Genetic connectivity of Australian white ibis: implications for management

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Declaration

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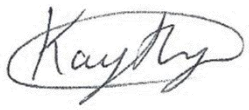
All other research described in this report is my own original work.



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ABSTRACT

Anthropogenic processes often impact genetic connectivity of wild populations. Widespread degradation across Australia's inland wetland network has contributed to severe declines for many waterbird species. Meanwhile, breeding colonies of the Australian white ibis (*Threskiornis molucca*) have increased along the coast. Resource availability may be influencing site fidelity among urban ibis, but whether this impacts on levels of gene flow between inland and coastal, urban areas remains unknown. This study uses single nucleotide polymorphisms (SNPs) to ascertain the structure of several urban and inland colonies of white ibis across south-eastern Australia. Associations between gene flow and several environmental factors were tested, including geographic distance, the Great Dividing Range, urbanisation intensity and surface water permanence. Additionally, effective population sizes were estimated along with the impact of various management scenarios on future genetic diversity. Spatial and regression analyses revealed no significant differences in allele frequencies, or relatedness, therefore suggesting widespread dispersal and gene flow between inland and coastal colonies. Furthermore, effective sizes were large enough to maintain genetic diversity into the future, even under various management scenarios. However, the lack of genetic partitioning found suggests that urban management of the ibis should not be undertaken in isolation of inland conservation efforts.

KEYWORDS *Threskinornis molucca*, genetic structure, urbanisation, waterbird management, wetlands

INTRODUCTION

Anthropogenic processes often impact wild populations, with patterns of dispersal and gene flow shifting along with ever-changing landscapes. The negative consequences that can arise from altered connectivity are well established and include inbreeding or outbreeding depression, reduced evolutionary potential and increased disease transmission (Frankham 2005). Designing management strategies at landscape scales can therefore benefit from genetic-based studies that provide insight into important genetic and demographic processes, assess long term population viability and help define appropriate management units (DeYoung and Honeycutt 2005). An understanding of these processes can also contribute to the development of effective management plans for species occupying both urban and native habitats (Luniak 2004).

Anthropogenic impacts on wetland dynamics and waterbird dispersal

Australia's waterbirds are well adapted to navigating the transient mosaic of wetlands that characterise the arid and semi-arid interior of the continent. Intermittent periods of wetland flooding and drying form the natural flow regimes of Australia's inland wetlands (Bino et al. 2015). Sequential flooding after periods of high rainfall triggers waterbird breeding in large colonies ('boom' periods), while dry phases decrease wetland productivity and force waterbirds to disperse towards more suitable habitats ('bust' periods) (Kingsford et al. 1999, Roshier et al. 2002, Kingsford and Auld 2005). Waterbirds are highly associated with fluctuating flow regimes (Kingsford et al. 2013, Colloff et al. 2018), making them useful indicators of wetland condition and the quality of important ecosystem services (Reid et al. 2013). As a result, fluctuations in waterbird abundance and biodiversity often reflect environmental changes.

The wetland habitats of inland Australia have undergone widespread degradation, and this has led to severe declines in abundance for many waterbird species. Freshwater wetlands are among the most threatened ecosystems (Lemly et al. 2000) and are degrading at a faster rate than terrestrial or marine ecosystems (Millenium Ecosystem Assessment 2005). Water resource development, pollution and climate change are among the key anthropogenic processes that have drastically altered wetland flow regimes (Poff et al. 1997). Climate change is expected to exacerbate these processes, for example by increasing the number of low or no-flow years (Young and Chiew 2011, Colloff et al. 2016). Many waterbirds are

being pushed to their biological and ecological limits of resilience (Bino et al. 2015). Colony success is being undermined by the breakdown of 'boom' and 'bust' cycles and deterioration of wetland quality (Leslie 2001). Waterbirds are also being affected by reduced connectivity between larger wetland systems (Paton et al. 2009), habitat degradation occurring in other parts of their range (Nebel et al. 2008, Hansen et al. 2015) and the aridification of key refugia sites (Davis et al. 2013). Drastic declines in waterbird numbers across inland Australia have been observed over the past few decades as the capacity of wetlands to support large colonies is decreased (Kingsford and Johnson 1998, Kingsford and Thomas 2004, Kingsford et al. 2017, Brandis et al. 2018). Although waterbird breeding has increased in situations where river flow has been reinstated (Kingsford and Auld 2005), inland colonies have continued to decline drastically in recent years (Porter et al. 2017). Coastal wetlands with abundant resources may therefore provide refuge for many species.

Dispersing towards coastal urban regions provides a more stable habitat for species affected by wetland degradation. Anecdotal evidence suggests that waterbirds make use of both habitats, moving from the ephemeral wetlands of inland Australia to more reliable coastal wetlands east of the Great Dividing Range (GDR) during 'bust' periods (Kingsford et al. 2010). While many waterbirds are facing range contractions due to wetland degradation and urbanisation (Kingsford and Norman 2002), a few are tapping into the wealth of anthropogenic resources available along the coast. For example, the Australian wood duck (*Chenonetta jubata*) (Kingsford 1992) and Australian white ibis (*Threskiornis molucca*) (Martin et al. 2010, Smith et al. 2013b) are now thriving in coastal regions, while inland populations continue to decline. With many more species making urban habitats their permanent home (Low 2017), there is a pressing need to understand how increasing urbanisation might affect the movement and genetic connectivity of waterbirds that traditionally rely on inland wetlands.

Genetic connectivity of Australia's waterbirds

Little is known about the impacts of urbanisation or resource availability on the genetic structure of waterbird populations across Australia. Previous research has explored the genetic structure of migratory waterbirds across countries (Robertson and Gemmell 2002, Szczys et al. 2005) and levels of introgression between waterbird species (Rhymer et al. 1994). Other research has investigated the east-west genetic divergence of avian populations across the southern regions of Australia, where the arid interior may play a role in limiting

gene flow. For example, the historical isolation of Australian musk duck populations (*Biziura lobata*) has been examined (Guay et al. 2010), as well as the genetic divergence of populations for several other avian species inhabiting mesic environments in south-east and south-west Australia, including the Australian magpie (*Cracticus tibicen*) (Baker et al. 2000, Toon et al. 2003) and various honeyeater, robin and lorikeet species (Toon et al. 2010, Dolman and Joseph 2012). The impact of biogeographical barriers in northern Queensland on genetic structure and divergence of populations has also been investigated for honeyeaters (and other Meliphagids) (Peñalba et al. 2017) and fairy wrens (Lee and Edwards 2008). Other studies have focussed on waterbird biodiversity and distribution in relation to seasonal water availability and river regulation levels (Kingsford et al. 2004a, Wen et al. 2016), as well as the impacts of urbanisation on site fidelity in the Australian white ibis (Martin et al. 2012). However, little is known about whether regional differences in resource availability, wetland stability or urbanisation intensity affects the genetic connectivity of waterbird colonies across Australia. In addition, the impact of physical barriers such as the GDR on gene flow between inland and coastal waterbird colonies has yet to be explored. Although there is scarce evidence to suggest the GDR restricts movement between inland and coastal waterbird colonies (Guay et al. 2012), knowledge of the relative impacts of physical barriers on gene flow between colonies would enhance our understanding of the landscape features affecting waterbird connectivity. Research examining these various impacts on the genetic connectivity of waterbirds inhabiting both inland and coastal wetlands would provide valuable information for their management and conservation.

Impacts of urbanisation on genetic diversity and connectivity

Many species faced with encroaching urbanisation are undergoing synurbanisation. This term was first coined to describe the adjustment of wild populations to urban conditions (Luniak 2004), particularly for species whose abundance and success in urban habitats is higher than in their rural or natural habitats (Francis and Chadwick 2012). Fundamental differences between urban and natural habitats may give rise to altered genetic connectivity and influence the long-term persistence of species occupying both habitats (Andrzejewski et al. 1978, Gehlbach 1996, Frankham 2005).

Urbanisation can alter the genetic connectivity of populations through the formation of source-sink population dynamics. Variation in habitat quality, both spatially and temporally, produces a gradient of habitat ‘suitability’ across the landscape (McIntyre and Barrett 1992,

McGarigal and Cushman 2002). This can lead to dynamic interactions between 'source' populations occupying optimal habitats and 'sink' populations exhibiting a local demographic deficit (Dias 1996, Kawecki 2008). Resource and mate competition can drive gene flow from source to sink populations (Loehle 2012), which along with increased dispersal out of unpredictable sink habitats, can help maintain connectivity between populations (Marten et al. 2006). While urban habitats with sub-optimal conditions often support sink populations, local adaptation to urban conditions may transition them into demographic sources (Fulgione et al. 2000, Björklund et al. 2009). This could in turn drive increased genetic differentiation between urban and non-urban populations over time, for example if urban-adapted individuals show a preference for their natal urban habitats (Davis and Stamps 2004, Minias et al. 2017). This trend has been observed for several avian species, where genetic differentiation is highest between urban and non-urban populations (refer to Table 1). Altered connectivity arising from source-sink dynamics in these cases has implications for gene flow and the genetic diversity of populations occupying both urban and non-urban habitats.

Whether urban colonies of Australian waterbirds function as demographic sources or sinks remains to be studied. It is well known that unregulated wetlands are more effective at supporting waterbirds compared to highly regulated wetlands (Kingsford et al. 2004b, Reid et al. 2013, Kingsford et al. 2017). In a rapidly changing landscape however, artificial wetlands and water bodies increasingly provide refuge for waterbirds (Murray and Hamilton 2010, Murray et al. 2013, Hamilton et al. 2017). The steady surplus of urban resources may compensate for sup-optimal flow conditions (Chard et al. 2017) and confer a fitness benefit for individuals migrating out of unstable inland habitats (Kuno 1981). Urban populations may thus be in the process of transitioning into demographic sources, driving gene flow out towards declining inland colonies. Additionally, many waterbirds have high dispersal propensities, which along with erratic migrations and varied foraging strategies, enable them to deal with a complex wetland system (Overton et al. 2009, Kingsford et al. 2010). Waterbirds may be buffered against the negative effects of reduced connectivity caused by increasing urbanisation, as high dispersal capabilities play a key role in maintaining connectivity (Slatkin 1987) and have been shown to be advantageous in the urban environment (Rochat et al. 2017). Overall, genetic research investigating levels of gene flow between these types of habitat can provide a better understanding of source-sink dynamics and would benefit waterbird conservation in Australia.

Table 1. Examples of urbanisation impacting genetic variation (GV) and differentiation (F_{ST} or similar measure) for avian species occupying both urban (U) and non-urban (N) habitats (n.s. = near/non-significant). Refer to Appendix A for a full reference list and scientific species names.

Species	GV (U) [a]	Differentiation [b]			Potential Driving Forces	Ref
		U/N	U/U	N/N		
Waterbirds						
Eurasian coot	↓ n.s.	↑	↑↑ [c]	=	N source for U; habitat differences; time since urbanisation	1
Mallard duck	↓ n.s.	↑	=	=	Alternate form (U); geographic differentiation; genetic drift.	2,3
Other Avian Species						
Common kestrel	=	↑[c]	↑	↓	Site fidelity (U); founder effects (U)	4
Dark-eyed Junco	↓	↑	-	=	Founder effects → genetic drift (U); ↑ sedentariness (U)	5
European blackbird	↓	↑	↑↑	↓	Founder effects (U); ↑ sedentariness (U); N source for U	6,7
Great tit	↑	↑	↑ [c]	-	Territorial competition (gene flow U → N); limited dispersal (U)	8
	↓[c]	↓	↓	-	Large N_e; recent nature of urbanisation (genetic lag)	9
Song sparrow	↓	↑	=	=	↓ dispersal (U); population history/demography; ↑ predation (U)	10

[a] GV indicates whether genetic variation within urban sites is significantly higher or lower than in non-urban.
[b] Arrows indicate the relative magnitude of significant genetic differentiation compared to other habitat pairs (e.g. ↑ = more differentiated), unless pairs are not significantly differentiated from each other (=).
[c] Result only applied to a portion of sites (e.g. sites with the highest urbanisation level or comparisons between sites that differ in the history of urbanisation), or some of the comparisons were non-significant.

Increased sedentariness and site fidelity might result from resource stability in urban regions and could alter connectivity among populations. Synurbic populations typically exhibit reduced individual territories and migratory behaviour in urban regions (Luniak 2004), likely driven by increased resource availability (Frederick and Ogden 1997). Resource availability has been linked to increased sedentariness, earlier breeding times and longer reproductive periods in urban populations of the European blackbird (Partecke and Gwinner 2007, Evans et al. 2012) and drastically reduced home range sizes in several other avian species (Groepper et al. 2008, Vangestel et al. 2010, Varner et al. 2014). For Australia's waterbirds, broad-scale movements are heavily influenced by fluctuating resource availability (Roshier et al. 2008). The presence of stable urban resources may reduce the need to disperse far in search of food and suitable breeding grounds, increasing sedentariness and reducing connectivity between

waterbird colonies. The role of urban resources in mediating waterbird movements and gene flow may subsequently require consideration in management plans for these species.

Altered connectivity caused by increasing urbanisation not only influences genetic connectivity, but also has implications for the genetic diversity of populations and their capacity to adapt to environmental changes. For species where urbanisation is associated with reduced dispersal, increasing urbanisation may place populations at risk of losing genetic diversity (Etterson and Shaw 2001, Hoffmann and Sgrò 2011, Oakley 2013). This can have negative impacts on fitness and adaptive responses, particularly for small, isolated populations (Frankham 2005). Lower genetic diversity among urban populations has been reported for a variety of avian species, even for species with high dispersal tendencies (Table 1). This trend is likely driven by reduced gene flow between populations, for example due to increased resistance to movement across urban landscapes (Delaney et al. 2010) or increased sedentariness within urban populations (Partecke and Gwinner 2007, Evans et al. 2009). Alternatively, urbanisation may lead to increased genetic diversity through adaptation to novel urban conditions, the provision of habitat heterogeneity and urban corridors facilitating gene flow for urban-adapted species (Fulgione et al. 2000, Desender et al. 2005, Björklund et al. 2009). Overall, these case studies suggest that certain features of the urban landscape, such as resource quality or human disturbance, impact the sharing of genetic material between populations. High dispersal rates among waterbirds could maintain similar levels of genetic diversity between urban and non-urban colonies. However, as other mechanisms can negatively affect gene flow (such as increased sedentariness) and contribute to lower genetic diversity within colonies, the impacts of urbanisation on the fitness and evolutionary potential of waterbirds should not be overlooked.

Genetic consequences of urban management

Management may be required for synurbic species that have become overabundant in urban regions. Anthropogenic disturbances can trigger the overabundance of certain native species (termed ‘super-dominant’ species), leading to a variety of negative ecological impacts that may intensify under land use and climate change (Pivello et al. 2018). This is especially a risk for generalist species capable of occupying and thriving in human-altered habitats (Pivello et al. 2018). Management of urban populations may be necessary in cases where overabundance causes serious negative ecological effects. For example, the noisy miner (*Manorina melanocephala*), an over-abundant native species in Australia, is thought to contribute to

reduced biodiversity through aggressive exclusion of other species in both urban and rural habitats, and has been found to exhibit heightened aggression and boldness in urban regions (Parsons et al. 2006, Lowry et al. 2011). When management is deemed necessary, there may be adverse impacts on genetic diversity, particularly if an historical bottleneck has occurred due to rapid population growth (Cooper and Herbert 2001).

Anthropogenic culling or harvesting often negatively impacts wild populations by altering connectivity and reducing effective population sizes. Urban management can drive habitats to become 'ecological traps', enticing individuals with favourable conditions but exerting a net negative impact on the regional meta-population via reduced survival rates (Doak 1995, Jonzén et al. 2005). Reductions of wild populations can also lead to decreased gene flow between subpopulations, loss of genetic diversity through genetic drift and smaller effective population sizes (N_e) (Allendorf et al. 2008). N_e is defined as the size of an idealised population that would contain an equivalent amount of genetic variation present in the study population, although various conceptually different versions of N_e can be estimated (Husemann et al. 2016). N_e plays a key role in governing evolutionary processes such as rates of genetic drift, loss of genetic variation and levels of inbreeding (Frankham 1995, England et al. 2006). Reducing N_e through intense management could have detrimental impacts on long-term population persistence and evolutionary potential (Frankham 2005). Declines in local subpopulations due to management may also indirectly impact subpopulations in other parts of a species range if they are connected via a regional meta-population (Allendorf et al. 2008). In Australia, urban management of synurbic waterbirds could reduce N_e and increase the risk of inbreeding and loss of evolutionary potential, which could in turn impact declining inland colonies if these two regions are genetically connected.

Synurbanisation of the Australian white ibis

The Australian white ibis (*Threskiornis molucca*, hereafter referred to as ibis) is a colonially breeding native species (Carrick 1962, Cowling and Lowe 1981) which has drastically increased in abundance along the east coast of Australia (Martin et al. 2010, Smith et al. 2013b). One speculation is that urban growth was ignited by several independent introductions to parks between the 1960s and 80s (Symonds 1999, Ross 2004) and fuelled by inland wetland degradation, drought and the availability of urban resources (Woodall 1985, Shaw 2000, Martin et al. 2007). The success of urban colonies of the ibis is likely facilitated

by its generalist foraging strategies (Marchant and Higgins 1990, Martin et al. 2011), wide dispersal capabilities, high reproductive rates, and behavioural and ecological flexibility (Evans et al. 2010) and has necessitated widespread management of urban colonies. Meanwhile, inland colonies of this species have continued to decline across eastern Australia, with fewer than 3000 reported annually over the past few years (Porter et al. 2006, Porter et al. 2017). The success of this species in urban regions and its declining but continued existence in inland regions makes it a good candidate to study the effects of urbanisation and wetland stability on the genetic connectivity of synurbic waterbirds in Australia.

