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1 **Population genetics informs the management of a controversial Australian waterbird**

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12 **Running head:** Australian white ibis genetics  
13

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37

38 **Key words**

39 Australian white ibis; Effective population size; Population genetics; Urbanisation; Waterbird conservation  
40

41 **Abstract**

42 Widespread degradation across Australia's inland wetland network has contributed to severe declines for many  
43 waterbird species. In contrast, breeding colonies of the Australian white ibis (*Threskiornis molucca*) have  
44 increased in urbanised areas along the coast, but the level of dispersal and gene flow between inland and coastal  
45 areas remain unknown. This study uses single nucleotide polymorphisms (SNPs) to ascertain the variables  
46 influencing genetic connectivity among several inland and urban colonies of white ibis across south-eastern  
47 Australia between 2015 and 2018. The contemporary effective population size was estimated, and this value was  
48 used in simulations to evaluate the impact of various management scenarios on future genetic diversity. We  
49 found no significant differences in allele frequencies between localities, or robust evidence of site fidelity,  
50 therefore suggesting widespread dispersal and gene flow between inland and urban colonies. Furthermore,  
51 effective sizes were large enough to maintain genetic diversity into the future under various realistic  
52 management scenarios. However, the lack of genetic partitioning found suggests that urban management of the  
53 ibis should not be undertaken in isolation of the conservation requirements of inland colonies.

54

55 **Introduction**

56 Increasing urbanisation around the globe has facilitated the growth of large populations of urban wildlife  
57 (Francis and Chadwick 2012; Low 2017; Luniak 2004). Wildlife management plans now need to accommodate  
58 synurbic species, i.e. those adapted to both urban and native habitats (Luniak 2004). For example, they may  
59 need to consider complex source-sink dynamics arising between native and urban populations (Marten et al.  
60 2006) or the risk of urban culling practices negatively impacting non-urban populations via 'ecological traps'  
61 (Hale and Swearer 2016). Altered connectivity between native and urban localities may arise due to  
62 anthropogenic barriers, differences in resource availability or the consequences of intense urban management,  
63 among other driving forces (Allendorf et al. 2008; Francis and Chadwick 2012). Although urban-adapted  
64 populations may respond favourably to increased urbanisation intensity, native populations may be impacted via  
65 inbreeding or outbreeding depression and reduced effective population sizes ( $N_e$ ) (Frankham 2005; Lowe and  
66 Allendorf 2010). In turn, the long-term persistence of these populations may be limited owing to reduced  
67 evolutionary potential and adaptive responses (Frankham 2005; Lowe and Allendorf 2010). Incorporating  
68 knowledge of these genetic processes into management plans can help define appropriate management units  
69 (DeYoung and Honeycutt 2005) and ensure that the management of urban populations is balanced with the  
70 conservation of native ones.

71

72 Australia's waterbirds are well adapted to navigating the transient mosaic of wetlands that characterise the  
73 interior of the continent, yet anthropogenic processes have complicated their conservation and management  
74 (Kingsford et al. 2010; Roshier et al. 2001). Critical flow thresholds reached during high rainfall and sequential  
75 flooding periods of inland wetlands ('boom' phases) support successful breeding in large colonies, while dry  
76 ('bust') phases encourage widespread dispersal (Kingsford et al. 1999; Roshier et al. 2002). However, water  
77 resource development, pollution and climate change have contributed to altered wetland flow regimes  
78 (Arthington and Pusey 2003). Colony success is being undermined by a breakdown of 'boom' and 'bust' cycles  
79 and the aridification of key refugia sites, leading to drastic declines in waterbird abundance across major  
80 wetland basins (Brandis et al. 2018; Davis et al. 2013; Kingsford et al. 2017; Leslie 2001). In a rapidly changing  
81 landscape, artificial wetlands increasingly provide habitat for waterbirds, with a few species becoming  
82 permanent urban residents (Martin et al. 2010; Murray et al. 2013).

83

84 The genetic diversity and extent of gene flow between these recently urbanised Australian waterbirds and their  
85 original wild populations is poorly understood. Previous research on Australia's waterbirds has explored trends  
86 in biodiversity and distribution in relation to seasonal water availability and river regulation levels (Kingsford et  
87 al. 2004; Wen et al. 2016), while genetic research has primarily explored the impacts of vast geographic  
88 barriers, such as deserts and oceans, on gene flow and genetic diversity (Guay et al. 2010; Robertson and  
89 Gemmell 2002; Szczyz et al. 2005). It remains less clear whether urbanisation and resource availability affect  
90 gene flow and connectivity in synurbic Australian waterbirds traditionally relying on inland wetlands.

91

92 The Australian white ibis (*Threskiornis molucca*, hereafter referred to as ibis) is a recently urbanised colonial  
93 Australian waterbird. Rapid growth in urban regions may have been ignited by several independent  
94 introductions to parks between the 1960s and 80s and fuelled by inland wetland degradation, drought and the  
95 availability of urban resources (Ross 2004). Management of urban ibis has become necessary owing to the  
96 diseases they carry (Epstein et al. 2006), potential negative impacts on other native species (Underwood and  
97 Bunce 2004), collisions with aircraft (Australian Transport Safety Bureau 2002) and their status as a public pest.

