Island.

424 The star-like mtDNA haplotype network detected for kākāpō, which centers
425 around haplotypes from the southwest South Island, could indicate a historical
426 population expansion from this region (Slatkin & Hudson, 1991; Rogers &
427 Harpending, 1992), possibly following the Last Glacial Maximum (~10,000 years
428 ago). Though different species show different patterns of post-glacial colonisation
429 (Trewick & Wallis, 2001; Wallis & Trewick, 2009; Dussex, Wegmann & Robertson,

430 2014; Weston & Robertson, 2015), many New Zealand forest species were limited to
431 refugia during glacial maxima (Leschen *et al.*, 2008; Shepherd & Lambert, 2008;
432 Marshall *et al.*, 2009), when glaciers covered much of the South Island (McGlone,
433 1985).

434 The timeframe of population decline and recovery are important
435 considerations in conservation management and specifically in kākāpō. The recent
436 population decline on Stewart Island (mode of 125 years ago) could explain a lag
437 between demographic decline and a genetic bottleneck signal, as it may take several
438 generations for genetic drift to act. This is especially true in kākāpō, where long
439 generation time might slow genetic drift associated with a population bottleneck
440 because of a shorter effective time of exposure to bottleneck (Hailer *et al.*, 2006;
441 White *et al.*, 2014b; Dussex *et al.*, 2015). In any case, a severe kākāpō population
442 decline happened relatively recently, which has implications for genetic diversity. If
443 a population becomes small quickly, genetic diversity may be lost more rapidly than
444 in a gradual reduction, although this depends on the measure of diversity (Nei,
445 Maruyama & Chakraborty, 1975; Allendorf, 1986; England *et al.*, 2003). Time to
446 recovery is also important, as the chances of losing genetic diversity are greater in a
447 severe and prolonged bottleneck than in the event of a quick demographic recovery
448 (Sutton *et al.*, 2011; Jansson *et al.*, 2012). A rapid population decline and loss of
449 historical genetic diversity in kākāpō are consistent with previous findings of low
450 contemporary genetic diversity (Robertson, Minot & Lambert, 2000; Robertson,
451 2006; Bergner *et al.*, 2014) and evidence of inbreeding depression (White *et al.*,
452 2014a). We recommend, in keeping with the current kākāpō recovery plan (Neill,
453 2008), prioritising an urgent increase in population size. Our findings also support
454 current methods of managed breeding to maintain existing genetic diversity using
455 pedigrees (Bergner *et al.*, 2014) and artificial insemination (Robertson *et al.*, 2011).

456 Estimates of historical N_e based upon museum samples can also provide
457 more accurate conservation targets than those based solely on contemporary
458 individuals (Leonard, Vilà & Wayne, 2004; Alter, Newsome & Palumbi, 2012). As

459 kākāpō are currently restricted to offshore islands, attaining historical South Island
460 population sizes is unlikely. A proposal to eliminate mammalian predators across
461 New Zealand (Russell *et al.*, 2015) might make mainland populations of kākāpō at
462 historical sizes achievable, but this remains a theoretical scenario. Mammalian
463 predators introduced by Europeans could have been a key driver of kākāpō decline,
464 suggesting that eliminating these predators should be priority in an eventual
465 mainland reintroduction.

466 To understand different histories of decline in endangered species, it is
467 important to consider both the unique traits of each species and differences in
468 human impacts. New Zealand avifauna with similar traits to kākāpō, including
469 flightlessness and large size, were disproportionately driven extinct by Polynesian
470 settlement (Bromham *et al.*, 2012). For example, Polynesians directly exploited both
471 adult Moa and their eggs for food (Anderson, 1989; Allentoft *et al.*, 2014; Holdaway
472 *et al.*, 2014). In contrast, while Polynesians hunted kākāpō for food and skins, our
473 study suggests that mammalian predation rather than human hunting may have
474 been more important in kākāpō decline. Indeed, different combinations of human
475 hunting and mammal predation may have led to the extinction of similarly sized
476 avian species, such as penguins (Boessenkool *et al.*, 2008) and Chatham Island
477 parrots (Wood *et al.*, 2014).

478 A historical genetic perspective is generally useful for guiding contemporary
479 management to minimise risk of extinction in endangered species (Leonard, 2008).
480 Genetic analysis of historical and contemporary samples has been used to
481 understand recent history and inform conservation of other iconic New Zealand
482 birds such as kea (*Nestor notabilis*, Dussex *et al.*, 2014; 2015), kiwi (*Apteryx* spp.,
483 Shepherd & Lambert, 2008; Shepherd *et al.*, 2012), and takahē (*Porphyrio*
484 *hochstetteri*, Grueber & Jamieson, 2011). However, despite close evolutionary
485 relatedness (kea and kākāpō) and similar traits (kākāpō, kiwi, takahē), each species
486 exhibited a different response to human colonisation, highlighting the importance of
487 considering each one separately. The genetic analysis of contemporary and

488 historical individuals allows an understanding of how much diversity has been lost,
489 as well as the probable timeframe and cause, resulting in a powerful approach for
490 disentangling the complicated history of decline in island endemic species.

491

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505 **Tables and Figures**

506 **Table 1:** Estimates of genetic diversity for the mtDNA control region and 13
507 microsatellite loci for historical and contemporary kākāpō with the sample size (n),
508 number of observed haplotypes (h), number of segregating sites (S), haplotype
509 diversity (Hd), nucleotide diversity (π), nucleotide diversity considering indels as
510 one mutational event (Indel π), mean number of alleles (A), allelic richness (R_s),
511 observed (Ho) and expected heterozygosity (He) with their respective standard
512 deviations (SD) in brackets.