Differences in wetland stability and resource availability between inland and urban regions may be a key factor contributing to the increasing urbanisation of this species, producing a source-sink dynamic. Like other waterbirds, ibis depend on critical flooding thresholds for successful breeding to occur (Overton et al. 2009, Brandis et al. 2011, Arthur et al. 2012, Brandis and Bino 2016, Colloff et al. 2018). Urban wetlands lack these natural flow regimes and provide poor-quality food sources as ibis increasingly depend on landfills (Smith 2009, Martin et al. 2011), the latter of which may be contributing to decreased hatching success (Smith et al. 2013a). This suggests urban colonies may function as demographic sinks, supported by an influx of inland individuals during the breeding season. This is supported by the fact that the 1.5% annual growth rate in urban regions cannot alone account for the drastic increase observed during the breeding season (Smith et al. 2013b, Thomas et al. 2014). However, as inland colonies continue to decline in recent years (Porter et al. 2017), urban colonies may be in the process of becoming demographic sources. For example, urban ibis exhibit higher reproductive and fledgling success, likely due to resource availability (Lowe 1985, Smith et al. 2013a, Smith et al. 2013b), which may facilitate this transition. Further research is required to assess levels of gene flow between inland and coastal colonies of the ibis and determine whether differences between these habitats may be driving the formation of a source-sink dynamic.

Increasing sedentariness and site fidelity among urban ibis may also alter genetic connectivity between colonies. Urban ibis have been found to exhibit site fidelity (Smith and Munro 2010, Martin et al. 2011, Martin et al. 2012), a daily foraging distance of up to 40km (Murray 2005, Martin et al. 2011) and extended breeding periods compared to their inland counterparts (Lowe 1985, Smith et al. 2013a). These characteristics may have been influenced by the availability of predictable food and water sources (Martin et al. 2012). It has been suggested

that urban colonies are comprised of both mobile and sedentary sub-units (Corben and Munro 2008, Thomas et al. 2014), possibly established due to contracting migration routes along the coast in response to abundant resources (Smith and Munro 2011). However, it is unknown what impact the increasing sedentariness of urban ibis may have on local or regional genetic connectivity. Examining differences in within-colony relatedness between inland and coastal regions and looking for patterns of isolation by distance would provide useful insight into the levels of local and regional gene flow for this species.

Lastly, urban management of the ibis may have detrimental impacts on regional genetic diversity and long-term persistence of inland colonies if these two regions are genetically connected. Management of urban ibis has been implemented since the mid-90s due to their disease risk (Epstein et al. 2006), displacement of other native species (Ross 2004, Underwood and Bunce 2004), collisions with aircraft (Shaw 1999, Corben and Munro 2006) and their status as a public nuisance. Management practices range from nest destruction and egg-oiling, to waste management, food restrictions and even the use of pyrotechnics at landfills to scare off birds (Shaw 1999, Ross 2004, Martin et al. 2007). Many colonies have been controlled to well under their original size using a combination of these management strategies (Shaw 1999, Bankstown City Council 2012, Camden Council 2013). As the ibis is a protected native Australian waterbird, there is a pressing need to manage urban populations to minimise public health risks and commercial damage, while ensuring that management does not have a detrimental impact on the species (Bomford and Sinclair 2002). If inland and coastal colonies are genetically connected, processes occurring in one part of this species range (such as urban management) will have implications for the whole population. Investigating the impact of urban culling on future genetic diversity would shed light on how current management practices may impact colonies in both inland and coastal regions.

Study Scope and Aims

As urban towns and cities support increasingly managed populations of Australian white ibis and inland colonies continue to decline, research into the genetic connectivity of inland and coastal colonies is needed to better inform current management and conservation practices. In this study, single nucleotide polymorphisms (SNPs) were used to ascertain the genetic diversity and structure of several inland and coastal colonies of ibis across south-eastern Australia. Allele frequency data and more highly resolving genotypic data, that will

potentially detect changes in dispersal over a few generations (Stow et al. 2001), were analysed. This study tested whether the Great Dividing Range acts as a barrier to gene flow as well as the prediction that wetland stability and urbanisation intensity are associated with levels of gene flow. Lastly, effective population sizes were estimated and the impact of various management strategies on future genetic diversity was explored.

STUDY AREA

Our study focussed on several coastal and inland ibis colonies from Brisbane, QLD in the north to Melbourne, VIC in the south (Refer to Figure 1 and Table 2 for site locations and abbreviations). Sites were located on either side of the GDR, including 5 to the east and 6 to the west of this mountain range. Catchments east of the GDR drain into the Pacific Ocean and are characterised by a temperate climate, dominant summer rainfall and runoff co-efficients of around 30% (Chiew and McMahon 2002). Catchments west of the GDR drain into the Murray-Darling River Basin and are characterised by drier conditions, intermittent flooding and runoff coefficients of less than 15% (Chiew and McMahon 2002).

Mean surface water permanence (± S.E.) within a 50km radius of each site for the inland and coastal regions were 5.55% (± 3.30) and 31.15% (± 5.77), respectively. This data was extracted from the Water Observations from Space dataset (WOfS) and represents the mean percentage of time surface water was present within 25 × 25m grid cells across Australia between 1987 and 2014 (Mueller et al. 2016). For example, surface water observed 80% of the time indicates the presence of a permanent water body (Mueller et al. 2016). A radius of 50km around each site was chosen to cover local dispersal and resource-use of urban ibis. Urban ibis have been recorded interacting with foraging and breeding sites within approximately 40km of their colonies based on data from one breeding season (Martin et al. 2011), and a similar study conducted over 4 years found high resighting rates (70% of females and 77% of males) at urban sites up to 50 km apart (Martin et al. 2012).

The urbanisation level of each site was extracted with a principle components analysis (PCA), following similar methods in Liker et al. (2008) and Seress et al. (2014). Seven urbanisation variables, including building density, public parks, landfill and managed water sources, were extracted as the proportion of area covered by each landscape feature within a 50km radius of each site, using the Catchment Scale Land Use of Australia (CLUM) 2017 dataset (Australian Bureau of Agricultural and Resource Economics and Sciences 2017). A

PCA of these variables in R Studio v3.5.0 (R Core Development Team 2015) yielded one principle component explaining 65.81% of the variance, which was used as the urbanisation score for each site. Urbanization scores ranged from -1.72 at LAC to 3.54 at LUH (Table 2). Mean urbanisation level for coastal and inland sites were 1.85 (± 0.86 S.E.) and -1.55 (± 0.09 S.E.). Higher scores indicated greater building densities and more public services, such as parks and landfill.

METHODS

Australian white ibis feathers were opportunistically collected between 2015 and 2018 from each of the 11 study sites. For most sites, feathers were obtained in collaboration with the Feather Map of Australia project (collected under Wildlife Act 1975 and National Parks Act 1975 Research Permit no. 10007719). Feathers obtained at Centennial Parklands (CEN) and River Gum Creek Reserve (RGC) were collected under NPWS Biodiversity Conservation Act 2016 Scientific License no. SL102028 and Crown Land (Reserves) Act 1978 Research Permit No. 10008663, respectively. Feathers were collected from the ground, in nests or plucked from chicks or deceased birds. Latitude and longitude were recorded for each site using GPS, and for individual feathers at RGC and CEN to avoid sequencing feathers that were found close together.

Table 2. Study sites with designated abbreviations, region (located in a catchment with rivers flowing east (coastal) or west (inland) of the GDR)), urbanisation and WOfS scores for each site and sample size (N, final sample size after sequencing and filtering shown in brackets).

Site	Code	Region	Urban Score [a]	WOFS Score [b]	N
Lucas Heights	LUH	Coastal	3.54	32.68	8 (8)
Centennial Parklands	CEN	Coastal	3.01	48.29	8 (8)
Boondall Wetlands	BOO	Coastal	2.40	37.57	11 (5)
River Gum Creek Reserve	RGC	Coastal	1.70	21.34	9 (6)
Goulburn Wetlands	GOU	Coastal	-1.38	15.89	10 (9)
Barmah Reed Beds	BAR	Inland	-1.51	3.90	10 (8)
Gwydir Wetlands	GWY	Inland	-1.52	1.91	7 (6)
Macquarie Marshes	MAC	Inland	-1.68	0.85	8 (5)
Lowbidgee	LOW	Inland	-1.69	3.70	11 (9)
Putta Bucca Wetlands	PBW	Inland	-1.15	21.82	6 (3)
Lachlan Reserve	LAC	Inland	-1.72	1.11	6 (1)

[a] Urbanisation score extracted from a PCA of several urban landscape features.

[b] Mean percentage time surface water was detected between 1987 and 2014 (Mueller et al. 2016) within a 50km radius of the site (grid cells not containing any water (i.e. 0%) were not included in the mean).

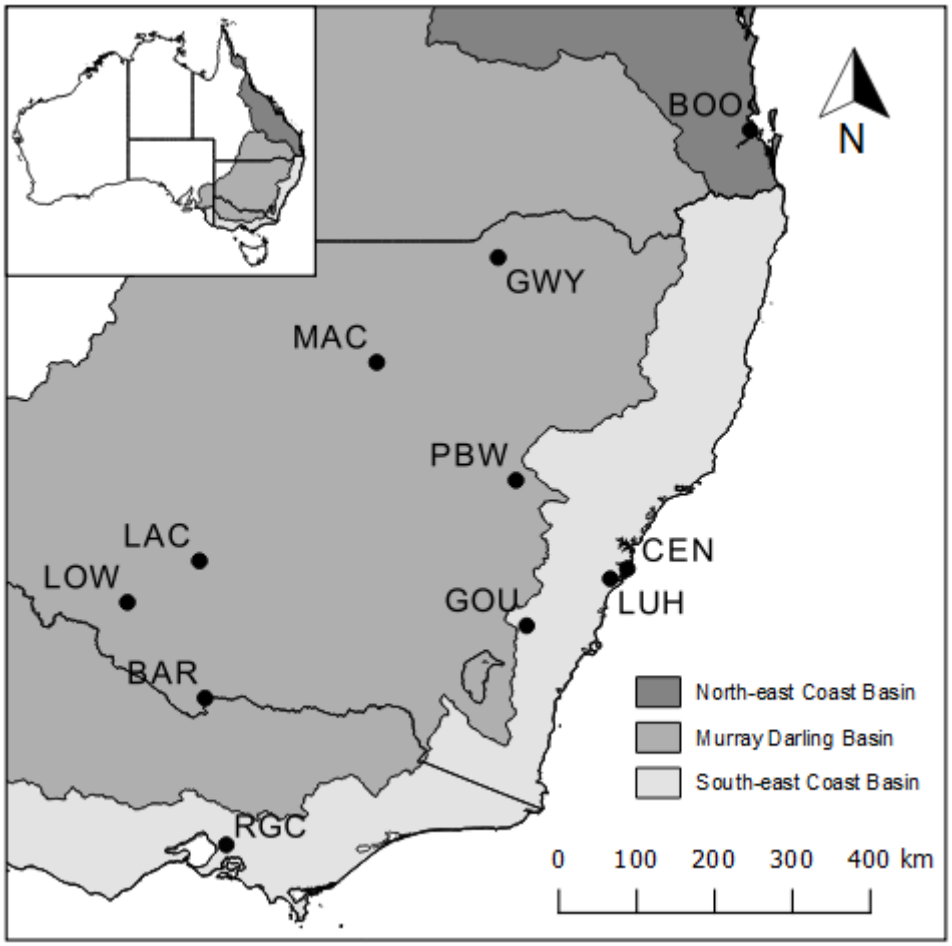

Figure 1. Map of 11 study sites across south-eastern Australia, depicting the north-east coast (dark grey) and south-east coast (light grey) river basins (contain rivers flowing east from the Great Dividing Range (GDR)), and the Murray Darling Basin (medium grey) (contains rivers flowing west from the GDR). Refer to Table 2 for a description of site abbreviations.

DNA Extraction and Sequencing

Tissue was extracted from tail and wing feathers by scraping internal material from the 10 mm basal tip of the calamus (Guilfoyle et al. 2017, Selås et al. 2017) along with external skin (following a rinse in DNAse/RNAse free distilled water). Tissue from smaller feathers was extracted by finely slicing the basal tip horizontally and chopping both calamus and internal material into smaller segments. Where possible, the superior umbilicus blood clot was also extracted, as it has previously been shown to provide a better source of DNA compared to skin cells from the calamus tip (Horváth et al. 2005, Selås et al. 2017). All material was finely chopped in lysis buffer to facilitate digestion. DNA extraction was performed using Isolate II Genomic DNA kits (Bioline). The standard protocol for DNA extraction was

followed with several modifications, including a longer digestion period (12-24hrs) (Bush et al. 2005, Arnold et al. 2017) with a higher ratio of proteinase K (35 µL, concentration 24 mg/mL, Bioline) and frequent vortexing every 5 minutes for the first hour of digestion. To maximise DNA yield, silica membranes were heated to 70ºC for 10 minutes prior to eluting samples and two 50 µL elution steps were performed. DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc.) and visualised for quality using gel electrophoresis.

DNA extracts were sent to Diversity Arrays Technology P/L (Canberra) for DartSeq™ genotyping, a genotyping-by-sequencing method based on single nucleotide polymorphisms (SNPs) (Jaccoud et al. 2001, Sansaloni et al. 2011, Kilian et al. 2012). This method genotypes several thousand SNPs across the genome on an Illumina platform (Sansaloni et al. 2011). A brief overview of this method is described below (for a detailed description see Kilian et al. (2012)).

To prepare the library, DNA was first checked for quality by incubating template DNA in a 1X solution of Multi-Core™ restriction enzyme buffer (Promega) at 37°C for 2 hours. Each sample was then processed with digestion and ligation reactions using *PstI* and *SphI* restriction enzymes and amplified using barcode and adaptor specific PCR primers. The following PCR conditions were used: 1 minute initial denaturation (94 °C), 30 cycles of 20 second denaturation (94 °C), 30 second annealing (58 °C) and 45 second extension (72 °C), and finally a 7 minute extension (72 °C). Following PCR, 10 µL of each sample were pooled together, diluted and denatured with NaOH. Sequencing of the library was conducted on an Illumina HiSeq®2500 platform. The sequencing (single read) was run for 77 cycles, resulting in 77 base pair long fragments prior to barcode removal. A subset of samples was run through the library preparation protocol and subsequent analyses to produce a set of technical replicates and assess the reproducibility of each SNP call.

Sequences were then converted to fastq format (Illumina HiSeq®2500 software) and samples were de-multiplexed based on their ligated barcode. Reads containing Phred Q-scores < 25 were removed, and reads were checked for contamination by alignment to the Bacterias_NCBI and Fungi_NCBI20170606 databases. SNPs were then called using the DArT proprietary pipeline DArTSoft14™ (Diversity Arrays Technology P/L), whereby sequence clusters were first called from pooled samples and then for individual samples. Only SNPs present in both homozygous and heterozygous forms were called, filtering out

monomorphic sequence clusters. The DArT pipeline also filters SNPs to retain those with an average ratio of read depth between alleles of 0.75, minimum read depth of 5 and reproducibility average > 90%.

Filtering Pipeline

SNPs were further filtered in R to retain loci with a reproducibility average of 1, a call rate greater than 0.95 (i.e. genotyped in at least 95% of individuals), a maximum average read depth of 86.4 across all genotypes (average read depth + 3 S.D.), a minimum average read depth of 10 across all genotypes and a minor allele frequency above 2% (MAF ≥ 0.02) to minimise rare alleles caused by genotyping errors. Additional filtering was performed to eliminate samples with more than 15% missing data, by applying a call rate filter of 0.85.

Samples were also filtered to remove highly related individuals, in order to more reliably estimate F_{ST} between groups (Szulkin et al. 2016). As some relatedness estimators perform poorly in some conditions, simulations were conducted with the software COANCESTRY v1.0.1.9 (Wang 2011) to choose the best estimator given the structure of the data. These simulations generated genotypes for 100 individuals for each of eight possible relationships (from monozygotic twins to unrelated) based on the allele frequencies and missing data rates per locus of the empirical dataset. The results of these simulations indicated that of the 5 moment and 2 likelihood estimators considered, the dyadic maximum-likelihood estimator outlined in Milligan (2003) performed best. Subsequently, relatedness was estimated using the R packages *gdsfmt* v1.6.10 and *SNPRelate* v1.14.0 (Zheng et al. 2012), implementing the dyadic MLE method with estimates truncated to the biologically meaningful range of 0-0.5 as outlined in Milligan (2003). This analysis was conducted on a linkage disequilibrium (LD) pruned SNP dataset to avoid the strong influence of SNP clusters in relatedness analyses. One sample from each pair of highly related individuals (where IBD > 0.1875) was removed (Anderson et al. 2010), based on the proportion of missing data. Sites containing fewer than 5 samples following relatedness filtering were removed, along with resulting monomorphic SNPs, for site-level analyses.

Loci potentially under selection were identified with F_{ST} and PCA based methods for detecting outlier loci, to ensure the assumption of neutrality is met for subsequent analyses, such as genetic connectivity analyses and N_e estimation, and to look for evidence of divergent selection. Two F_{ST}-based methods were employed to identify loci that lie outside

the distribution of F_{ST} values expected under genetic drift. First, the method by Whitlock and Lotterhos (2015) was conducted using the R package *OutFLANK* v0.2. Prior to identifying outliers, the dataset was trimmed to remove SNPs with very low heterozygosity ($H_E < 0.1$) which may cause the distribution to deviate from the Chi-square distribution (retaining 971 SNPs). The false discovery rate (FDR) was set at 0.05, such that SNPs with q-values lower than this threshold were considered as outliers. Secondly, the Bayesian simulation method by Beaumont and Balding (2004) was conducted with the program BayeScan v2.1 (Foll and Gaggiotti 2008). The datafile was converted to the correct format using PGDSpider v2.1.1.5 (Lischer and Excoffier 2011) and run with the following default parameters: burn-in = 50000, thinning interval = 10, sample size = 5000, pilot runs = 20, length of each pilot run = 5000, prior odds for neutral model = 10, and FDR = 0.05. The prior odds setting indicates that the selection model is 10 times less likely than the neutral model, the recommended setting for thousands of loci to lower the chance of false discoveries. Both *OutFLANK* and BayeScan methods were run with site locations as the 'populations' (with LAC grouped with LOW due to its low sample size), as well as by region (inland or coastal).