98

99 Management practices such as nest destruction, egg-oiling and limiting access to food sources at parks and  
100 landfill, have been employed by local councils, though regional impacts remain unknown and these strategies  
101 may counteract efforts by fragmenting colonies and encouraging breeding elsewhere (Martin et al. 2007; Ross  
102 2004; Shaw 1999). Urban ibis counts now reach into the tens of thousands across eastern Australia (Porter et al.  
103 2015), while inland colonies have continued to decline, with fewer than 3000 reported annually over the past  
104 few years (R.T. Kingsford et al., University of New South Wales, unpublished data, Porter et al. 2017).

105

106 For the ibis, it is unknown what impact urbanisation may have on local or regional gene flow, whether inland  
107 and urban colonies are interbreeding or whether urban management has genetic consequences. Movement of ibis  
108 across the Great Dividing Range is likely given their high dispersal propensity and the fact that the 1.5% growth  
109 rate in urban regions cannot account for increases along the coast during the breeding season (Smith et al.  
110 2013b), which predominantly occurs between June and February in urban regions (Smith et al. 2013a). High  
111 resighting rates within 100km of the eastern coastline also suggest that ibis use the coastline as a landmark for  
112 migration (Smith and Munro 2011), which may facilitate gene flow across the eastern states. However, the  
113 broad-scale movements of waterbirds are strongly influenced by fluctuating resource availability (Roshier et al.  
114 2008). The presence of stable urban resources may be linked to higher reproductive and fledgling success, site  
115 fidelity, and shorter daily foraging distances among urban ibis (Martin et al. 2012; Martin et al. 2011; Smith et  
116 al. 2013a). It has also been suggested that urban colonies may be comprised of sedentary subunits exhibiting site  
117 fidelity and mobile subunits that interbreed with inland colonies (Corben and Munro 2008; Thomas et al. 2014).  
118 Altered connectivity among urban and native colonies may arise as a result of these differences, and thus  
119 management strategies may need to consider the possible genetic consequences on both urban and inland  
120 colonies.

121

122 In this study, we use single nucleotide polymorphisms (SNPs) to ascertain the genetic diversity and structure of  
123 several inland and urban colonies of ibis across south-eastern Australia. We test the prediction that relatedness, a  
124 proxy for site fidelity, is higher within urban colonies, and that water permanence and urbanisation intensity are  
125 associated with altered levels of gene flow among colonies. We also estimate effective population sizes and use  
126 simulations to explore the impact of various management strategies on future genetic diversity. Finally, we  
127 discuss the implications of our findings with respect to the scale of management currently undertaken for this  
128 species.

129

## 130 **Materials and Methods**

### 131 *Sample collection and site classification*

132 Australian white ibis feather samples were collected non-invasively from five urban and six inland sites between  
133 2015 and 2018 (Fig 1, Table 1, Research permits 10007719, 10008663 and SL102028). Urban sites were  
134 predominantly suburban parks or wetlands, except for Lucas Heights (LUH) which functions as an important  
135 ibis foraging site (Corben and Munro 2008). The inland sites were traditional breeding sites for waterbirds,  
136 including Gwydir Wetlands (GWY), Lowbidgee Wetlands (LOW) and Macquarie Marshes (MAC), and are  
137 target sites for environmental water management (Brandis and Bino 2016). Although breeding seasons are  
138 variable for the ibis and flooding-dependent for inland colonies, most samples were collected within the peak

139 urban breeding season (June to February)  $\pm$  1 month, which covers an extension of the inland breeding season  
140 and may occur year-round depending on resource availability (Smith et al. 2013a).

141

142 An urbanisation level and mean surface water permanence score was assigned to each site using the Catchment  
143 Scale Land Use of Australia (CLUM) 2017 dataset (Australian Bureau of Agricultural and Resource Economics  
144 and Sciences 2017) and the Water Observations from Space dataset (WOfS) (Mueller et al. 2016), respectively.  
145 Urbanisation scores were obtained from a principal components analysis (PCA) of seven landscape variables  
146 (density of urban, rural, public parks or other building types, landfill and managed water) measured as the  
147 proportion of area covered by each landscape feature within a 50km radius of the site, covering local foraging  
148 distances of urban ibis (Martin et al. 2011). A PCA of these variables in R Studio v3.5.0 (R Core Development  
149 Team 2019) yielded one principal component explaining 65.81% of the variance, which was used as the  
150 urbanisation score for each site (Table 1). Water permanence scores represent the mean percentage of time that  
151 surface water was present within 25  $\times$  25m grid cells across Australia between 1987 and 2014 (Mueller et al.  
152 2016), which was averaged across a 50km radius for each site.

153

#### 154 *DNA extraction and next-generation sequencing*

155 Feathers were rinsed in RNase/DNase-free water and stored at room temperature. Tissue was extracted from  
156 the 10 mm basal tip of the calamus of each feather along with the superior umbilicus blood clot when present to  
157 maximise DNA yield (Guilfoyle et al. 2017; Selås et al. 2017). Genomic DNA was extracted with the Isolate II  
158 Genomic DNA kit (Bioline), following the standard protocol with several modifications. To facilitate digestion  
159 of feather-derived tissue, a higher ratio of proteinase K (35  $\mu$ L, concentration 24 mg/mL, Bioline) and a longer  
160 digestion period (12-24 hours) (Bush et al. 2005) were used. Two 50  $\mu$ L elution steps were performed, with  
161 Silica membranes heated to 70°C to maximise DNA yield.