513

514

	mtDNA						Microsatellites			
	<i>n</i>	<i>h</i>	<i>S</i>	<i>Hd</i>	π	Indel π	<i>A</i>	<i>Rs</i>	<i>Ho</i>	<i>He</i>
Historical	51	17	21	0.82	0.00104	0.003	6.15 (2.08)	5.43 (1.81)	0.5 (0.15)	0.64 (0.11)
Contemporary	45	3	8	0.56	0.00114	0.002	3.08 (1.04)	1.44 (0.14)	0.43 (0.17)	0.43 (0.14)

515

516

517 **Table 2:** Prior and posterior distributions of parameters for scenarios of the
 518 ‘European bottleneck’ for Stewart Island (a) and the ‘constant population size’ for
 519 the mainland (b) that obtained the highest posterior probability when comparing
 520 scenarios of bottleneck in kākāpō. Timing of events is in generations, assuming a
 521 generation time of 25 years in kākāpō.

522 (a)

Parameter	Prior distribution	Posterior mode	5% HPD	95% HPD
$N_{e-St-pre-EU}$	uniform [10 - 5×10^5]	1,623	100	143,486
N_{e-St}	uniform [2 - 500]	2.30	1.50	49.30
tEU (bottleneck time)	uniform [1 - 20]	5.07	4.56	17.66
μ rate (microsatellite)	uniform [10^{-6} - 10^{-3}]	3.7×10^{-5}	1×10^{-5}	9.71×10^{-5}
μ rate (mtDNA)	uniform [10^{-8} - 10^{-7}]	4.8×10^{-8}	1×10^{-8}	9.3×10^{-8}

523

524 (b)

Parameter	Prior distribution	Posterior mode	5% HPD	95% HPD
$N_{e-main-pre-EU}$	uniform [10^2 - 5×10^5]	46,764	4,915	163,789
μ rate (microsatellite)	uniform [10^{-6} - 10^{-3}]	6.11×10^{-5}	1.21×10^{-5}	9.9×10^{-5}
μ rate (mtDNA)	uniform [10^{-8} - 10^{-7}]	3.2×10^{-8}	3.2×10^{-8}	1×10^{-7}

525

526 **Table 3:** Estimates of historical and contemporary effective population size (N_e)

527 based on genetic data in kākāpō using the Linkage Disequilibrium (LD) and
 528 Approximate Bayesian Computation (ABC) methods. Estimates of contemporary
 529 number of adults (N_a) and number of breeding adults (N_b) are based on monitoring
 530 data.

Parameter	Method	Estimate
$N_{e-main-pre-EU}$	ABC	46,764 (4,915-163,789)
$N_{e-St-pre-EU}$	ABC	1,623 (100-143,486)
N_{e-St}	ABC	2.30 (1.50-49.30)
$N_{e-contemporary}$	LD	25.9 (16-45.8)
N_a	Empirical	56 (40.59 - 71.41)
N_b	Empirical	18 (7.75 - 28.25)

531

532 **Fig. 1:** (a) Geographic distribution of historical and contemporary kākāpō
 533 populations (adapted from Miller *et al.* 2003; Department of Conservation 1996).
 534 The hatched area shows locations of kākāpō fossil or subfossil remains, and the grey
 535 shaded area shows the maximum historical kākāpō distribution after 1840.
 536 Breeding populations of contemporary kākāpō are restricted to the three offshore
 537 islands denoted by stars, Hauturu (Little Barrier) Island, Whenua Hou/Codfish
 538 Island and Anchor Island. These contemporary kākāpō populations consist of both
 539 founders and their descendants, but only founders are included in this study.
 540 Distribution of (b) historical and (c) contemporary (founder) kākāpō mtDNA control
 541 region haplotypes. Sample sizes are shown within the circles and colours
 542 correspond to unique haplotypes.

543 **Fig. 2:** Haplotype networks for contemporary and historical kākāpō based on 420
 544 bp of mtDNA control region sequence. Colours correspond to geographic locations;
 545 numbers within each circle correspond to haplotype number from Fig. 1 and sample
 546 size. All haplotypes differ by a single difference unless noted on the line connecting
 547 the two. Empty circles represent haplotypes absent from our historical samples.
 548 Dotted lines represent inferred connections to haplotypes not present in the same

549 time period; dashed lines connect haplotypes through time.

550 **Fig. 3:** Individual clustering assignment for kākāpō with (a) a priori clustering of
551 individuals ($n = 96$) with the lowest $\ln(K)$ implemented in STRUCTURE ($K\text{-max} = 2$)
552 and (b) without a priori clustering with a DAPC. Results obtained for $K\text{-max} = 3$
553 shown for comparison. Vertical bars represent individuals, and colours correspond
554 to specific clusters. (c) Clustering assignments for historical kākāpō only ($K\text{-max} = 3$
555 and $K\text{-max} = 2$). (d) DAPC analysis for kākāpō with a priori clustering where each
556 point represents an individual, and ellipses represent 95% confidence limits for
557 population-level groups. The one contemporary individual from Fiordland (Richard
558 Henry) clusters with historical samples (contemporary red bar in barplots and black
559 square in scatterplot).

560

561

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