A third PCA-based outlier detection method was implemented with the R package *PCAdapt* v4.0.3 (Luu et al. 2017), which identifies outlier loci with respect to how they relate to population structure. This method does not rely on predefined population groupings and is not affected by the presence of admixed individuals, where other methods such as BayeScan are (Luu et al. 2017). The analysis was run by first performing a PCA to identify the number of genetic clusters present in the dataset, obtaining z-scores by regressing each SNP (with MAF ≥ 0.05) against the K principle components selected and computing the Mahalanobis distance test statistic. These distances are then transformed into p-values for multiple-hypothesis testing and then into q-values to control the FDR. An FDR α of 0.05 was set as for *OutFLANK*. The more conservative method of controlling the FDR, the Bonferroni correction, was also implemented (Storey and Tibshirani 2003), as well as using an LD-pruned subset of SNPs as the presence of LD may affect the ascertainment of population structure and outlier detection using this method.

Finally, the R package *dartR* v1.0.5 (Gruber et al. 2018) was used to identify loci deviating significantly from Hardy-Weinberg equilibrium (HWE) in multiple sites, which may be attributed to genotyping errors, with a Bonferroni correction applied to correct for multi-test

errors. HWE testing was also applied to samples pooled from all sites, to assess deviation from HW proportions potentially due to population stratification.

Investigation of Genetic Diversity, Differentiation and Relatedness

Measures of within-population genetic variation used in this study were computed for sites with n > 5 (i.e. excluding LAC and PBW) and included: observed heterozygosity (H_O), expected and unbiased expected heterozygosity accounting for sample size (H_E and uH_E, respectively), mean number of private alleles across loci (N_p) and the fixation index ($F = 1 - (H_O/H_E)$) which were all calculated using the excel add-in GenAlEx v6.5 (Peakall and Smouse 2012). Allelic richness (Ar), the harmonic mean number of alleles across loci corrected for sample size (Hughes et al. 2008), was calculated for each site using the R package *Hierfstat* v0.44-22 (Goudet 2005), with 95% confidence intervals about the mean. The mean inbreeding coefficient (F_{IS}) across loci for each site was calculated in GENEPOP v4.7.0 (Rousset 2008), with 95% bootstrapped confidence limits (999 bootstraps) computed in *Hierfstat*. Finally, significant differences in the distribution of multi-locus heterozygosities between regions was calculated for samples pooled from all 11 sites, using a two-sample Kolmogorov-Smirnov test in the base R package *stats* v3.5.1.

Genetic differentiation between sampling locations (with n > 5) was estimated with Weir and Cockerham's θ (θ_{WC}) (Weir and Cockerham 1984), using the R package *diveRsity* v1.9.90 (Keenan et al. 2013), with 95% bootstrapped confidence intervals (1000 bootstraps). Goudet's G-statistic Monte Carlo test was also run (999 simulations) to assess the significance of the global F_{ST} estimate. Exact G tests were run in GENEPOP to assess significant deviation in pairwise site genic (allelic) differentiation (MC parameters: dememorization: 10000, batches: 20, iterations per batch: 5000). Pairwise F_{ST} was also calculated between the two regions, inland and coastal. A one-way analysis of variance (ANOVA) was used to assess significant differences in mean pairwise F_{ST} within and between regions, using a natural-log transformation to normalise the data (with a constant (a) added to remove negative values, ln (Y + a)). Finally, an analysis of molecular variance (AMOVA) was conducted in GenAlEx, to examine partitioning of genetic variation due to regional, site and individual differences, with missing data interpolated.

To further elucidate the genetic structure of ibis colonies, a Principle Coordinates Analysis (PCoA) was conducted in GenAlEx using a genetic distance matrix computed for all pairs of

samples, with missing data interpolated. To identify the number of genetic clusters present in the dataset, k-selected clustering was performed with the R package *Adegenet* v2.1.1 (Jombart 2008, Jombart and Ahmed 2011) using the function 'find.clusters' to run successive k-means with an increasing number of clusters. The dataset was transformed with a PCA prior to running the analysis (retaining 100 PC's) and the optimal value of k was chosen based on the lowest BIC score.

To confirm the number of genetic clusters present, a Bayesian clustering approach was implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). This method assumes a model in which K populations are each characterised by a set of allele frequencies (Pritchard et al. 2000). No prior site information was supplied and the program was run under the admixture model with correlated allele frequencies (Falush et al. 2003). The setting 'correlated allele frequencies' accounts for correlations between loci that may arise due to migration or shared ancestry (admixture LD) and assumes that allele frequencies are likely to be similar for different populations (Falush et al. 2003). Optimal run length was determined by trialling a series of burn-in/MCMC parameter settings (5000/50000, 10000/100000, 50000/500000 and 100000/1000000) for multiple runs of $K = 2$. Variance between runs was highest for the first two parameter settings and similarly low for the last two. To save computational time, the third parameter setting (50000/500000) was chosen. Ten replicates for each putative group ($K = 1$ to $K = 10$) were performed. To identify the most likely value of K, the natural log probability (LnP(K)) over multiple runs was plotted following the method by Evanno et al. (2005) as implemented in STRUCTURE HARVESTER v0.6.94 (Earl 2012). STRUCTURE results were aligned and visualised using CLUMPAK (Kopelman et al. 2015).

Relatedness

Relatedness estimated from genetic data operates under the assumption that for related individuals, alleles have a higher probability of identity-by-descent (IBD) than unrelated individuals. Maximum likelihood estimation of relatedness (Milligan 2003, Choi et al. 2009) was carried out using the R packages *gdsfmt* and *SNPRelate* on an LD-pruned subset of 217 SNPs (sliding window = 200, LD threshold = 0.2) and compared with estimates obtained using the complete dataset of 1860 SNPs. Average pairwise relatedness within each site and region was then calculated using GenAlEx, using the MLE-computed pairwise matrix of

relatedness. For each site, 95% confidence intervals around the null hypothesis of random mating were estimated based on 9999 permutations and 9999 bootstraps.

Spatial Structure

To infer potential isolation by distance and determine whether genetic correlation (r) between individuals decreases with increasing distance, we conducted a spatial analysis of genetic variation with a multi-locus spatial autocorrelation analysis in GenAlEx. Individual pairwise geographic and genetic distance matrices were computed with missing data interpolated. Spatial autocorrelations were run with 9999 bootstrap iterations to compute 95% confidence intervals around the mean r within each distance class, and 9999 permutations to compute 95% confidence intervals about the null hypothesis of 'no spatial structure' ($r = 0$).

The detection of spatial structure involves a trade-off between distance class size (spatial resolution) and sample size per distance class (Banks and Peakall 2012). However, relatively few pairs of individuals (n = 64 pairs) fell within the 1-50km range representing local dispersal distances for urban ibis (Martin et al. 2011, Martin et al. 2012). These pairs of individuals were grouped with those falling in the 51-150km distance class (total n = 145 pairs) and a multiple distance class run confirmed that grouping these distances classes together did not mask the detection of finer-scale structure. We also grouped distance classes above 900 km due to low sample sizes. This resulted in seven distance class bins (0, 150, 300, 450, 600, 750, 900 and 1400km), where sample size per bin > 100. A similar analysis was run for the inland and coastal regions independently to examine spatial structure on either side of the GDR, with monomorphic SNPs removed for each region prior to analysis. Low sample sizes for within-region analyses required larger bin sizes to be used, resulting in a coarser spatial scale.

A heterogeneity test was also run for each region to determine if the correlograms deviated significantly from randomly shuffled data from across the geographic space, following the methods of Smouse et al. (2008). A heterogeneity test between the inland and coastal regions was also performed, with distance class bins chosen to compromise on the range of pairwise distances observed within each region while maximising sample size per bin. This resulted in five uneven distance class bins (0, 200, 600, 800 and 1000km). A significance level of 1% was applied to minimise type 1 error rates as recommended by Banks and Peakall (2012).

Structure across environmental gradients

To examine trends in pairwise F_{ST} across environmental gradients and determine whether genetic differentiation increases with habitat differences, we conducted a linear regression analysis on pairwise F_{ST} and two environmental variables, including their interactions, with the R package *FinePop* v1.3 (Kitada et al. 2017). The linear regression method employed by Kitada et al. (2017) accounts for correlations in F_{ST} values between sites, to avoid the bias resulting from these correlations that is inherent in partial Mantel tests (Guillot and Rousset 2013). Two estimates of genetic differentiation were tested including Weir and Cockerham's θ (θ_{WC}) and the empirical Bayes G_{ST} estimator (EBF_{ST}) suitable for species with high levels of gene flow (Kitada et al. 2007), calculated using the function 'FstBoot' in *FinePop* (with 999 bootstraps for both methods). Two site-level environmental variables were examined, including urbanisation score and mean surface water permanence (refer to Table 2). Geographic distances computed in GenAlEx were also incorporated into the models to account for correlations between distance and pairwise F_{ST}. A total of 11 models were tested using the function 'FstEnv' in *FinePop*, and the best fit model with the minimum Takeuchi information criterion (TIC) was selected. The full model was:

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

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

Estimating Effective Population Size (N_e)

Preliminary estimates for effective population size (N_e) were obtained using the bias-corrected linkage disequilibrium (LD) method of Waples and Do (2008) under the 'random mating model', as implemented in NeEstimator v2.1 (Do et al. 2014). Estimation of N_e using this method operates under the assumption that for neutral, physically unlinked loci in a closed, randomly mating population, LD (or the correlation of gene frequencies) driven by drift occurs at a rate inversely proportional to N_e (Langley 1977, Laurie-Ahlberg and Weir 1979, Hill 1981, Waples et al. 2016). In the model, drift is assumed to be solely responsible for the signal in the data rather than mutation, migration or selection, and the population is assumed to be stable and panmictic (Hill 1981).

Obtaining raw estimates of N_b

N_e was estimated using pooled samples from all sites, with known juveniles and chicks removed from the dataset prior to analysis, resulting in a subset of 66 samples. The two individuals removed were collected between 2017-2018 and were unlikely to reach sexual maturity within the sampling period. However, as most feathers were collected non-invasively, juveniles may still be present in the dataset used for this analysis. Rare alleles were screened out with two P_{crit} values, 0.02 and 0.05, to ensure only alleles that are actually present in the population are used in the analysis (Waples and Do 2010). A P_{crit} of 0.05 reduces bias at the cost of a reduction in precision, while the converse is often true for lower P_{crit} values (0.01 or 0.02), which tend to upwardly bias estimates (Waples and Do 2010, Waples et al. 2014). The 95% confidence intervals were calculated using both the parametric method and the bias-corrected jack-knife method of Waples and Do (2008). Output from NeEstimator was interpreted as the effective number of breeders ($\widehat{N}_b$) in one reproductive cycle which produced the cohorts that were sampled, rather than the effective population size ($\widehat{N}_e$) per generation, due to the presence of overlapping generations for this species (Waples and Do 2010). N_b provides information on how the variance in reproductive success, for example between age classes or sexes, affects genetic drift in a breeding event or season (Wang et al. 2016).

Bias adjustment for overlapping generations

As LD estimators of N_e assume discrete generations (Waples and Do 2010), raw output from NeEstimator ($Raw\widehat{N}_b$) was corrected for bias due to overlapping generations, using the formulas derived by Waples et al. (2014). Ibis reach sexual maturity within 2-3 years (Marchant and Higgins 1990, Smith and Munro 2011) and are classified as adults at 4 years of age (Smith et al. 2013b). As ibis are long-lived (reaching up to 26 years of age (Australian Bird and Bat Banding Scheme 2018)) and are known to breed year round if resources are abundant (Lowe 1985, Beilharz 1988), it is likely the dataset contains a broad range of adult ages from multiple cohorts. Using random samples of adults (multiple cohorts), for which the number of cohorts is not close to the generation length of the species, could underestimate N_e if $N_b >> N_e$, causing a downward bias when multiple cohorts are present (Waples et al. 2014). To first estimate the degree of bias present in $Raw\widehat{N}_b$ due to overlapping generations,

the ratio N_b/N_e was predicted from two life history traits using equation 1 below (Waples et al. 2013).

$$N_b/N_e = 0.485 + 0.758\log(AL/\alpha) \quad [1]$$

Here, AL = adult lifespan ($\omega - \alpha + 1$; ω = maximum age) and α = age at sexual maturity. For the ibis, α was taken as the average of recorded ages at sexual maturity, 2.5 (Marchant and Higgins 1990, Smith and Munro 2011), while AL was calculated based on the maximum recorded age of the white ibis, 26 years (Australian Bird and Bat Banding Scheme 2018), giving $AL = 24.5$. To adjust $\widehat{N}_b$ for this inherent bias, equation 2 below was applied and subsequently equation 3 was applied to the output from equation 2 to yield a preliminary bias-adjusted estimate of N_e (Waples et al. 2014).

$$\widehat{N}_{b(Adj2)} = \frac{raw\widehat{N}_b}{1.103 - 0.245 \times \log(AL/\alpha)} \quad [2]$$

$$\widehat{N}_{e(Adj2)} = \frac{\widehat{N}_{b(Adj2)}}{0.485 + 0.758 \times \log(AL/\alpha)} \quad [3]$$

Bias adjustment for the presence of physical linkage

Another assumption of LD estimators is the independent inheritance of loci (probability of recombination = 0.5). The use of several thousand SNPs inevitably contains a portion of physically linked loci, particularly for SNPs obtained via genotyping-by-sequencing methods which may contain more LD than expected by chance (Waples et al. 2016). This introduces the risk of pseudo-replication (Waples and Do 2010), which could downwardly bias and lower the precision of N_e estimates (Do et al. 2014, Waples et al. 2016). A bias-correction using genome size and karyotype has been developed to address this issue, which assumes that larger genomes are more likely to contain a larger portion of unlinked loci and thus the bias due to physical linkage is lower (Waples et al. 2016). To adjust $\widehat{N}_e$, equations 4 and 5 below were applied to $\widehat{N}_{e(Adj2)}$ (Waples et al. 2016).

$$\widehat{N}_{e(Adj3)} = \frac{\widehat{N}_{e(Adj2)}}{0.098 + 0.219 \times \ln(Chr)} \quad [4]$$

$$\widehat{N}_{e(Adj4)} = \frac{\widehat{N}_{e(Adj2)}}{-0.910 + 0.219 \times \ln(cM)} \quad [5]$$

Here, Chr is the haploid chromosome number for the species and cM is the genome size in centimorgans. As these aspects of genome architecture are unknown for the Australian white ibis, estimates for the closely related Sacred ibis (*Threskiornis aethiopicus*) were used (haploid chromosome number = 34, genome size = 1.25 pg) (Venturini et al. 1986). Genome size in pg was converted to an approximate size in cM by first converting pg to Mb (1.25 pg $\approx$ 1222.5 Mb (Cavaller-Smith 1985, Dolezel et al. 2003)) and then converting Mb to cM using estimated recombination rates (cM/Mb). Recombination rates are unknown for close relatives of the ibis, although such estimates fall within a relatively narrow range for several other avian species (see Table 3). Using the average recombination rate across these species (2.17 cM/Mb) gave an approximate genome size of 2652.8 cM for the ibis. It should be noted that recombination rate varies considerably across the genome (Kong et al. 2002, Jensen-Seaman et al. 2004, Myers et al. 2006, Wahlberg et al. 2007), is not always highly conserved between closely related species (Dawson et al. 2007) and may even differ between the sexes (Hansson et al. 2005). As such, subsequent analyses were based on the more reliable bias-adjustment of equation 4.

Table 3. Estimates of recombination rate (cM/Mb) for other avian species. Genome sizes obtained from the online database of animal genome sizes (Gregory, 2018).

Species Name	**Common Name**	**Genome size (pg)**	**cM/Mb**	**Reference**
Gallus gallus domesticus	Chicken	1.25-1.28	3.11[a]	Groenen et al. (2009)
			2.80-2.90[b]	Romanov et al. (2014)
Taeniopygia guttata	Zebra finch	1.25	1.29-1.60[b]	Romanov et al. (2014)
			1.5[c]	Backström et al. (2010)
Anas platyrhynchos	Mallard duck	NA	2.00	Pigozzi and del Priore (2016)

[a] Average genetic size of a 1Mb bin
[b] Depending on the presence of evolutionary breakpoint regions
[c] Average across the genome (not including microchromosomes)

Future Genetic Diversity under Urban Management

To predict the impact of urban management on the future genetic diversity of ibis across south-eastern Australia, forward simulations were run with the program BOTTLESIM v2.6 (Kuo and Janzen 2003). Two effective population sizes (rounded to the nearest even number) were trialled: the bias-adjusted estimate based on P_{crit} = 0.05, and its lower jack-knifed confidence limit. Simulations were first run under constant population sizes (i.e. no urban management), and then under various management scenarios of 2%, 5% and 10% reductions

each year, both with and without population growth of 1.5% factored in (Smith et al. 2013b). Under these management scenarios, simulations were also run by maintaining the population at Sydney's regional management target of 6500 ibis (Ecosure Pty Ltd 2010). Assuming an N_e/N ratio of 0.1 (Frankham 1995, Frankham et al. 2014), this sustained the effective size at 650 after the initial population culls. Additionally, single bottlenecks of 50% and 90% were trialled. The following settings were employed in all scenarios: dioecy with random mating, 1:1 sex ratio, 85 years (~28 generations), 500 iterations, longevity = 26 years, age at sexual maturity = 3 years and generational overlap = 100%. Individuals from different sampling localities were grouped together as one population for these simulations.