162

163 Single nucleotide polymorphisms (SNPs) were developed and genotyped with DArTSeq™ genotyping at  
164 Diversity Arrays Technology P/L (Canberra) on an Illumina HiSeq®2500 platform (Jaccoud et al. 2001; Kilian  
165 et al. 2012; Sansaloni et al. 2011). Preliminary quality control was performed by DArT P/L, and additional  
166 filtering steps were carried out with the base and *dartR* v1.0.5 (Gruber et al. 2018) packages in R to retain loci  
167 with a reproducibility average of 1, call rate greater than 0.95, maximum average read depth of 86.4 across all  
168 genotypes (average read depth + 3 S.D.), minimum average read depth of 10 across all genotypes and minor  
169 allele frequency above 2% (MAF  $\geq$  0.02) to minimise rare alleles caused by genotyping errors, and applied a  
170 sample call rate of 0.85. Additionally, duplicates were removed using *SNPRelate* v1.14.0 (Zheng et al. 2012)  
171 and *COLONY2* v 2.0.6.5 (Jones and Wang 2010), and a putatively neutral dataset was obtained by identifying  
172 loci under selection using three independent methods with *OutFLANK* v0.2 (Whitlock and Lotterhos 2015),  
173 *BayeScan* v2.1 (Foll and Gaggiotti 2008) and *PCAdapt* v4.0.3 (Luu et al. 2017). Finally, deviation from Hardy-  
174 Weinberg equilibrium (HWE) locally within sites was assessed to identify possible genotyping errors (Waples  
175 2014), with a Bonferroni correction applied to correct for multi-test errors. Refer to Electronic Supplementary  
176 Material for a detailed description of the library preparation process and SNP calling employed by DArT P/L  
177 (see also Kilian et al. (2012)), and for details on the outlier tests.

178

#### 179 *Genetic structure analyses*

180 Population structure was analysed by calculating  $F_{ST}$  with the R package *diveRsity* v1.9.90 (Keenan et al. 2013),  
181 performing a principal coordinates analysis (PCoA) in the program GenAlEx v6.5 (Peakall and Smouse 2012)  
182 with missing data interpolated, and a Bayesian clustering approach implemented in STRUCTURE v2.3.4  
183 (Pritchard et al. 2000). Pairwise  $F_{ST}$  between sites was calculated using Weir and Cockerham's  $\theta$  ( $F_{ST(WC)}$ )  
184 (Weir and Cockerham 1984), with 95% bootstrapped confidence intervals (1000 bootstraps). Goudet's G-  
185 statistic Monte Carlo test (999 simulations) assessed the significance of the global  $F_{ST}$  estimate. The PCoA was  
186 based on standardised covariance of genetic distances for codominant markers. The STRUCTURE analysis was

187 performed with the following settings: no prior population information, admixture model, correlated allele  
188 frequencies and optimal burn-in/MCMC parameter settings of 50000/500000. Ten replicates for each putative  
189 group ( $K = 1$  to  $K = 10$ ) were run and the most likely value of  $K$  was identified following the method by Evanno  
190 et al. (2005) in STRUCTURE HARVESTER v0.6.94 (Earl 2012). Global observed heterozygosity ( $H_O$ ),  
191 unbiased expected heterozygosity ( $uH_E$ ) (Hughes et al. 2008) and the inbreeding coefficient ( $F_{IS}$ ) were  
192 calculated.

193

194 Recent gene flow between localities and across various spatial scales were estimated with pairwise genotypic  
195 correlations as a proxy for relatedness ( $r_{xy}$ ) using COANCESTRY and GenAlEx. Mean relatedness for inland  
196 and urban sites were computed with the dyadic ML estimator in COANCESTRY and group differences were  
197 tested based on 9999 bootstraps. Spatial genetic structure was examined globally and for inland and urban sites  
198 separately with a multi-locus spatial autocorrelation analysis in GenAlEx, using seven uneven distance class  
199 bins (0, 150, 300, 450, 600, 750, 900 and 1400km). 95% confidence intervals were computed around the mean  $r$   
200 within each distance class (9999 bootstraps) and to test the hypothesis of ‘no spatial structure’ (9999  
201 permutations). A heterogeneity test was run for each spatial autocorrelation analysis using a significance level of  
202 1% to minimise type 1 error rates (Banks and Peakall 2012; Smouse et al. 2008).

203

204 To determine whether genetic differentiation increases with habitat differences, a linear regression analysis was  
205 conducted on pairwise  $F_{ST}$  between localities and two environmental variables (urbanisation score and mean  
206 surface water permanence), including their interactions, with the R package *FinePop* v1.3 (Kitada et al. 2017).  
207 Two estimates of genetic differentiation were calculated including Weir and Cockerham’s  $\theta$  ( $F_{ST(WC)}$ ) and the  
208 empirical Bayes  $G_{ST}$  estimator ( $EBF_{ST}$ ) suitable for species with high levels of gene flow (Kitada et al. 2007).  
209 This method also accounted for correlations between geographic distance and  $F_{ST}$ . Out of 11 models (listed in  
210 Table 2), the best fit model with the minimum Takeuchi information criterion (TIC) was selected (Takeuchi  
211 1976). The full model was:

$$212 \quad F_{ST} = \beta_1 D + \beta_2 U + \beta_3 W + \beta_4 D \times U + \beta_5 D \times W + \beta_6 U \times W + \beta_7 D \times U \times W$$

213 Here,  $D$  is the Euclidian distance between sites, and  $U$  and  $W$  are the absolute differences in urbanisation score  
214 and mean surface water permanence between pairs of sites, respectively. The parameters  $\beta_i$  are the partial  
215 regression coefficients.

216

### 217 *Effective population size*

218 The effective population size,  $N_e$ , was calculated for ibis across south-eastern Australia using the bias-corrected  
219 linkage disequilibrium (LD) method of Waples and Do (2008) under the ‘random mating model’, as  
220 implemented in NeEstimator v2.1 (Do et al. 2014). Because the allele frequency data pooled across all sampling  
221 sites did not exhibit significant sub-structuring, the whole dataset was treated as a single population. Multiple  
222  $P_{crit}$  values (threshold at which rare alleles were screened out) were trialled to investigate the balance between  
223 increasing precision and reducing bias (Waples and Do 2010). The 95% C.I.’s were calculated using both the  
224 parametric and bias-corrected jack-knife method of Waples and Do (2008).

225 While estimating  $N_e$  using samples taken from a single cohort of mixed-age individuals is the most reliable  
226 method for iteroparous species (Waples et al. 2014), the ibis feathers samples most likely came from multiple  
227 cohorts and this can compound the bias caused by overlapping generations (if the number of cohorts is not close  
228 to the generation length, and  $N_b$  differs considerably from  $N_e$ ). Known juveniles were excluded from the  
229 analysis to reduce the number of generations included in the analysis, and the output from NeEstimator was  
230 interpreted as the effective number of breeders ( $Raw\hat{N}_b$ ) in one reproductive cycle which produced the cohorts  
231 that were sampled, rather than the effective population size per generation (Waples and Do 2010). Following  
232 this, the overlapping generations bias was predicted and accounted for using two life history traits; adult lifespan  
233 ( $AL = \omega - \alpha + 1$ ;  $\omega$  = maximum age) and age at sexual maturity ( $\alpha$ ), using equations derived by Waples et al.

234 (2013) and listed in Waples et al. (2014). For the ibis,  $\alpha$  was taken as the average of recorded ages at sexual  
235 maturity, 2.5 (Marchant and Higgins 1990; Smith and Munro 2011), and  $\omega$  was taken as the maximum age  
236 recorded from banding data, 26 (Australian Bird and Bat Banding Scheme 2018). The naïve estimate of  $N_e$  was  
237 calculated from  $\hat{N}_b$  and then adjusted to account for the downward bias caused by physical linkage between loci,  
238 which may introduce more LD than expected by chance, using Equation 1a in Waples et al. (2016). As the  
239 karyotype of the Australian white ibis is unknown, the estimate for the closely related Sacred ibis (*Threskiornis*  
240 *aethiopicus*) was used (haploid chromosome number = 34) (Venturini et al. 1986). All equations used are listed  
241 in Table S1 in the Electronic Supplementary Material. Finally, because genetic estimates of  $N_e$  are prone to poor  
242 accuracy and precision when insufficient samples or loci are used and the census size is large (Waples and Do  
243 2010), simulations were conducted with NeOGen v1.3.0.6.a1 (Blower et al. 2019) to assess the precision of  $N_e$   
244 estimates under various sampling strategies (see Electronic Supplementary Material for methods used).

#### 245 *Forward simulations*

246 To predict the impact of urban management on the future genetic diversity of ibis across south-eastern Australia,  
247 forward simulations were performed with the program BOTTLESIM v2.6 (Kuo and Janzen 2003) using the  
248 bias-adjusted estimate of  $N_e$  based on  $P_{crit} = 0.05$  and its lower jack-knifed confidence limit (i.e. the most  
249 conservative estimate), rounded to the nearest even number to maintain even sex ratios. Simulations were first  
250 run under constant population sizes (i.e. no urban management), and then under different scenarios of high and  
251 low intensity management. High-intensity management scenarios involved culling 50% or 90% of the  
252 population in year 1 and keeping the population size stable in subsequent years. Low-intensity management  
253 scenarios involved culling 5% or 10% of the population each year (from year 1) before maintaining at either  
254 50% or 90% of the original size. Management scenarios were based on *in situ* impacts of urban management  
255 practices on ibis colonies (Table S2), but assumed that management impacts were widespread across the range  
256 of south-east Australian ibis. The following settings were employed in all scenarios: dioecy with random  
257 mating, 1:1 sex ratio, 85 years, 500 iterations, longevity = 24 years, age at sexual maturity = 3 years, and  
258 generational overlap = 100%. Estimates of genetic diversity (OA, observed number of alleles) were averaged  
259 over loci for each year of the simulation.

260

## 261 **Results**

### 262 *SNP filtering*

263 Following SNP and sample level filtering (Table S3), the final datasets consisted of 1,860 SNPs for 68 samples  
264 for general analyses and 1,809 SNPs for 64 samples for site-level analyses. No loci were detected as outliers by  
265 all three outlier detection methods, yielding a putatively neutral dataset. HWE testing within sampling sites  
266 found 11 loci deviating significantly from HW proportions which were retained in the final dataset because they  
267 did not deviate in more than one site. Preliminary analyses excluding all potential outliers and HW-deviant loci  
268 confirmed that retaining these loci in the final dataset did not significantly impact results.