RESULTS

Sequencing and Filtering

A total of 73,876 SNPs was obtained through DArT sequencing, and following SNP level filtering, 4,737 SNPs were retained for 76 individuals (see Appendix C for details on the filtering pipeline). Three samples were removed given their high genetic distance to other samples (range 13221-14576) suggesting a species origin other than the white ibis, and another two were removed due to missing data. Three additional samples were removed due to high degrees of relatedness with other samples. These individuals exhibited consistently high IBD (0.94-0.97 prior to truncation in *SNPRelate*) and low kinship coefficients (both k_0 and $k_1 = 0$), regardless of whether a stringently LD-pruned subset of SNPs or the full dataset were used. These samples, which were found within the same sites, most likely represent monozygotic twins and thus have been removed from all subsequent analyses. No other close relatives were found.

Only 11 loci deviated significantly from HWE within a single site following the Bonferroni correction. As these loci did not deviate in more than one site, it is unlikely that these deviations are caused by genotyping errors or null alleles and were not removed from the dataset. HWE testing applied to the dataset of pooled samples identified 36 loci deviating significantly from HW proportions, with the Bonferroni correction. Preliminary analyses indicated that results did not differ significantly when these loci were included or removed, and so these loci were retained in the final datasets.

Outlier detection analyses found 3 SNPs using the F_{ST}-based method of *OutFLANK*, which identified a different set of 3 SNPs for each H_E threshold of 0.05 and 0.1, although the former yielded a slightly worse fit to the Chi-square distribution. The 3 outliers found were all due to LAC (where n = 1) exhibiting significantly different allele frequencies to all other sites, and thus these were not considered as true outliers. When repeated with LAC grouped with its nearest neighbouring site, LOW, only 1 outlier was found under a H_E threshold of 0.05 and none were found under 0.1. Additionally, no outliers were identified with *OutFLANK* when region was used as the 'population' grouping to calculate F_{ST}. BayeScan found no outliers based on site or region groupings. The PCA-based method of *PCAdapt* found a range of different outliers (1 – 147) depending on the FDR criteria used and the level of LD allowed in the analysis, all based on K = 1 as determined by the PCA. No SNPs were found in common between the three different methods used for any of the different settings trialled. Given the lack of consensus on the presence of outlier loci in this dataset, the dataset was assumed to be selectively neutral and no loci were removed. Additionally, the exclusion of these loci (155 in total) made no significant difference to genetic structure analyses (see Appendix C for details on outlier tests and PCoA results with all potential outliers excluded).

The final dataset used for site-level analyses consisted of 64 individuals from nine sampling localities and 1,809 SNPs, while the dataset used for all other analyses (e.g. at the regional scale) consisted of 68 individuals from eleven sampling localities and 1,860 SNPs.

Is there evidence of genetic structuring among colonies and regions?

Mean unbiased expected heterozygosity (uH_E) and observed heterozygosity (H_O) across all loci and populations was 0.169 (SE ± 0.001) and 0.149 (SE ± 0.001), respectively. The distribution of multi-locus heterozygosity did not differ significantly between inland and coastal regions based on the Kolmogorov-Smirnov test (D = 0.156, p > 0.05). For a summary of basic statistics for each site, refer to Appendix D.

Global F_{ST} across all loci and populations was 0.0032, which did not deviate significantly from 0 (p = 1.00), suggesting that site locations explain only 0.32% of the observed genetic variation. Pairwise F_{ST} between sites ranged from 0 (LOW/RGC) to 0.0191 (BOO/MAC) (Figure 2). No pairwise population genic differentiation deviated significantly from 0 (p = 1.00 for all pairs). F_{ST} across all loci between the inland and coastal regions was 0.0005, which did not deviate significantly from 0 (p = 1.00) and explained less of the observed

genetic variation than population groupings. There were no significant differences between mean pairwise F_{ST} within nor between regions (i.e. pairs across the GDR) (F(2) = 0.596, p = 0.557) (Figure 3). Furthermore, the AMOVA with within-individual variation suppressed revealed that no proportion of genetic variation is attributable to regional differences, while up to 1% is attributable to between-population differences (Figure 4).

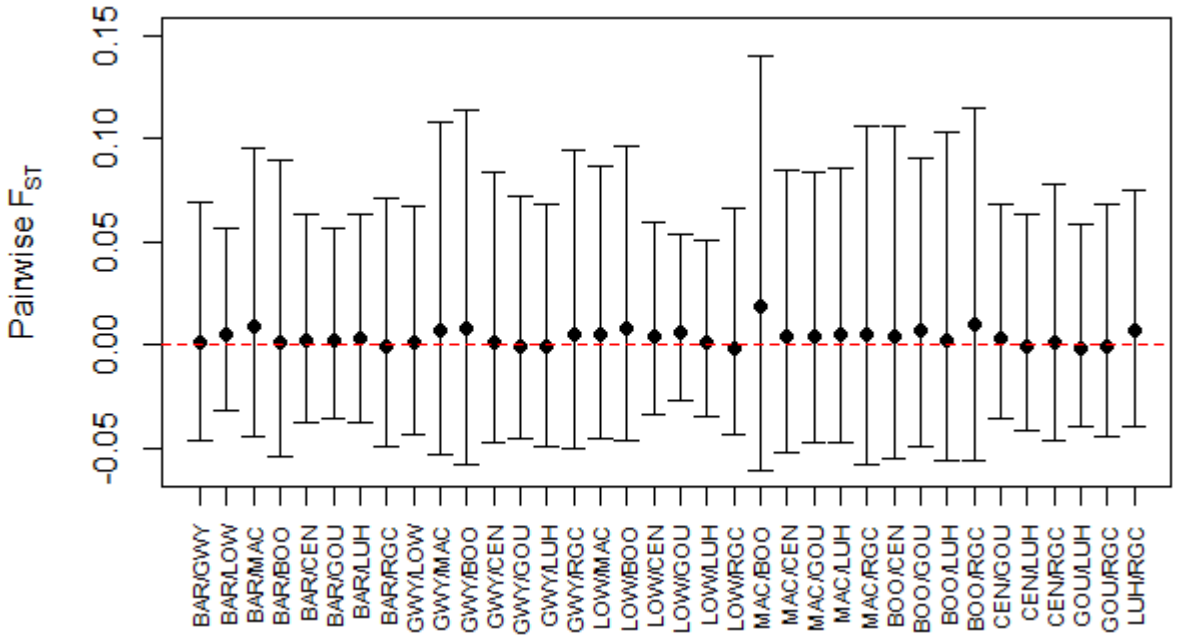

Figure 2. Pairwise F_{ST} (θ_{WC}) between sampling localities, with 95% bootstrapped confidence intervals (based on 1000 bootstraps). For abbreviations of sites, see Table 2.

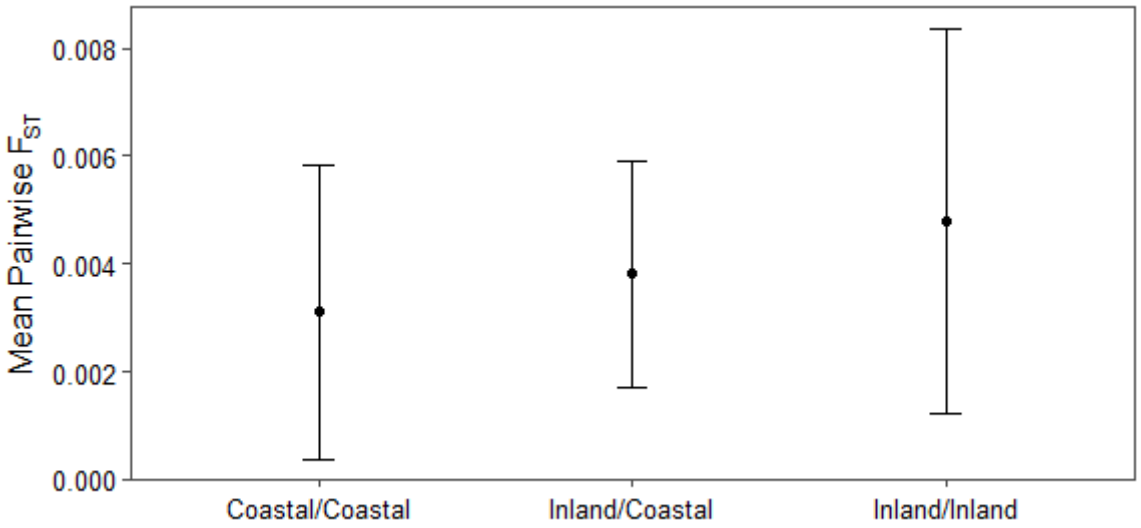

Figure 3. Mean pairwise F_{ST} grouped by region (with standard 95% confidence intervals about the mean). Inland/Coastal represents pairwise F_{ST} comparisons across the Great Dividing Range.

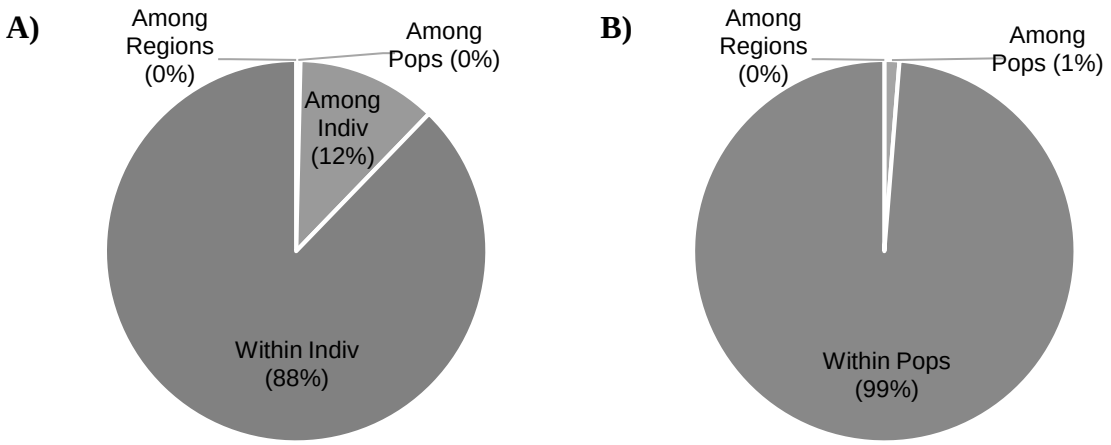

Figure 4. Analysis of molecular variance (AMOVA) with A) within-individual genetic variation included (Within Indiv) and B) within-individual genetic variation suppressed.

The PCoA confirmed the lack of genetic sub-structuring among samples, with the first and second principle coordinates of the PCoA explaining 2.43 and 2.35% of the genetic variation, respectively (Figure 5). Additionally, k-selected clustering and STRUCTURE analyses suggested the presence of a single genetic cluster containing all individuals in the dataset (Figure 6). The values of K which exhibited the highest log probabilities (LnP(K)) in the STRUCTURE analysis were $K = 1$ and $K = 2$ (see Appendix D for detailed output).

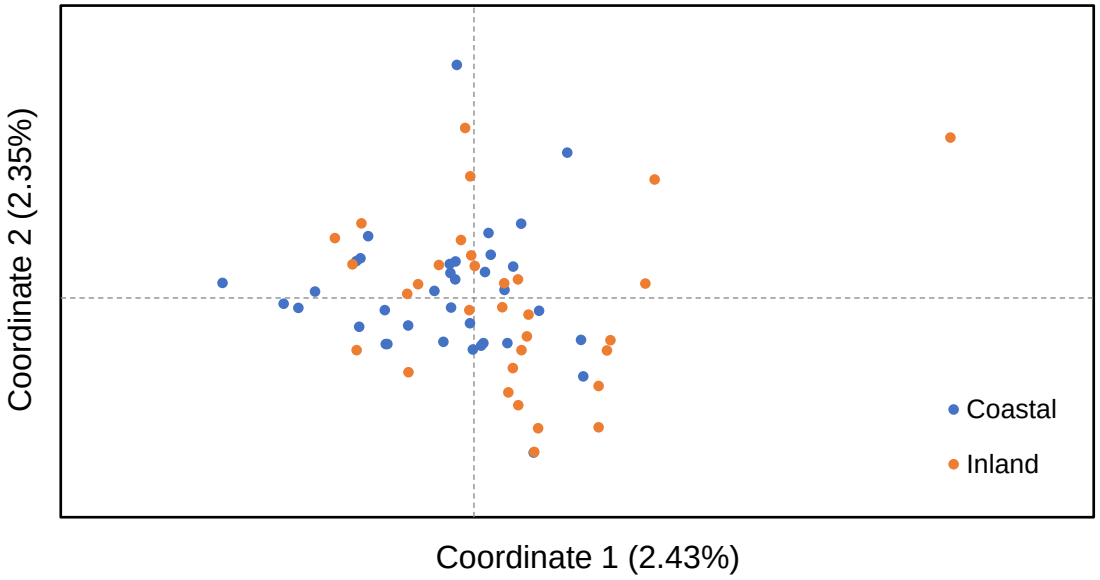

Figure 5. Plot of the 1st and 2nd principle coordinates identified by a principle coordinate analysis (PCoA), with individuals colour coded by region of origin.

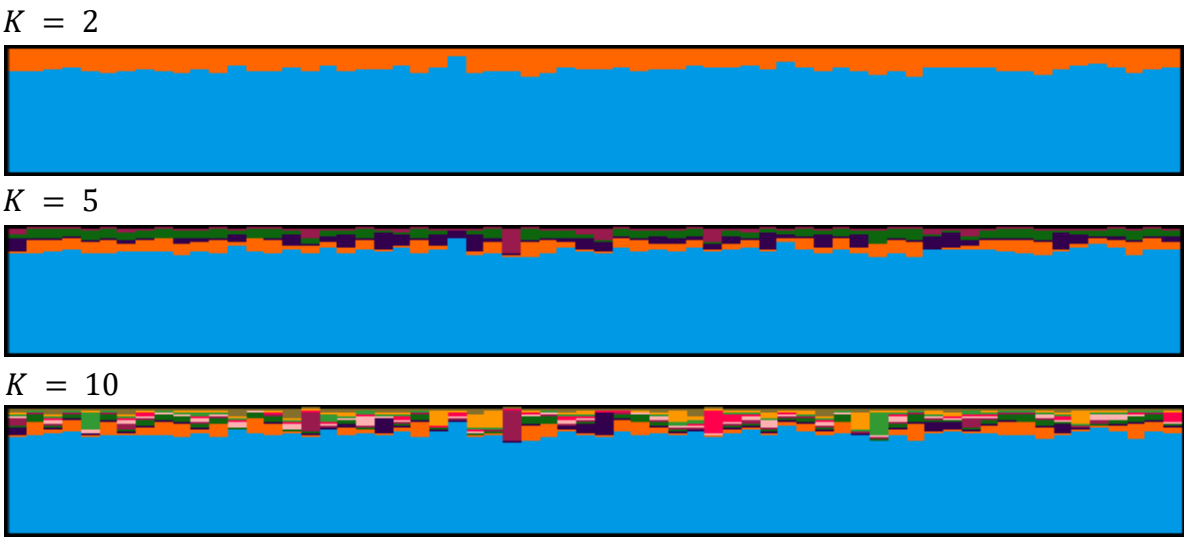

Figure 6. Aligned STRUCTURE bar plot for three values of K. Individuals are represented by a single vertical line, while colours represent inferred genetic clusters.

Relatedness

Pairwise relatedness (r_{xy}) was low between most pairs of individuals (μ = 0.014, S.E. ± 0.0005). Average r_{xy} did not differ significantly within nor between regions using the LD-pruned subset of SNPs (Figure 7) and was not significantly correlated with the difference in urbanisation scores between sites. Average within-population r_{xy} for the coastal region deviated significantly from the null hypothesis of random mating only for analyses using 1860 SNPs and not the LD-pruned subset. Mean within-population r_{xy} (± 95% CI) based on the LD-pruned dataset was 0.013 (± 0.002) among inland samples and 0.015 (± 0.002) among coastal samples (Figure 8A). Based on the full dataset, mean r_{xy} (± 95% CI) was 0.003 (± 0.0006) and 0.004 (± 0.0005) within inland and coastal regions respectively (Figure 8B).

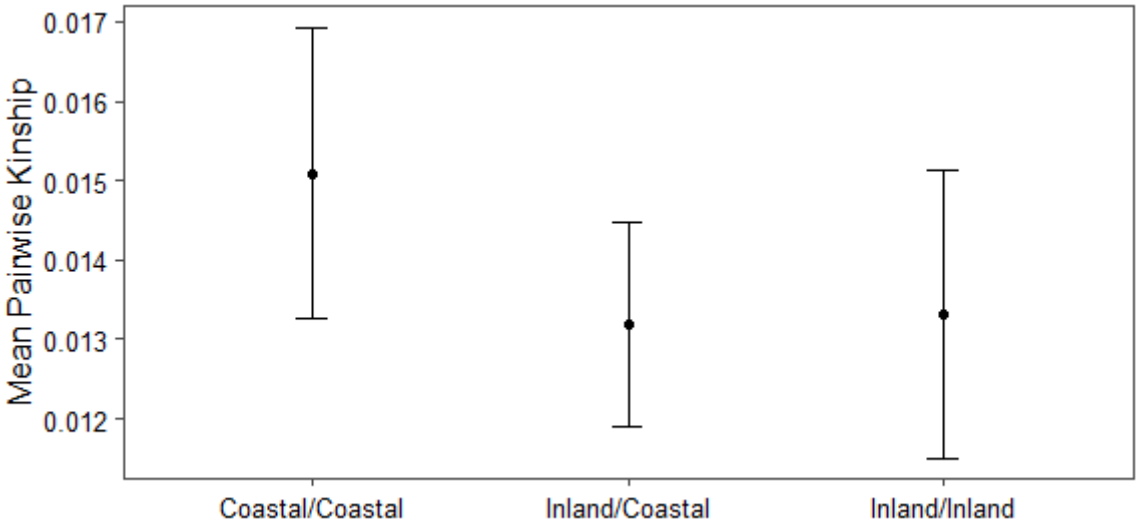

Figure 7. Mean pairwise relatedness within and between regions based on an LD-pruned subset of SNPs (error bars indicate standard 95% confidence intervals about the mean, Inland/Coastal represents relatedness between pairs on either side of the GDR).