269

### 270 *Genetic structuring and the impacts of environmental variables*

271 Global  $F_{ST}$  was 0.0032 and did not deviate significantly from 0 ( $p = 1.00$ ), and pairwise  $F_{ST}$  between sites were  
272 all non-significant, ranging from  $<0.001$  to 0.0191 (Table S4). The PCoA and STRUCTURE analyses also  
273 indicated a lack of sub-structuring, with the first and second principal coordinates of the PCoA explaining 2.48  
274 and 2.40% of the genetic variation, respectively (Fig S1) and STRUCTURE identifying a single genetic cluster  
275 ( $K = 1$ ) containing all individuals in the dataset. Mean unbiased expected heterozygosity ( $uH_E$ ) and observed  
276 heterozygosity ( $H_O$ ) across all loci and populations was 0.169 (SE  $\pm$  0.001) and 0.149 (SE  $\pm$  0.001),  
277 respectively, and the global inbreeding coefficient  $F_{IS}$  was 0.124 (additional statistics listed in Table S5).

278

279 Pairwise relatedness ( $r_{xy}$ ) was low overall (mean = 0.007, S.E.  $\pm$  0.0003) and was significantly greater among  
280 urban samples than among inland samples at the 99% confidence level (mean =  $0.009 \pm 0.0002$  and  $0.007 \pm$

281 0.0001, respectively). For the pooled dataset, spatial autocorrelation analyses provided evidence for a weak  
282 correlation between geographic distance and genotypic similarity ( $r$ ), with  $r$  ( $\pm$  95% C.I.) being significantly  
283 greater than 0 at distance classes 0 km ( $r = 0.005 \pm 0.004$ ,  $p < 0.05$ ) and 150 km ( $r = 0.004 \pm 0.004$ ,  $p < 0.05$ ).  
284 The heterogeneity test indicated positive spatial structure at the correlogram level for the pooled dataset ( $\omega$   
285 = 42.6,  $p < 0.01$ , Fig S2), but not when examining spatial structure for inland and urban samples separately.

286

287 Finally, the linear regression between genetic differentiation ( $\theta_{WC}$  or  $EBF_{ST}$ ) and three environmental variables  
288 (distance, urbanisation and surface water permanence) identified the model incorporating all three  
289 environmental variables and their interactions (model =  $F_{ST} \sim -1 + (\text{distance} + \text{urban} + \text{water})^2$ ) to be the best fit  
290 model ( $R^2 = 0.218$ , TIC = 92.310 for  $\theta_{WC}$ ;  $R^2 = 0.066$ , TIC = 98.676 for  $EBF_{ST}$ ; Table 2). However, this model  
291 was a weak fit for the data ( $p > 0.05$  for both  $\theta_{WC}$  and  $EBF_{ST}$ ), and only the interaction between urbanisation  
292 score and mean surface water permanence was significant ( $Z = -1.97$ ,  $p < 0.05$ ). The model with the second best  
293 fit only included distance ( $R^2 = 0.083$ , TIC = 100.799 for  $\theta_{WC}$ ;  $R^2 = 0.029$ , TIC = 102.252 for  $EBF_{ST}$ ; Table 2).  
294  $\Delta$ TIC between these two models was 8.489 for  $\theta_{WC}$  and 3.576 for  $EBF_{ST}$ .

295

### 296 *Effective population size across south-eastern Australia*

297 Our estimated  $N_e$  for pooled samples (after bias adjustments and using  $P_{crit} = 0.05$ ) was 3448.3 (95% jack-  
298 knifed CI = 1202.8 -  $\infty$ , Table 3). Trials using a range of  $P_{crit}$  values showed that a  $P_{crit}$  of 0.05 (i.e. reduced  
299 bias) gave similar  $N_e$  estimates to those obtained with less stringent criterion (i.e. increased precision), except  
300 for a  $P_{crit}$  of 0.02 (Fig 2). The ratio  $N_b/N_e$  calculated from life-history traits was 1.236. Simulations in NeOGen  
301 showed that under 2015 estimates of census size, the sample and locus numbers used were too small to provide  
302 an accurate estimate of the effective population size of ibis across south-eastern Australia. The bias-adjusted  
303 estimate of  $N_e$  was closer to the lower bounds of the NeOGen simulations, which ranged from 3269 – 4060  
304 when 66 samples and 1860 loci were used (Table 3 and Fig S3).

305 Simulations run under constant initial effective sizes ( $N_i$ ) of both 3448 and 1202 (rounded to the nearest even  
306 number) indicated that more than 99% of the initial genetic variation would be retained after 85 years (Fig 3).  
307 Under different management scenarios, the percentage of retained genetic diversity only fell below 90% for the  
308 lower limit (1202) under the 90% bottleneck scenarios (Fig 3b). For these scenarios, the percentage of observed  
309 number of alleles (OA) retained fell below 90% after 60 years for the single bottleneck, 64 years for the 10%  
310 yearly scenario, and 79 years for the 5% yearly scenario. In all scenarios of urban management, the observed  
311 number of alleles deteriorated faster than observed heterozygosity, as expected by theory (Allendorf 1986).

312

### 313 **Discussion**

314 We found no evidence of significant genetic partitioning among Australian white ibis sampled across most of  
315 their south-east Australian distribution. Analyses at various spatial and temporal scales indicated high levels of  
316 gene flow between densely inhabited urban centres and inland wetlands, where the ibis is in decline (Porter et al.  
317 2017). In addition, there was no strong evidence that distance, urbanisation intensity or temporal variability in  
318 surface water availability affected gene flow among colonies. We estimated an effective size ( $N_e$ ) of  $\approx$  3448,  
319 and forward simulations of various management-induced population declines show that genetic erosion is  
320 unlikely under realistic scenarios. Nonetheless, high levels of gene flow among ibis colonies highlight the need  
321 to consider the implications of current urban management strategies for the conservation issues facing inland  
322 colonies.