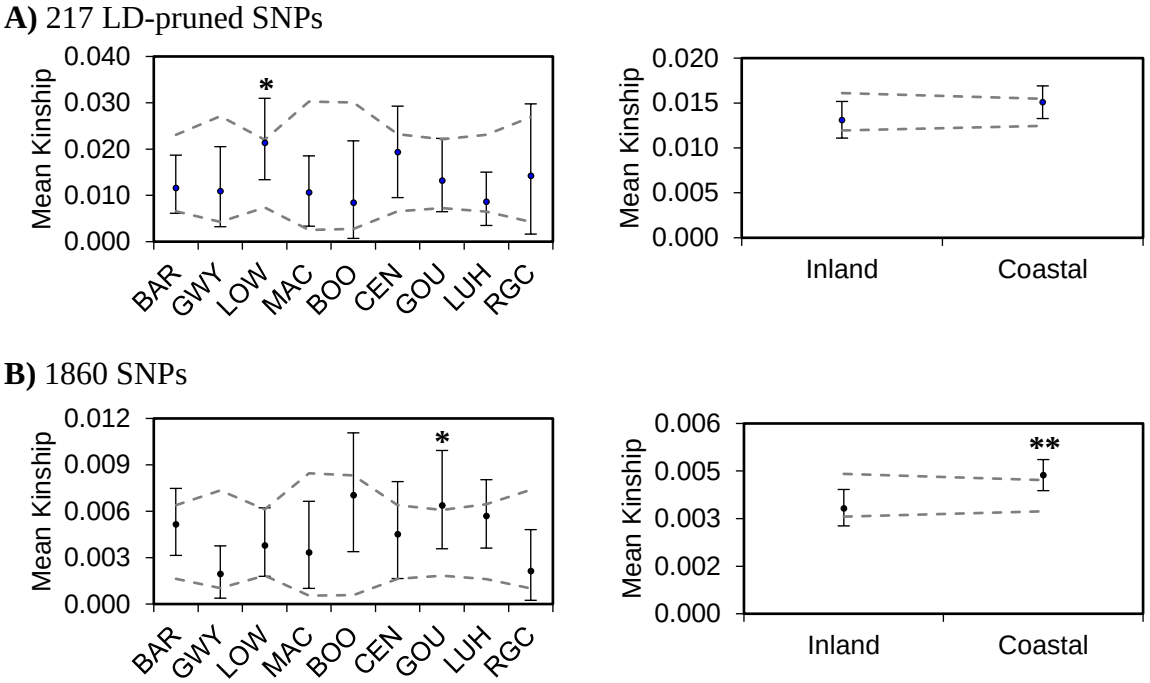

Figure 8. Mean within-population pairwise relatedness based on A) 217 LD-pruned SNPs and B) the full dataset of 1860 SNPs (Error bars indicate 95% confidence intervals about the mean based on 9999 bootstrap replicates, while grey dashed lines indicate 95% confidence intervals around the null hypothesis of no difference between populations (random mating) based on 9999 permutations; *$p < 0.05$, **$p < 0.01$). For abbreviations of sites, see Table 2.

Spatial Autocorrelation

For the whole region, genetic correlation (r) was significantly greater than random data (r = 0) at distance classes 0 (r = 0.005, $p < 0.05$) and 150 (r = 0.004, $p < 0.05$); and the heterogeneity test indicated statistically credible positive spatial structure (omega = 42.6, $p < 0.01$) (Figure 9). However, as genetic correlation was lower than 0.01, the results likely indicate a very weak correlation. For the inland region, distance class 0 exhibited significantly greater genetic correlation (r = 0.006 ± 0.006, $p < 0.05$), however the spatial correlogram was only near-significant under a 1% significance level (omega = 25.3, p = 0.024). For the coastal region, none of the distance classes were significant and the heterogeneity tested suggested no significant positive spatial structure was present. A pairwise population heterogeneity test also indicated that spatial correlograms for inland and coastal regions were not significantly different from one another (omega = 7.52, p = 0.67, Figure 10).

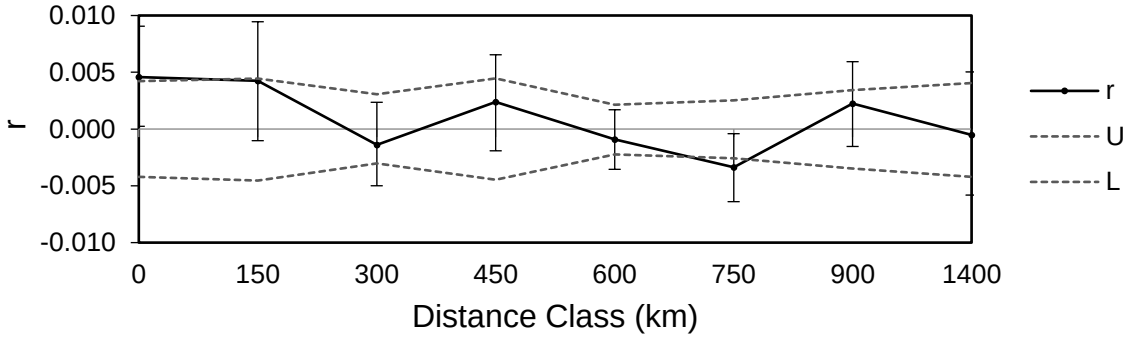

Figure 9. Spatial correlogram showing mean genetic correlation (r) at different distance class bins (U and L represent corrected upper and lower 95% confidence intervals about the null hypothesis of 'no spatial structure' as determined by 9999 permutations; error bars within each distance class represent 95% confidence intervals about genetic correlation (r), as determined by 9999 bootstrapped resampling; number of comparisons per distance class = 209, 145, 320, 179, 550, 462, 257 and 156).

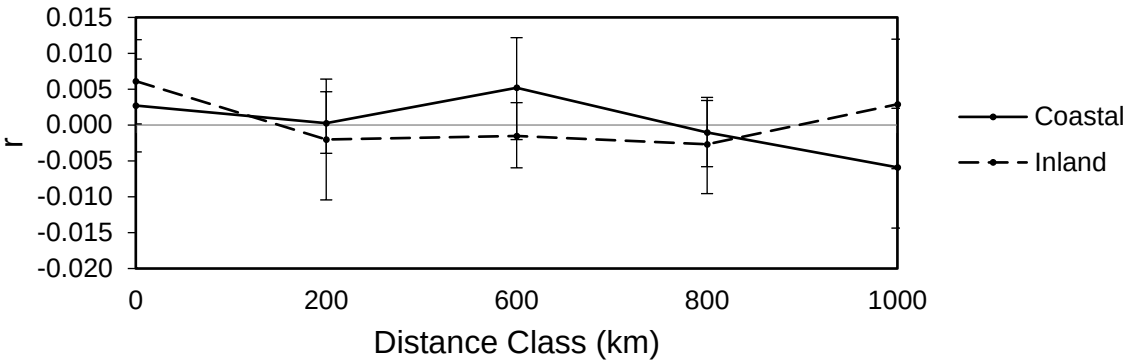

Figure 10. Heterogeneity test comparing the spatial correlograms of inland and coastal regions (based on 9999 permutations and 9999 bootstraps).

Does urbanisation intensity or surface water permanence influence genetic differentiation?

The best fit linear regression model, for both θ_{WC} and EBF_{ST}, with the lowest TIC score was the model incorporating all three environmental variables (distance, urbanisation level and mean surface water permanence) and their interactions (model = F_{ST} ~ -1 + (distance + urban + water)^2; Table 4). However, this model indicated only a weak, non-significant correlation (R^2 = 0.218 and 0.066, respectively) and correlation coefficients were typically lower when EBF_{ST} was used. All correlation coefficients were non-significant, except for the interaction between urbanisation score and mean surface water permanence for θ_{WC} (Z = -1.97, p < 0.05).

Table 4. Linear regression results (R^2 and TIC scores) for 11 models based on two different measures of genetic differentiation (θ_{WC} and EBF_{ST}) (distance = the geographic distance between sites, urban = the absolute difference in urbanisation score between sites; and water = the absolute difference in mean surface water permanence between sites).

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

What is the effective population size across South-Eastern Australia?

Estimates of the effective number of breeders and effective population sizes obtained with a P_{crit} of 0.05 are listed in Table 5. Trials using a range of P_{crit} values indicated that estimates obtained with a P_{crit} of 0.05 (i.e. reduced bias) were close to those obtained with less stringent criterion (i.e. increased precision) (Waples and Do 2010, Waples et al. 2014), except for P_{crit} of 0.02 which appeared to upwardly bias the estimate (Figure 11). Because of this, estimates of effective size obtained using a P_{crit} of 0.05 were used in subsequent analyses (refer to Appendix D for effective size estimates obtained using a P_{crit} of 0.02).

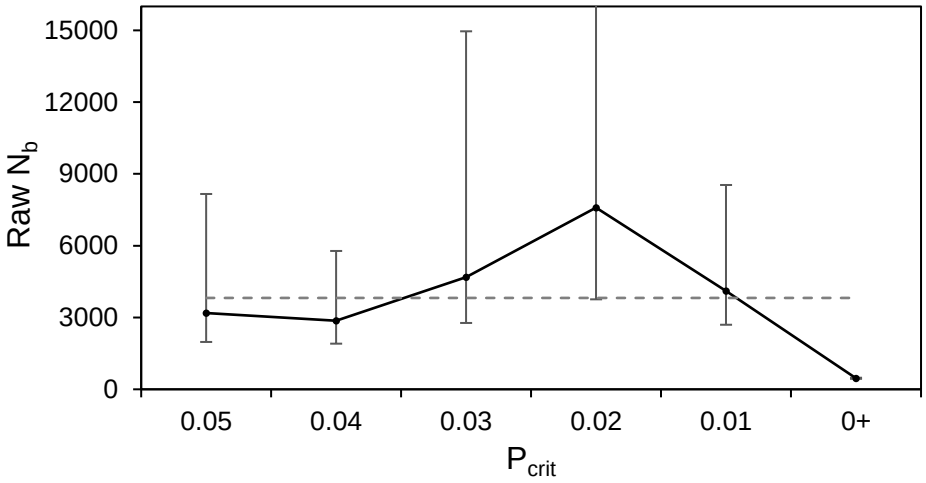

Figure 11: $Raw\widehat{N}_b$ with parametric 95% confidence intervals obtained for a range of P_{crit} values. Upper limit is undefined (i.e. infinite) for P_{crit} of 0.02. The dashed line indicates the average effective size across P_{crit} values.

$Raw\widehat{N}_b$ for the whole region was 3191.4 based on a P_{crit} of 0.05. The bias correction based on overlapping generations yielded a slightly higher $\widehat{N}_{b(Adj2)}$ compared to $Raw\widehat{N}_b$, although subsequent conversion to $\widehat{N}_{e(Adj2)}$ was lower than $Raw\widehat{N}_b$ ($N_e < N_b$ for over half of the species examined in Waples et al. (2013)). The ratio N_b/N_e calculated from life-history traits was 1.236, indicating that $\widehat{N}_b$ is likely to underestimate the true N_b for a single cohort and create a downward bias as more cohorts are added (Waples et al. 2014). As this ratio is > 0.6, it is likely a good indicator of the bias in $Raw\widehat{N}_b$ when multiple consecutive cohorts are used (Waples et al. 2014). After LD-bias correction using the haploid chromosome number for Sacred ibis ($Chr = 34$), $\widehat{N}_{e(Adj3)}$ for the whole region was 3448.3 (95% jack-knifed CI = 1202.8 - ∞). $\widehat{N}_{e(Adj3)}$ was similar for both the coastal and inland regions, estimated at 2594.6 (95% CI = 508.5 - ∞) and 2499.6 (95% CI = 494.6 - ∞), respectively (see Appendix D). The LD-bias adjustment increased all naïve N_e estimates ($\widehat{N}_{e(Adj2)}$) by 14.9%. Finally, every estimate had an infinite upper bound for the jack-knifed confidence limits (i.e. a negative estimate interpreted as 'no upper bound' or infinite).

Table 5. Estimates of effective size for the South-East Australian region, based on a P_{crit} of 0.05, before ($Raw\widehat{N}_b$) and after correcting for bias due to overlapping generations ($\widehat{N}_{b(Adj2)}$, $\widehat{N}_{e(Adj2)}$) and physical linkage ($\widehat{N}_{e(Adj3)}$, $\widehat{N}_{e(Adj4)}$), with 95% jack-knifed and parametric confidence intervals (∞ = no upper bound).

Bias-Correction	**Estimate**	**Jack-knifed 95% CI**	**Parametric 95% CI**
$\boldsymbol{Raw\widehat{N}_b}$	3191.4	1113.2, ∞	1978.4, 8162.8
$\boldsymbol{\widehat{N}_{b(Adj2)}}$	3710.3	1294.2, ∞	2300.1, 9490.0
$\boldsymbol{\widehat{N}_{e(Adj2)}}$	3001.0	1046.8, ∞	1860.4, 7675.8
$\boldsymbol{\widehat{N}_{e(Adj3)}}$ **(*Chr*)**	3448.3	1202.8, ∞	2137.7, 8820.0
$\boldsymbol{\widehat{N}_{e(Adj4)}}$ **(*cM*)**	3675.6	1282.1, ∞	2278.6, 9401.3

What impact will urban management have on regional genetic diversity?

Simulations under constant effective sizes indicated more than 99% of the initial genetic variation is retained after 85 years, for both 3448 and 1202 initial effective population sizes (Figure 12A). However, the rate at which genetic diversity deteriorated differed under the 2%, 5% and 10% management scenarios tested. Under the scenario of culling 2% each year (with and without population growth of 1.5%) neither initial N_e (N_i) led to the percentage of retained genetic diversity dropping below the recommended level of 90% to avoid inbreeding depression (Figure 12B). Under the 5% scenario genetic diversity dropped below 90% after 82 years for N_i = 3448 (without population growth), and 81 and 62 years for N_i = 1202 (with and without population growth, respectively) (Figure 12C). Under the 10% scenario, genetic diversity retained dropped below 90% in all cases, after 34-50 years (Figure 12D). Effective sizes during these years ranged from 34 to 66, depending on the management scenario, N_i and population growth. Maintaining the effective size at Sydney's management target of 650 (assuming N_e/N = 0.1) indicates more than 97% of genetic diversity is likely to be retained after 85 years under all management scenarios, both with and without population growth factored in. Under the extreme scenarios of culling 50 and 90% in year 1, the percentage of retained genetic diversity only fell below the 90% threshold for N_i = 1202 (Figure 13). In all scenarios of urban management, the observed number of alleles deteriorated faster than observed heterozygosity, as expected by theory (Allendorf 1986).

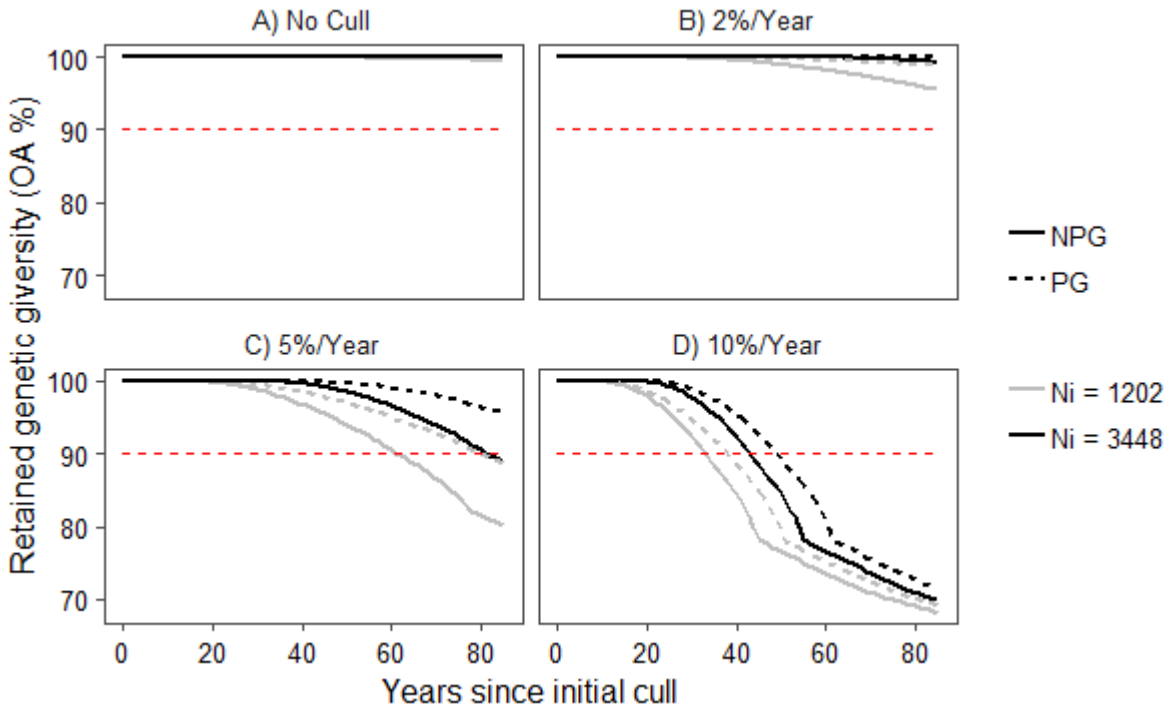

Figure 12. Percentage retained genetic diversity (OA, the observed number of alleles) after 85 years, under various management strategies for an initial N_e of 3448 (black) or 1202 (grey), both with population growth (PG) and without (NPG) (dashed and solid lines, respectively). Under the 10% Cull/Year scenario, effective size remained at 10 after 45-62 years due to rounding (to the nearest even number). Red dashed line indicates the recommendation of maintaining retained genetic diversity above 90% to avoid inbreeding depression.