323

324 Our analyses did not support our hypothesis that differences in resource availability alter genetic connectivity  
325 among ibis colonies. Low genetic differentiation based on allele frequency data (e.g. global  $F_{ST} = 0.0032$ )  
326 suggests a lack of longer-term barriers to dispersal and/or founder effects because genetic parameters like  $F_{ST}$   
327 are mostly a consequence of genetic drift occurring across many generations (Epps and Keyghobadi 2015).



328 However, individual-based genotypic approaches (e.g. pairwise relatedness,  $r_{xy}$ ) have the potential to detect  
329 influences on dispersal and gene flow at shorter time-scales because genotypes are typically reshuffled with  
330 each new generation or breeding event (Landguth et al. 2010; Stow et al. 2001). While the positive  $F_{IS}$  results  
331 support some local breeding within colonies, the results of our spatial autocorrelation analyses suggest there is  
332 only a weak relationship between distance and recent kinship among urban ibis ( $r_{xy}$ , the probability that alleles  
333 are identical by descent), suggesting that ibis disperse equally across coastal and inland regions. The patterns of  
334 ongoing gene flow detected in the current study are consistent with previous studies predicting regular dispersal  
335 between inland and semi-sedentary coastal colonies during the breeding season (Corben and Munro 2008;  
336 Thomas et al. 2014). Further, methodological constraints of the current study are unlikely to overlook the  
337 presence of a true biogeographic barrier to gene flow for the ibis. The number of samples per locality for site-  
338 level analyses (5 – 9) fell within the recommended range (4 – 6) when using large numbers of biallelic genetic  
339 markers (> 1000) to infer genetic differentiation between localities (e.g. Weir and Cockerham's  $F_{ST}$ ) (Willing et  
340 al. 2012).

341

342 Altered gene flow between urban and non-urban populations has been reported for a variety of avian species  
343 occupying both habitats. Previous research has generally found estimates of  $F_{ST}$  between urban or between  
344 urban and native populations to be higher than between native populations (Table S6, Fig S4). These differences  
345 may arise due to founder effects, different selective pressures between urban and native habitats, genetic drift,  
346 urban landscape resistance and/or increased urban sedentariness (Baratti et al. 2015; Minias et al. 2017; Rasner  
347 et al. 2004). Yet, we observed low genetic differentiation between all pairs of urban and inland colonies for the  
348 Australian white ibis ( $F_{ST(WC)}$  ranged from <0.001 to 0.0191), similar to previous work on the American white  
349 ibis (*Eudocimus albus*) detecting high gene flow between colonies 600km apart (Stangel et al. 1991). Given the  
350 lack of genetic sub-structuring found among south-east Australian ibis, we were unable to disentangle the  
351 relative magnitude of gene flow from inland to urban colonies and vice versa, however reduced abundance  
352 observed in some urban regions during the wet-season as a result of reduced anthropogenic food sources  
353 provides support for bi-directional gene flow (Chard et al. 2017). Thus, in conjunction with their high dispersal  
354 propensity, widespread gene flow between ibis colonies may be facilitated by fluctuations in both hypervariable  
355 inland conditions and urban food sources. Such fluctuations could drive large scale colony shifts and reduce  
356 genetic differentiation between colonies, as has been observed for several colonial waterbirds in the Northern  
357 Hemisphere (Den Bussche et al. 1999; Reudink et al. 2011; Stangel et al. 1991). Very few migrants are needed  
358 to homogenise allele frequencies among locations and can result from migratory individuals remaining in non-  
359 migratory colonies (Oomen et al. 2011) or individuals seeking better breeding prospects (Del Lama et al. 2002).  
360 Thus, dynamic wetland conditions driving small- and large-scale colony shifts likely plays a larger role in  
361 mediating gene flow for the ibis than urbanisation intensity, site fidelity or physical barriers.

362

363 The effective population size ( $N_e$ ) we estimated for south-east Australian ibis reflects a relatively large mean  
364 effective size across several recent generations. Two independent surveys employing different sampling  
365 approaches counted 24,165 (Porter et al. 2015) and 25,002 ibis (Department of Planning Industry and  
366 Environment 2015) across the eastern states in 2015. Considering that  $N_e/N$  ratios for many taxa are small on  
367 average (Frankham et al. 2014), the  $N_e$  obtained in the present study reflects the large census sizes estimated for  
368 east Australian ibis. Although relating  $N_e$  to  $N$  has many caveats (Palstra and Fraser 2012), birds on average  
369 exhibit relatively low  $N_e/N$  ratios (0.65) and relatively high  $N_b/N$  ratios (0.86) (Waples et al. 2013). The  
370  $N_b/N_e$  ratio estimated from life-history traits for the ibis (1.236) was also consistent with ratios for other avian  
371 species which exhibit a mean ratio of 1.35 (Waples et al. 2013). Other caveats include using relatively small  
372 sample sizes to estimate  $N_e$  or random mixed-age samples of adults which may downwardly bias  $N_e$  estimates  
373 (Schlesselmann and Robertson 2020). Our simulations in NeOGen indicated that accurate confidence intervals  
374 around the true  $N_e$  were difficult to ascertain with current sample sizes, with our current estimate of  $N_e$  being  
375 closer to the lower bound in the simulations. However, empirical estimates of the lower bound of  $N_e$  can still be  
376 informative, with simulations showing a low likelihood of the true lower bound being lower than the estimated  
377 one, even with small samples sizes and large population sizes (Waples and Do 2010). Additionally, the use of  
378 several thousand SNPs likely counters sample size biases by enabling the detection of a weak signal (Waples  
379 and Do 2010). Thus, while more accurate estimates of effective size could be obtained with increased sample