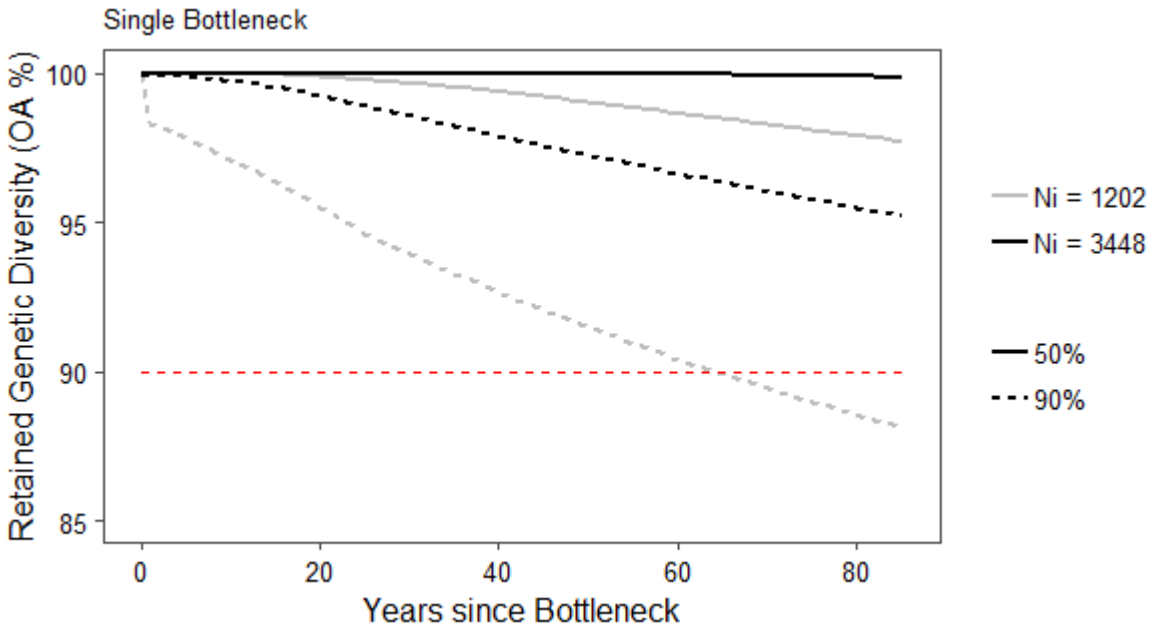

Figure 13. Percentage of retained genetic diversity (OA, observed number of alleles) after 85 years after culling 50% and 90% of the initial N_e in year 1 and maintaining the resulting N_e in subsequent years. Red dashed line indicates the recommendation of maintaining retained genetic diversity above 90% to avoid inbreeding depression.

DISCUSSION

No evidence of genetic partitioning was found in Australian white ibis sampled across most of their south-east Australian distribution. Analyses at the genic and genotypic scales indicated high levels of recent gene flow between the flourishing urban centres and degraded inland wetlands, where the Australian white ibis is in severe decline (Porter et al. 2017), (refer to Figure 14). Consequently, management practices implemented to control urban colonies have implications for the whole population across south-eastern Australia.

Genetic differentiation between sites and regions was consistently low at various spatial and temporal scales, suggesting widespread gene flow between inland and coastal colonies. There was no strong evidence that geographic distance, the GDR, urbanisation intensity or temporal surface water permanence affected gene flow between sites or regions. While low genetic diversity can mask the detection of fine-scale population structure, the lack of significant genetic structure found in the present study is unlikely to be affected by the levels of genetic diversity observed (global uH_E = 0.169). SNPs are known to yield lower estimates of genetic diversity, such as expected heterozygosity, than microsatellites (Jones et al. 2007, Guichoux

et al. 2011), but have still enabled the detection of population structure in previous studies where H_E ranged from 0.1 to 0.3 (Emanuelli et al. 2013, Pazmiño et al. 2017). Small sample sizes may also affect the estimation of genetic distances between populations (Vignal et al. 2002), however this is unlikely to influence the results of this study given the lack of structure found using analyses that did not depend on pre-defined populations. The results of this study thus provide strong support for the existence of an open, panmictic population of Australian white ibis across south-eastern Australia, although the true extent of panmixia across other parts of their range would require further study. Our findings based on genetic data are consistent with previous studies utilising banding and abundance data, which postulated regular dispersal between inland and semi-sedentary coastal colonies during the breeding season (Corben and Munro 2008, Thomas et al. 2014). In conjunction with previous research, the evidence for genetic panmixia found in the present study contributes to our growing understanding of the regional movements of this recent urban coloniser.

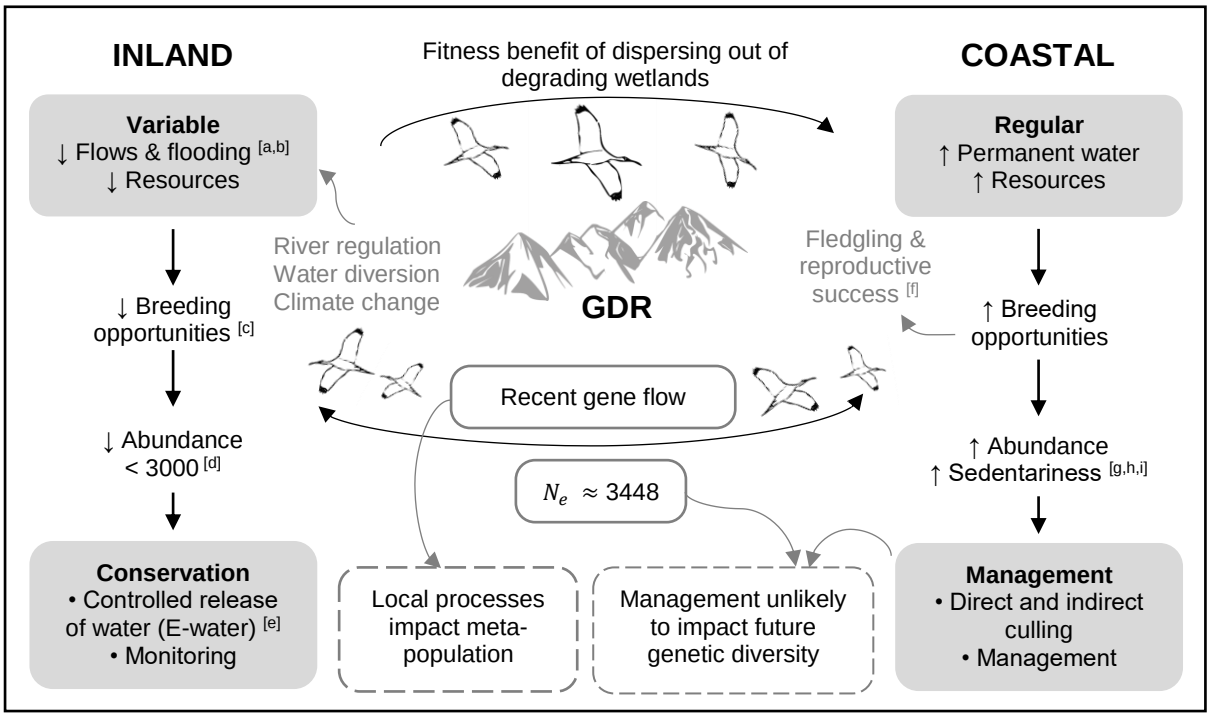

Figure 14. Differences in wetland degradation and resource availability between inland and coastal wetlands may have driven increases in urban Australian white ibis colonies, while inland colonies have continued to decline. This has resulted in a dichotomy between how the ibis is managed on either side of the Great Dividing Range. The results of this study suggest cohesion between these two approaches may be necessary.

References: [a] Lemly et al. (2000); [b] Vörösmarty et al. (2010); [c] Brandis et al. (2018); [d] Porter et al. (2017); [e] Kingsford and Auld (2005); [f] Smith et al. (2013a); [g] Smith and Munro (2010); [h] Martin et al. (2011); [i] Martin et al. (2012).

Dynamic food and water conditions, including fluctuations in rainfall and wetland productivity between the breeding and non-breeding seasons, may counteract the effects of urbanisation by sustaining gene flow between inland and coastal ibis colonies. Increasing sedentariness in urban regions, possibly driven by stable anthropogenic resources (Smith and Munro 2010, Martin et al. 2012), may have been expected to drive greater genetic differentiation between inland and coastal colonies if these two regions were not interbreeding. Yet, the results of this study are in stark contrast to many previous studies exploring the effect of urbanisation on the genetic connectivity of avian species with high dispersal propensities (Table 1). For example, genetic differentiation was higher between long-established urban and rural populations of the Eurasian coot (*Fulica atra*) (Minias et al. 2017). Dynamic wetland conditions may explain the lack of genetic partitioning found. In a study on the American white ibis (*Eudocimus albus*), low genetic differentiation (Wrights F_{ST} = 0.019) was found between colonies 600km apart, possibly driven by dynamic water conditions promoting long-distance dispersal (Stangel et al. 1991). For the Australian white ibis, previous research suggests that higher rainfall in urban areas during the non-breeding season may influence low rates of dispersal out of urban centres, due to declines in anthropogenic food sources during this period (Chard et al. 2017). Fluctuating conditions in both inland and coastal regions could be facilitating interbreeding between colonies and are likely to play a larger role overall in mediating gene flow for the ibis than urbanisation intensity, site fidelity or physical barriers.

The measures of genetic connectivity examined in this study provide insight into genetic processes occurring at different time scales. For dynamic landscapes, it can take time for environmental changes affecting population size, structure or connectivity to be reflected in genetic parameters (Bolliger et al. 2014). For example, low genetic variation in Australian grey nurse sharks (*Carcharias taurus*) was better explained by limited historical dispersal rather than contemporary human-induced bottlenecks (Stow et al. 2006). In cases where contemporary environmental changes are driving genetic changes, populations may be in a transient state before reaching equilibrium (Slatkin 1993). It is important to consider the population and genetic parameters which can influence this time lag and thus the temporal scale at which we can infer gene flow between inland and coastal colonies.

The ability to detect changes in dispersal using genetic data are influenced by the natural history of the organism, and the timing of the perturbation that altered connectivity. This is

because gene co-ancestries within individuals, and within and between sub-populations, accumulate at different rates (Wang 1997). Very recent isolation may not allow enough time for drift to result in the accumulation of allele frequency differences (e.g. between-patch measures of genetic diversity such as F_{ST}), because this typically takes many generations to occur (Keyghobadi et al. 2005, Epps and Keyghobadi 2015). Individual based approaches, such as those based on genotypic similarity (e.g. relatedness estimates), may be better suited to infer recent gene flow patterns because genotypes are typically reshuffled with each new generation or breeding event (Peery et al. 2008, Landguth et al. 2010, Lepais et al. 2010, Moore et al. 2014, Schunter et al. 2014). Additionally, population parameters can contribute to the time lag between altered connectivity and genetic responses. The presence of overlapping generations (Lloyd et al. 2013) and larger effective population sizes (Latter 1973) can result in longer time lags, while high dispersal rates (Nei et al. 1977) and increases in population connectivity (Landguth et al. 2010) can lead to shorter time lags. Contrasting genic and genotypic-based methods can provide insight into gene flow patterns at different time scales.

In the present study, we examined genetic distance at different time scales by assessing both genetic differentiation (F_{ST}) and pairwise relatedness (r_{xy}). The low and non-significant global F_{ST} calculated across the sampling distribution likely reflects historically high levels of gene flow. Relatedness between individuals was also not significantly different between regions, albeit slightly higher among coastal individuals, suggesting similarly high levels of contemporary gene flow within and between regions. Although relatedness was low overall, estimating relatedness without using a reference population to calculate allele frequencies often yields an average relatedness among individuals of zero (Wang 2014). However, as marker-based estimations of relatedness are more accurately interpreted as correlation coefficients between individuals rather than the true probability of IBD, the relative rather than absolute relatedness is more relevant, as it is in the present study (Wang 2014). Future studies utilising genetic data collected from known parent-offspring pairs along with larger sample sizes could more accurately elucidate contemporary gene flow patterns among inland and coastal breeding sites.

The effective population size determined for south-east Australian ibis also provides insight into genetic processes occurring at recent time scales and suggests that colonies are likely to maintain genetic variation over time. The estimate of N_e we obtained for the sampling

distribution ($\widehat{N}_{e(Adj4)}$ = 3448.3) likely reflects the mean effective size across several recent generations, given that the LD method is a genotypic analysis relying on a process that accumulates over several generations (Sunnucks 2000, Wang et al. 2016). This estimate of N_e, including its lower 95% confidence limit of 1202, is well above the recent recommendations by Frankham et al. (2014) that $N_e \geq 100$ is required to limit the total fitness loss to $\leq 10\%$ and avoid inbreeding depression, and $N_e \geq 1000$ is required to maintain evolutionary potential in the long term. Because of this, ibis across south-eastern Australia are unlikely to be susceptible to genetic erosion through drift and inbreeding.

The N_e estimates obtained in this study reflect census size estimates for the ibis. N_e for many taxa is often much lower than the census size, N, due to differences among individuals in their contribution of genetic material to the next generation (Frankham 1995, Palstra and Fraser 2012). Birds on average exhibit relatively high N_b/N ratios (0.86) and relatively low N_e/N ratios (0.65) (Waples et al. 2013), although this ratio is undetermined for the ibis. Furthermore, relating N_e to N has many caveats that require careful consideration, particularly as there is not often a simple linear relationship between the two (Palstra and Fraser 2012). However, the Eastern Australian Aerial Waterbird Survey counted 24,165 ibis across the eastern states in 2015 (including NSW, VIC and QLD) (Porter et al. 2015), suggesting the effective size obtained in the present study reflects similarly large census sizes. Furthermore, the high N_b/N_e ratio (1.236) based on life history traits for the ibis is consistent with ratios for other species with early maturity and long life-spans, with N_b per season being much larger than N_e per generation, as well as for other avian species, which exhibit a mean ratio of 1.35 (Waples et al. 2013). Future studies could obtain accurate adult census sizes (N_a) for the study region and examine the relative magnitude in N_e and N_a to identify ecological factors driving differences between these parameters and better inform management (Kalinowski and Waples 2002).

The presence of undefined upper bounds for the N_e estimates obtained in this study highlights an issue associated with determining effective sizes for large populations. Estimating N_e for large populations using small sample sizes is known to yield unreliable results and undefined upper limits (Waples and Do 2010). An 'infinite' upper bound suggests that all the genetic variation observed can be explained by sampling a finite number of individuals (i.e. sampling error) rather than parents (and thus genetic drift) (Laurie-Ahlberg and Weir 1979, Nei and Tajima 1981, Waples and Do 2010). Previous studies show that the precision of N_e decreases

with larger population sizes because the signal for estimating N_e is weaker, although the use of thousands of SNPs may increase the capacity of the indicator to detect a weak signal (Waples and Do 2010). The lack of upper boundaries found in this study are consistent with simulations for large population sizes, e.g. N = 5000 (Waples and Do 2010). More accurate estimates of effective population size for the ibis may be garnered with additional samples and more informative markers (Waples and Do 2010). However, simulations also indicate that under typical sampling conditions, the chances of underestimating the effective size of a large population is low, and lower bounds are generally informative even if upper bounds are undefined (Waples and Do 2010). For this reason, the effective size for the whole region is unlikely to be lower than 1000.

Given the effective size estimated for the ibis, colonies are unlikely to experience genetic erosion under current management targets. The ibis population target in a city varies between jurisdictional boundaries. Sydney's target for example is around 6,500 (Sydney Regional Ibis Management Plan) compared to 1500-2500 in the Gold Coast and Tweed Heads region (Jess Baglin, Ecosure, personal communication). Forward simulations did not support the *a priori* prediction that current urban management regimes would have a net negative impact on regional genetic diversity if ibis were widely dispersing. Under the 'no management' scenario, sustaining the south-east population at an N_e of 1202-3448 is sufficient to maintain more than 99% of the current genetic variation over the next 85 years (~28 generations). This is well above the recommended level of 90% to avoid inbreeding depression in the short term (Frankham et al. 2014). Under the realistic management scenario of culling 2% each year, more than 95% of initial genetic diversity would be retained. Additionally, genetic diversity was maintained above 90% even when sustaining the effective size for the whole population at Sydney's regional target of 650, assuming an N_e/N ratio of 0.1 (Frankham, 1995, Frankham et al., 2014). These results suggest that current management targets are high enough to provide a balance between controlling the negative impacts of this species in urban regions while ensuring genetic diversity of the whole population is sufficient to maintain evolutionary potential in the long term.

The evidence for widespread gene flow found in the present study may provide insight into this species' capacity to recolonise inland wetlands. Large scale gene flow between waterbird colonies is known to increase the chances of recolonising previously extirpated wetlands without human assistance (Friesen 1997). For example, recolonization of habitats by the

double-crested cormorant (*Phalacrocorax auritus*) is likely facilitated by high dispersal rates and large-scale gene flow (Vermeer and Rankin 1984, Friesen 1997). Similarly, ibis colonies may quickly recommence breeding in large colonies among inland wetlands if flow regimes and wetland quality are restored. Over time, fewer individuals may disperse to seek the resources of urban wetlands, although there is speculation that a portion of the population would still continue to breed semi-permanently in urban regions due to site fidelity (Smith and Munro 2011). This study provides a baseline with which to assess the impact of wetland restoration on the genetic connectivity of ibis colonies across the south-east of Australia. Future studies could also incorporate genetic data from other waterbird species to better understand gene flow across the Australian wetland network. Complex environmental changes, along with variation in individual species biology, can produce diverse and distinctive responses even between closely related species (Eldridge et al. 2014). Comparisons between species would shed light on potentially different genetic responses among waterbirds to landscape level processes such as wetland restoration or urbanisation.

Finally, management of ibis at the local scale may not be appropriate given the lack of genetic partitioning observed. There is currently a dichotomy between managing urban and conserving inland colonies of the ibis (see Figure 14). Colony-level management of waterbirds exhibiting high levels of gene flow across large spatial scales is typically not recommended (Friesen 1997). Appropriate management practices can be clarified by defining management units around genetic boundaries (DeYoung and Honeycutt 2005) best accomplished by assessing gene flow between locations rather than genetically defining populations (Stow and Magnusson 2012). The evidence of genetic panmixia found across south-east Australian sites suggests that urban management should not be conducted only at a site-level scale within urban regions or in isolation of inland conservation efforts. Although the current urban management regime is unlikely to affect future genetic diversity, it is important to consider its impacts in conjunction with reduced breeding opportunities among inland colonies and other processes affecting this species, such as widespread wetland degradation (Brandis et al. 2018). A broad scale management plan, although logistically challenging as it requires collaboration across many jurisdictional boundaries, would enable the most effective monitoring of regional or local processes which may impact the whole ibis population. For example, disease transmission could impact survival rates of ibis across the entire south-east Australian range (Hess 1996). Future studies could explore other potential

implications of widespread gene flow to advise the management of colonies across the eastern states.

MANAGEMENT IMPLICATIONS

The ibis is widely considered a pest by many and contributes to numerous public health and air safety issues, necessitating wide-scale management in urban areas. However, the lack of genetic partitioning found between urban colonies and their declining inland counterparts, raises important implications that need to be considered in urban management plans. Given their connectivity, the management of urban colonies should not be conducted in isolation of impacts occurring in other parts of its range, such as water resource development and wetland degradation. This study provides a baseline for monitoring changes in genetic structure and diversity of connected Australian white ibis colonies across south-eastern Australia over time in the face of wetland degradation and increasing urbanisation.

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APPENDIX A. DEFINITIONS OF NOTATIONS USED

- N_e = True effective population size per generation
- N_b = True effective number of breeders in one reproductive cycle
- r^2 = Index of linkage disequilibrium (LD)
- P_{crit} = Criterion for excluding rare alleles
- $\widehat{N}_e$ = Estimate of N_e based on genetic data
- $\widehat{N}_b$ = Estimate of N_b based on genetic data
- $\widehat{N}_{b(Adj2)}$ = Estimate of N_b adjusted to account for overlapping generations/age structure
- $\widehat{N}_{e(Adj2)}$ = Estimate of N_e from N_b
- $\widehat{N}_{e(Adj3)}$ = Estimate of N_e adjusted to account for physical linkage based on karyotype
- $\widehat{N}_{e(Adj4)}$ = Estimate of N_e adjusted to account for physical linkage based on genome size
- α = Age at maturity
- ω = Maximum age
- AL = Adult life span $(\omega - \alpha + 1)$
- Chr = Haploid chromosome number for the species
- cM = Genome size for the species in centimorgans

APPENDIX B. TABLE REFERENCES

Table B1. References and common names for species listed in Table 1.

Reference		Common Name	Scientific Name	Country
1	Minias et al. (2017)	Eurasian coot	*Fulica atra*	Poland
2	Baratti et al. (2009)	Mallard duck	*Anas platyrhynchos L.*	Italy
3	Baratti et al. (2015)			
4	Rutkowski et al. (2010)	Common kestrel	*Falco tinnuculus*	Poland
5	Rasner et al. (2004)	Dark-eyed Junco	*Junco hyemalis thurben*	U.S.A.
6	Evans et al. (2009)	European blackbird	*Turdus merula*	Western Palaearctic
7	Evans et al. (2012)			
8	Björklund et al. (2009)	Great tit	*Parus major*	Spain
9	Perrier et al. (2017)			France
10	MacDougall-Shackleton et al. (2011)	Song sparrow	*Melospiza melodia*	Canada

APPENDIX C. FILTERING PIPELINE

Table C1. SNP filtering pipeline showing the resulting number of SNPs and total sample size retained at each filtering step.

Filter	No. SNPs	N
Raw DArTseq data	73,876	76
Reproducibility average ≥ 1	64,898	76
SNP Call Rate > 0.95	8,745	76
Read depth (>10, <86.4)	7,174	76
Removal of secondaries	6,509	76
Minor Allele Frequency (MAF) > 0.02	4,737	76
Sample Call Rate > 0.85	4,737	74
Removal of non AWI samples (and resulting monomorphic SNPs)	1,876	71
Removal of highly related samples (IBD > 0.1875) (and resulting monomorphic SNPs)	1,860	68
Removal of sites for which n < 5 (and resulting monomorphic SNPs)	1,809	64

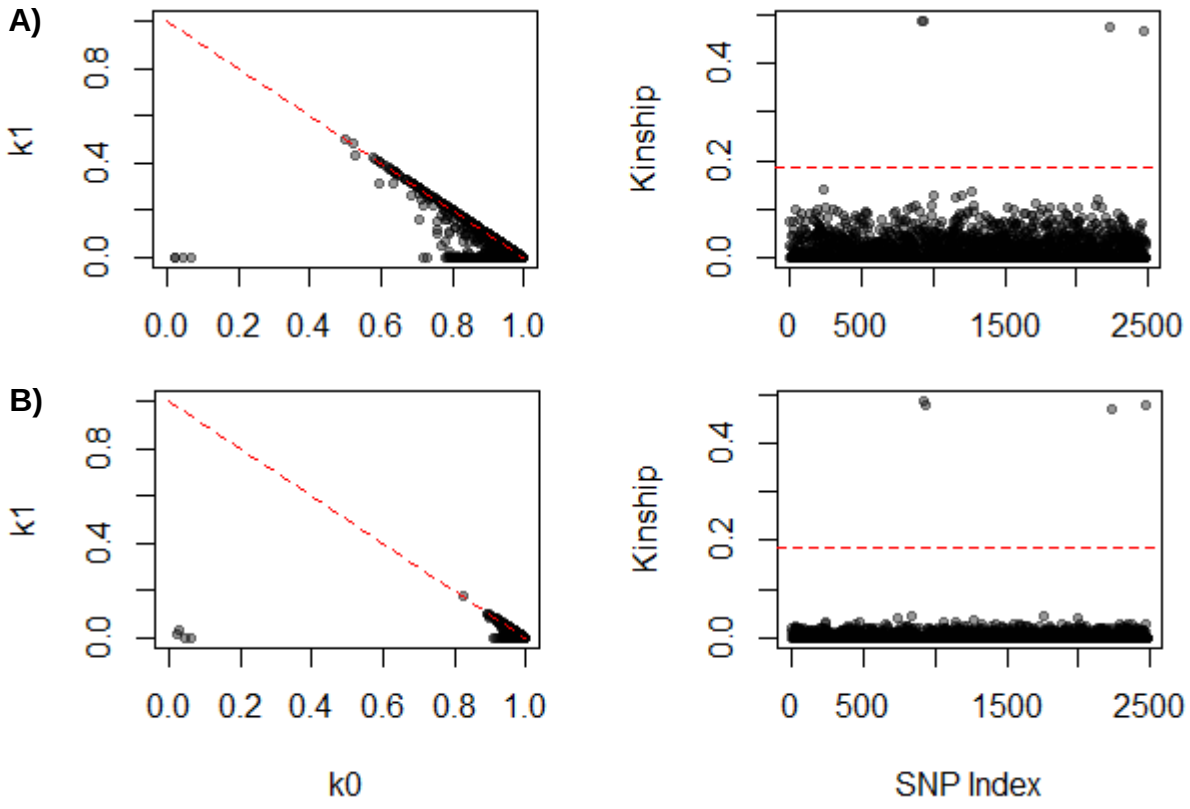

Figure C2. Plots depicting relatedness between pairs of individuals prior to their removal, based on A) an LD-pruned subset of SNPs and B) the full dataset (1867 SNPs) (red dashed line indicates the threshold of 0.1875 for the two figures on the right).

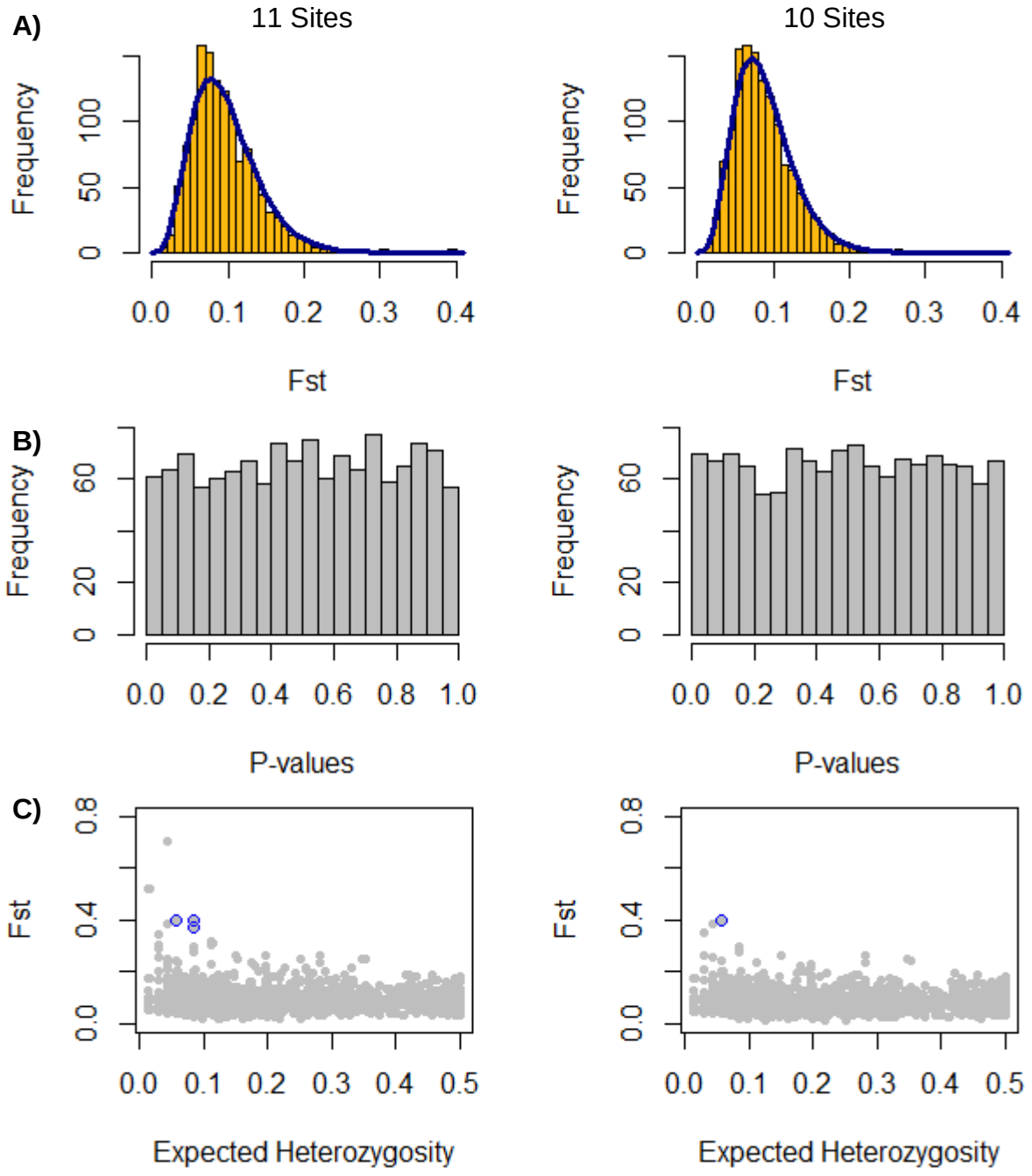

Figure C3. Results of the F_{ST}-based method of identifying outlier loci as implemented in *OutFLANK* compared between using the 11 sites as population groupings (left) and grouping LAC with its nearest neighbouring site LOW due to low sample size (right). Plots depict A) the distribution of F_{ST} values plotted against the Chi-square distribution, B) the frequency distribution of p-values and C) SNPs plotted by H_e and F_{ST}, with loci identified as outliers circled in blue (loci to the left of these points fall below the H_E threshold of 0.05 and were excluded from the algorithm).

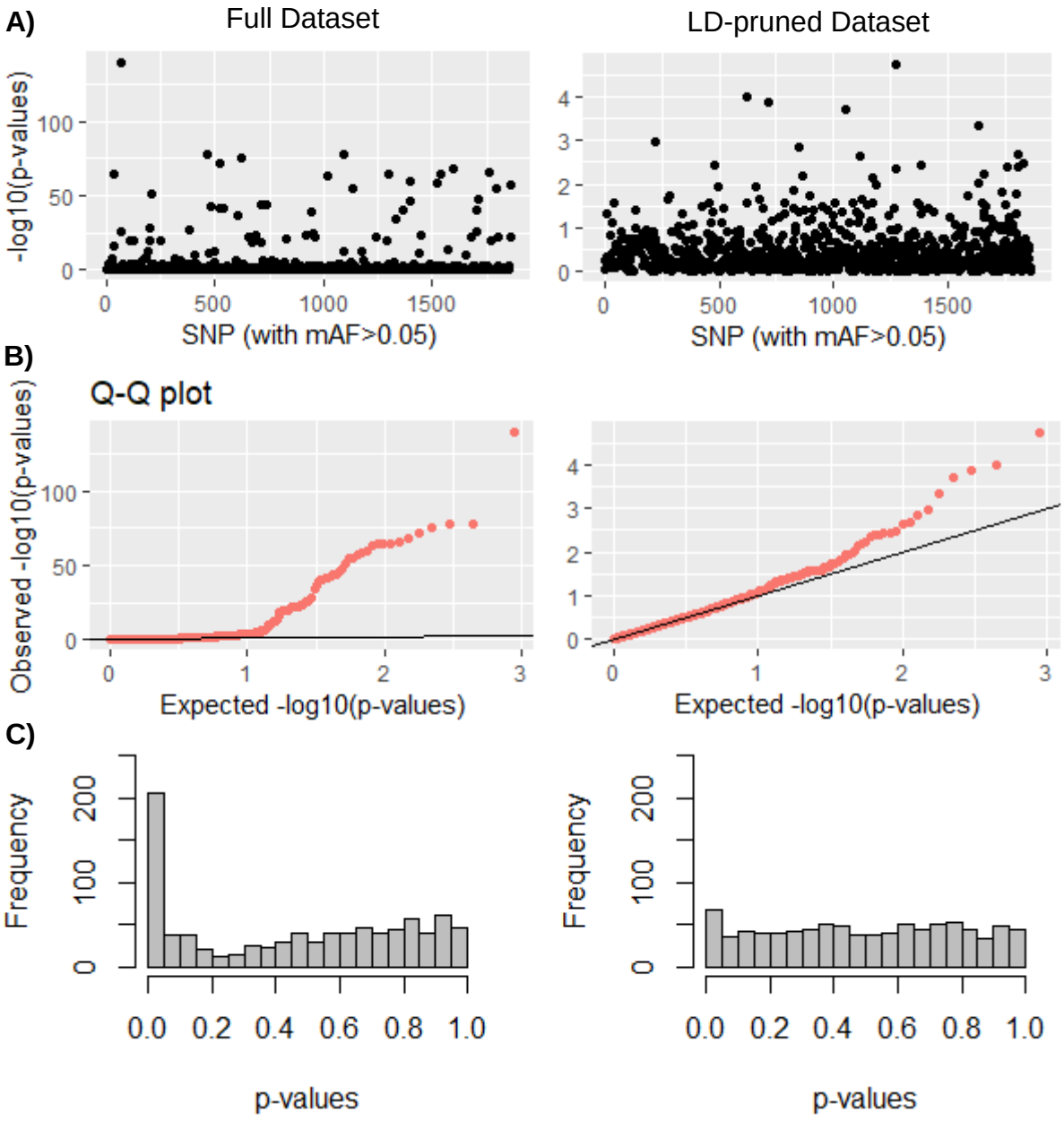

Figure C4. Results of the *PCAdapt* method of identifying outlier loci between the full dataset (left) and LD-pruned dataset (right), depicting A) Manhattan plots of -log10(p-values) across loci, B) Q-Q plots showing the expected uniform distribution of p-values (black line) and C) the frequency distribution of p-values.