380 sizes, based on empirical and simulation data the effective size of ibis colonies across south-eastern Australia is  
381 unlikely to be lower than 1000.

382

383 Lower estimates of effective size for the ibis are well above the recommended limits to avoid loss of genetic  
384 diversity due to inbreeding or genetic drift. Our estimates of  $N_e$  are above the recommendations by Frankham et  
385 al. (2014) that  $N_e \geq 100$  is required to limit the total fitness loss to  $\leq 10\%$  and avoid inbreeding depression in  
386 the short term, and  $N_e \geq 1000$  is required to maintain evolutionary potential in the long term. Forward  
387 simulations also suggested that conservative reductions in population size are unlikely to affect the evolutionary  
388 potential of this species via genetic erosion. If inland ibis interbreed with urban colonies on a seasonal basis,  
389 then local egg-based culling practices are likely to have a minimal negative impact on genetic diversity across  
390 their south-eastern Australia distribution.

391

392 However, given the scale of gene flow observed, it is important to consider the impacts of current management  
393 regimes in conjunction with the processes threatening inland colonies, such as reduced breeding opportunities  
394 (Brandis et al. 2018). Overall, a broad scale management plan would enable the most effective monitoring of  
395 regional or local processes which may impact the meta-population and has been recommended for several other  
396 waterbird species (Del Lama et al. 2002; Reudink et al. 2011; Stangel et al. 1991). In addition to increased  
397 collaboration across jurisdictional boundaries, wildlife managers tasked with monitoring or controlling  
398 Australian white ibis numbers should be aware that the scale at which ibises move across the eastern states may  
399 have implications for the success of local management strategies. While logistically challenging, undertaking a  
400 collaborative broad-scale management approach for the Australian white ibis would enable the most effective  
401 monitoring of regional or local processes which may impact the whole population. This would be critical to  
402 ensure that inland colonies do not fall into an 'ecological trap' (Hale and Swearer 2016) by breeding along the  
403 coast during periods of intense urban management. Future work could expand sampling to other parts of this  
404 species range in addition to employing eco-evolutionary simulation models to disentangle the effects of  
405 management on inland and urban colonies in light of their different ecological, life-history and dispersal traits.  
406 In the face of these complex processes, this study provides a baseline with which to monitor changes in gene  
407 flow of Australian white ibis colonies over time.

408

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415

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631

632

633 **Figure Captions**

634

635 **Fig 1** Map of 11 study sites where Australian white ibis feather samples were collected non-invasively between  
636 2015 and 2018. Shaded areas depict the three major river basins samples were collected within, with North-east  
637 Coast and South-east Coast basins comprising coastal rivers, and the Murray-Darling Basin comprising inland  
638 rivers. Sites, in decreasing urbanisation intensity, included: Lucas Heights (LUH), Centennial Parklands (CEN),  
639 Boondall Wetlands (BOO), River Gum Creek Reserve (RGC), Goulburn Wetlands (GOU), Barmah Reed Beds  
640 (BAR), Gwydir Wetlands (GWY), Macquarie Marshes (MAC), Lowbidgee (LOW), Putta Bucca Wetlands  
641 (PBW), and Lachlan Reserve (LAC)

642

643 **Fig 2** Estimated effective number of breeders ( $Raw\hat{N}_b$ ) of Australian white ibis sampled across south-eastern  
644 Australia, with parametric 95% confidence intervals obtained for a range of  $P_{crit}$  values (dashed line = average  
645  $Raw\hat{N}_b$  across  $P_{crit}$  values). The upper limit is undefined (infinite) for  $P_{crit}$  of 0.02

646

647 **Fig 3** Percentage of retained genetic diversity (OA, the observed number of alleles) over time (85 years) after  
648 reducing the initial  $N_e$  of 3448 (solid line) or 1202 (dashed line, rounded to nearest even number to maintain  
649 equal sex ratios) by bottlenecks of either A) 50% or B) 90% in BOTTLESIM v2.6. Scenarios included: no  
650 bottleneck = sustaining effective sizes at current estimates; 5% yearly = reducing  $N_e$  by 5% each year until  
651 reaching 50% or 90% bottlenecks; 10% yearly = reducing  $N_e$  by 10% each year until reaching 50% or 90%  
652 bottlenecks; and single bottlenecks = reducing  $N_e$  by 50% or 90% in year 1

653 **Tables**

654 **Table 1** Study sites where Australian white ibis samples were obtained between 2015 and 2018, with designated  
 655 abbreviations, urbanisation score, water permanence score and sample size (final sample size after sequencing  
 656 and filtering shown in brackets)

Site	Code	Sample size	Urbanisation <sup>a</sup>	Water permanence <sup>b</sup>
Lucas Heights	LUH	8 (8)	3.54	32.68
Centennial Parklands	CEN	8 (8)	3.01	48.29
Boondall Wetlands	BOO	11 (5)	2.40	37.57
River Gum Creek Reserve	RGC	9 (6)	1.70	21.34
Putta Bucca Wetlands	PBW	6 (3)	-1.15	21.82
Goulburn Wetlands	GOU	10 (9)	-1.38	15.89
Barmah Reed Beds	BAR	10 (8)	-1.51	3.90
Gwydir Wetlands	GWY	7 (6)	-1.52	1.91
Macquarie Marshes	MAC	8 (5)	-1.68	0.85
Lowbidgee	LOW	11 (9)	-1.69	3.70
Lachlan Reserve	LAC	6 (1)	-1.72	1.11
Pooled samples		94 (68)		

657 <sup>a</sup> Urbanisation score extracted as the PC1 score of a PCA of seven urban landscape features within a 50km  
 658 radius of each site.

659 <sup>b</sup> Mean percentage of time surface water was detected between 1987 and 2014 (Mueller et al. 2016) within a  
 660 50km radius of each site (excludes grid cells lacking water (0%)).



661 **Table 2** Linear regression results ( $R^2$  and TIC scores) for 11 models based on two different measures of genetic  
 662 differentiation ( $\theta_{WC}$  and  $EBF_{ST}$ ) among Australian white ibis localities both east and west of the Great Dividing  
 663 Range.

Model	$\theta_{WC}$		$EBF_{ST}$	
	$R^2$	TIC	$R^2$	TIC
$F_{ST} \sim -1 + \text{distance}$	0.083	100.799	0.029	102.252
$F_{ST} \sim -1 + \text{urban}$	0.004	103.155	0.001	103.184
$F_{ST} \sim -1 + \text{water}$	0.030	102.780	0.000	103.720
$F_{ST} \sim -1 + (\text{distance} + \text{urban})^2$	0.098	106.530	0.043	106.847
$F_{ST} \sim -1 + \text{distance} + \text{urban}$	0.090	104.578	0.029	105.909
$F_{ST} \sim -1 + (\text{distance} + \text{water})^2$	0.135	107.900	0.057	107.483
$F_{ST} \sim -1 + \text{distance} + \text{water}$	0.124	104.967	0.029	106.187
$F_{ST} \sim -1 + (\text{urban} + \text{water})^2$	0.126	120.140	0.002	114.938
$F_{ST} \sim -1 + \text{urban} + \text{water}$	0.048	120.185	0.002	114.928
$F_{ST} \sim -1 + (\text{distance} + \text{urban} + \text{water})^2$	0.218	92.310	0.066	98.676
$F_{ST} \sim -1 + \text{distance} + \text{urban} + \text{water}$	0.141	129.405	0.031	149.343

664 *Distance* is the geographic distance between sites, *urban* is the absolute difference in urbanisation score between  
 665 sites, and *water* is the absolute difference in mean surface water permanence between sites

666 **Table 3** Rounded estimates of effective size for Australian white ibis sampled across south-east Australia, both  
667 before ( $Raw\hat{N}_b$ ) and after correcting for bias due to overlapping generations ( $\hat{N}_{b(Adj)}$ ,  $\hat{N}_{e(Adj)}$ ) and physical  
668 linkage ( $\hat{N}_{e(Chr)}$ ) under two different  $P_{crit}$  thresholds (0.02 and 0.05), with 95% jack-knifed (JK) and parametric  
669 confidence intervals ( $\infty$  = no upper bound). Effective sizes estimated in NeOGen are based on 1860 simulated  
670 loci, two different sampling schemes (SS1 = sub-sampling allowed a small proportion of juveniles, S = 68; SS2  
671 = juveniles excluded when sub-sampling, S = 66) and assuming a census size of 24,165.

$P_{crit}$	LDNe	JK 95% CI	Parametric 95% CI
<i>0.05</i>			
$Raw\hat{N}_b$	3191	1113, $\infty$	1978, 8163
$\hat{N}_{b(Adj)}$	3710	1294, $\infty$	2300, 9490
$\hat{N}_{e(Adj)}$	3001	1047, $\infty$	1860, 7676
$\hat{N}_{e(Chr)}$	3448	1203, $\infty$	2138, 8820
$N_{e(Sim_{SS1})}$	9110	3474, 18054	
$N_{e(Sim_{SS2})}$	8344	3269, 15983	
<i>0.02</i>			
$Raw\hat{N}_b$	7592	1444, $\infty$	3761, $\infty$
$\hat{N}_{b(Adj)}$	8827	1678, $\infty$	4372, $\infty$
$\hat{N}_{e(Adj)}$	7139	1358, $\infty$	3536, $\infty$
$\hat{N}_{e(Chr)}$	8204	1560, $\infty$	4063, $\infty$
$N_{e(Sim_{SS1})}$	8733	4064, 13765	
$N_{e(Sim_{SS2})}$	8005	3823, 13557	

672

673 **Summary for online Table of Contents:** A population genetics study of Australian white ibis (*Threskiornis*  
674 *molucca*) across south-eastern Australia revealed high levels of recent gene flow between urban centres where  
675 the ibis is an overabundant native, and degraded inland wetlands, where the ibis and many other waterbird  
676 species are in severe decline. Management practices implemented to control urban colonies may have  
677 implications for declining native populations.