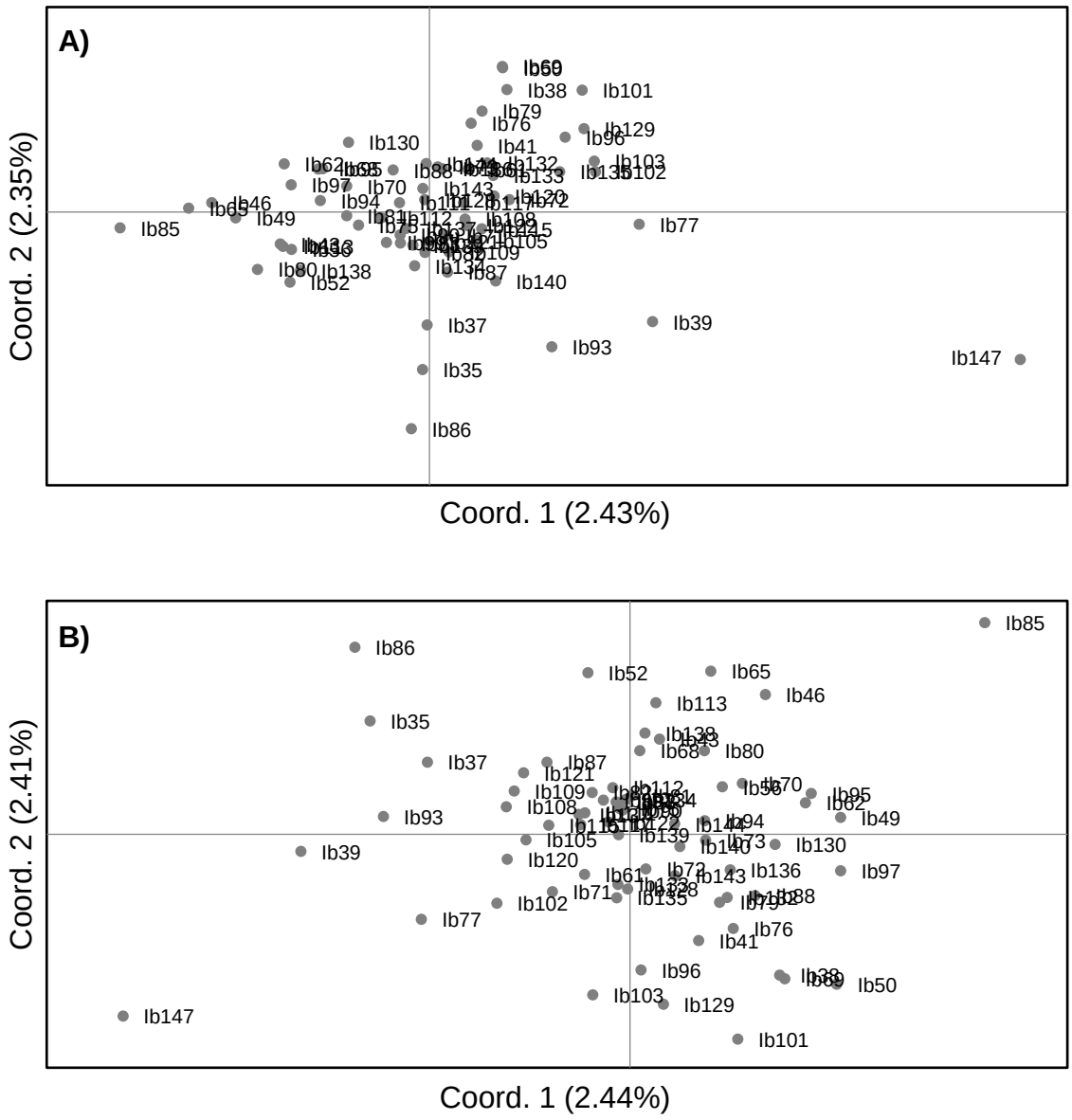

Figure C5. Principle coordinates analysis for A) the full dataset of 1860 SNPs (cumulative variance explained by coordinate 1 = 69.43 %) and B) the trimmed dataset removing all potential outliers (1705 SNPs) (cumulative variance explained = 61.34%), none of which produced a consensus set of outliers.

APPENDIX D. SUMMARY STATISTICS AND ADDITIONAL ANALYSES

Table D1. Estimates of genetic diversity within and among sites (N_p = number of private alleles, A_r = allelic richness adjusted for sample size, H_O = observed heterozygosity, H_E = expected heterozygosity, uH_E = unbiased expected heterozygosity, F_{IS} = inbreeding coefficient and F = fixation index ($1 - H_O/H_E$) (± S.E. in brackets).

	N_p	A_r	H_O	H_E	uH_E	F_{IS}	F
Global	*NA*	1.294	0.149 (0.001)	0.156 (0.001)	0.169 (0.001)	0.124	0.014 (0.003)
Inland/Natural							
BAR	0.024 (0.004)	1.311	0.159 (0.004)	0.162 (0.004)	0.173 (0.004)	0.088	0.001 (0.008)
GWY	0.030 (0.004)	1.300	0.156 (0.004)	0.157 (0.004)	0.172 (0.004)	0.097	-0.018 (0.008)
LOW	0.023 (0.004)	1.308	0.152 (0.004)	0.159 (0.004)	0.169 (0.004)	0.104	0.022 (0.008)
MAC	0.011 (0.002)	1.263	0.136 (0.004)	0.145 (0.004)	0.162 (0.005)	0.176	0.018 (0.009)
Coastal/Urban							
BOO	0.010 (0.002)	1.280	0.146 (0.005)	0.153 (0.004)	0.171 (0.005)	0.164	0.011 (0.010)
CEN	0.021 (0.003)	1.290	0.139 (0.004)	0.154 (0.004)	0.164 (0.004)	0.162	0.057 (0.008)
GOU	0.028 (0.004)	1.316	0.153 (0.004)	0.162 (0.004)	0.172 (0.004)	0.113	0.030 (0.008)
LUH	0.021 (0.003)	1.299	0.150 (0.004)	0.159 (0.004)	0.170 (0.004)	0.124	0.026 (0.008)
RGC	0.013 (0.003)	1.282	0.151 (0.004)	0.150 (0.004)	0.164 (0.004)	0.088	-0.024 (0.008)

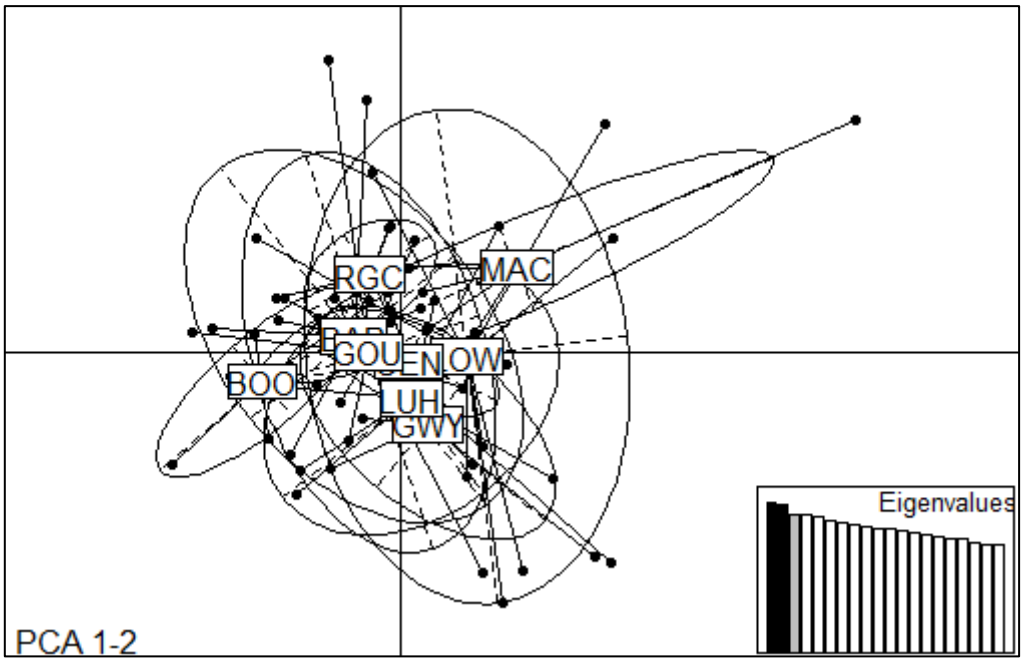

Figure D1. PCA describing genetic variation across samples with 95% confidence intervals around sampling locations. Bottom panel shows eigenvalues explaining decreasing proportions of the total genetic variation (1st and 2nd principle components shaded black, 3rd principle component shaded grey).

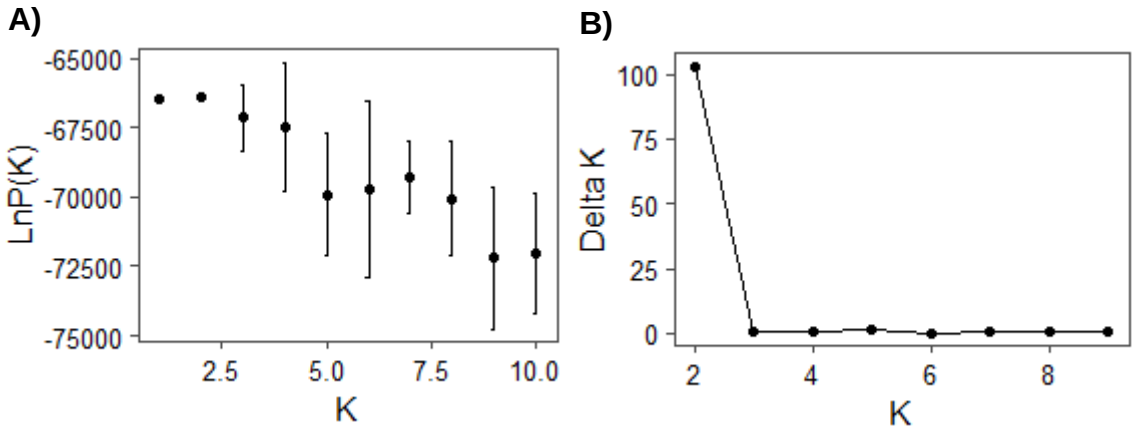

Figure D2. Plots of A) delta K, the rate of change in the log probability of data between successive values of K and B) LnP(K), the mean likelihood of the natural log probability of data (± S.D.); for ten iterations of each value of K tested (from K = 1 to K = 10).

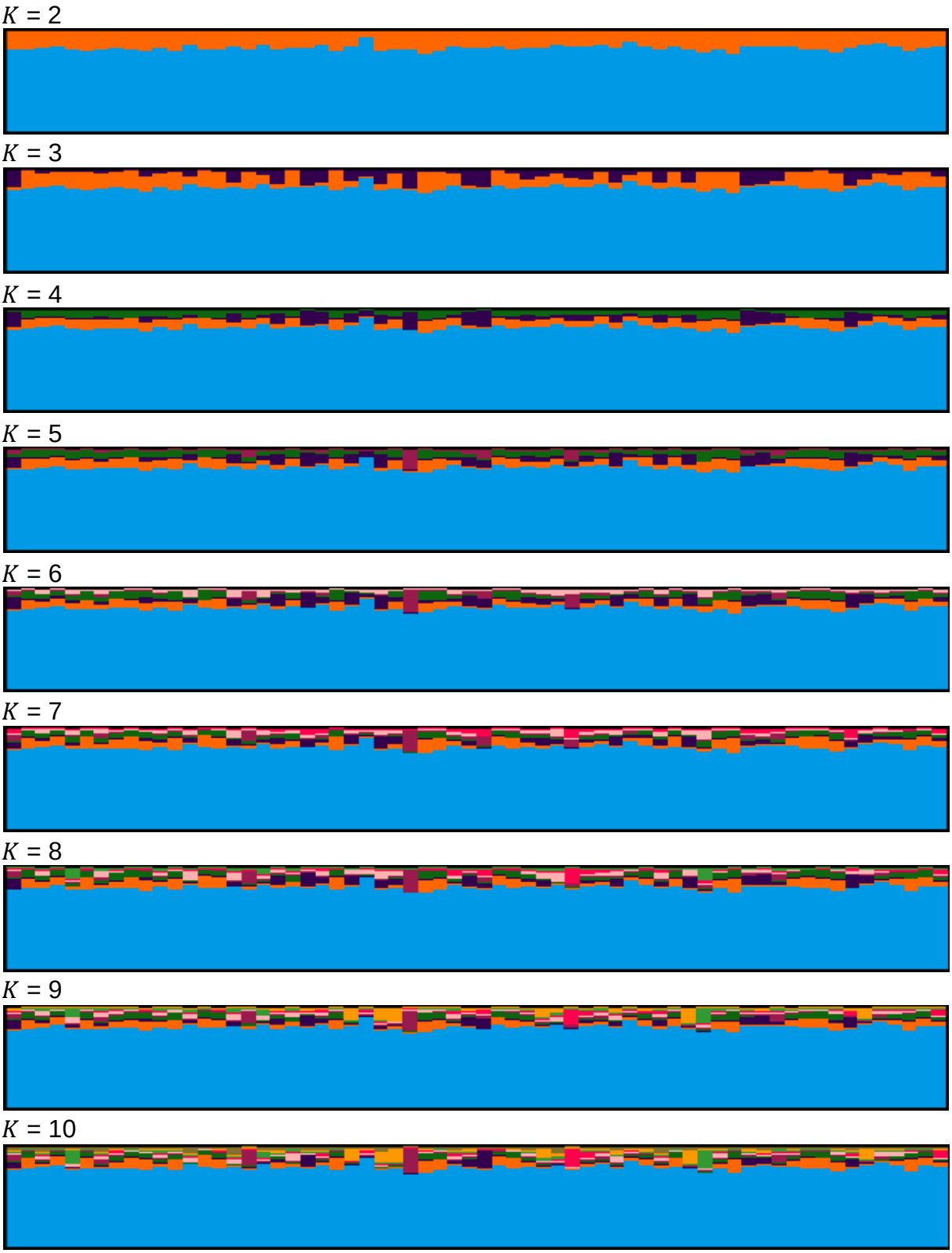

Figure D3. Aligned STRUCTURE results for K = 1 to K = 10.

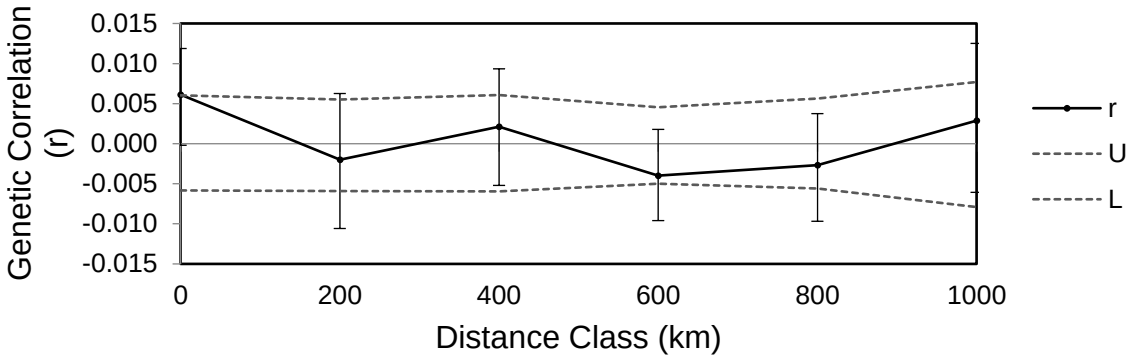

Figure D4. Spatial correlogram for the inland region (32 individuals and 1651 SNPs). (sample size per distance class bin = 92, 81, 71, 104, 100, 48).

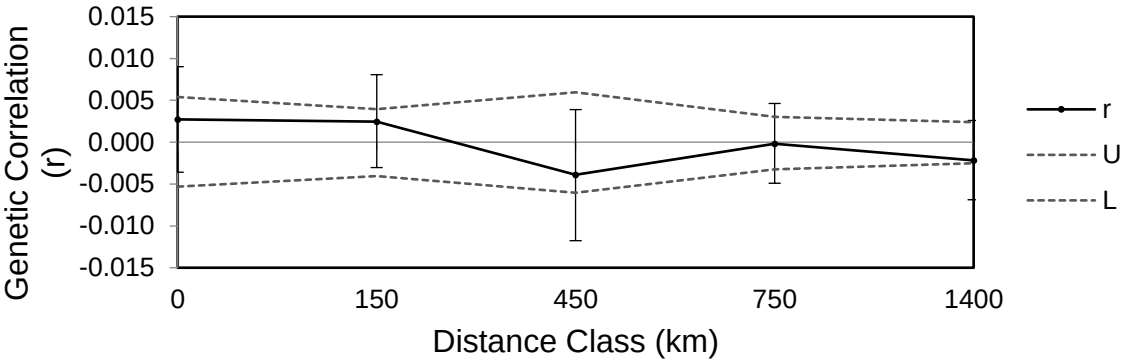

Figure D5. Spatial correlogram for the coastal region, based on 36 individuals and 1619 SNPs. (sample size per distance class bin = 117, 136, 72, 150, 155).

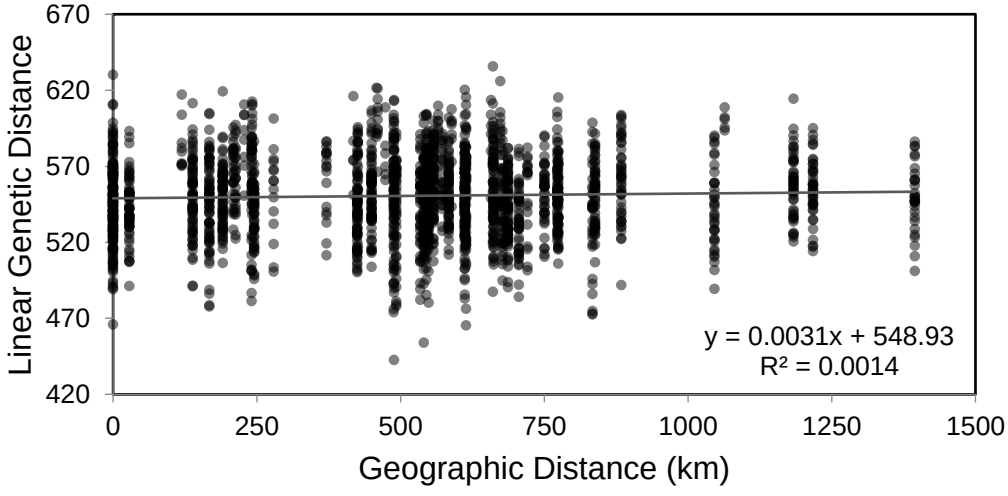

Figure D6. Mantel test for pooled samples from all sites across the south-east Australian sampling region (based on 9999 permutations).

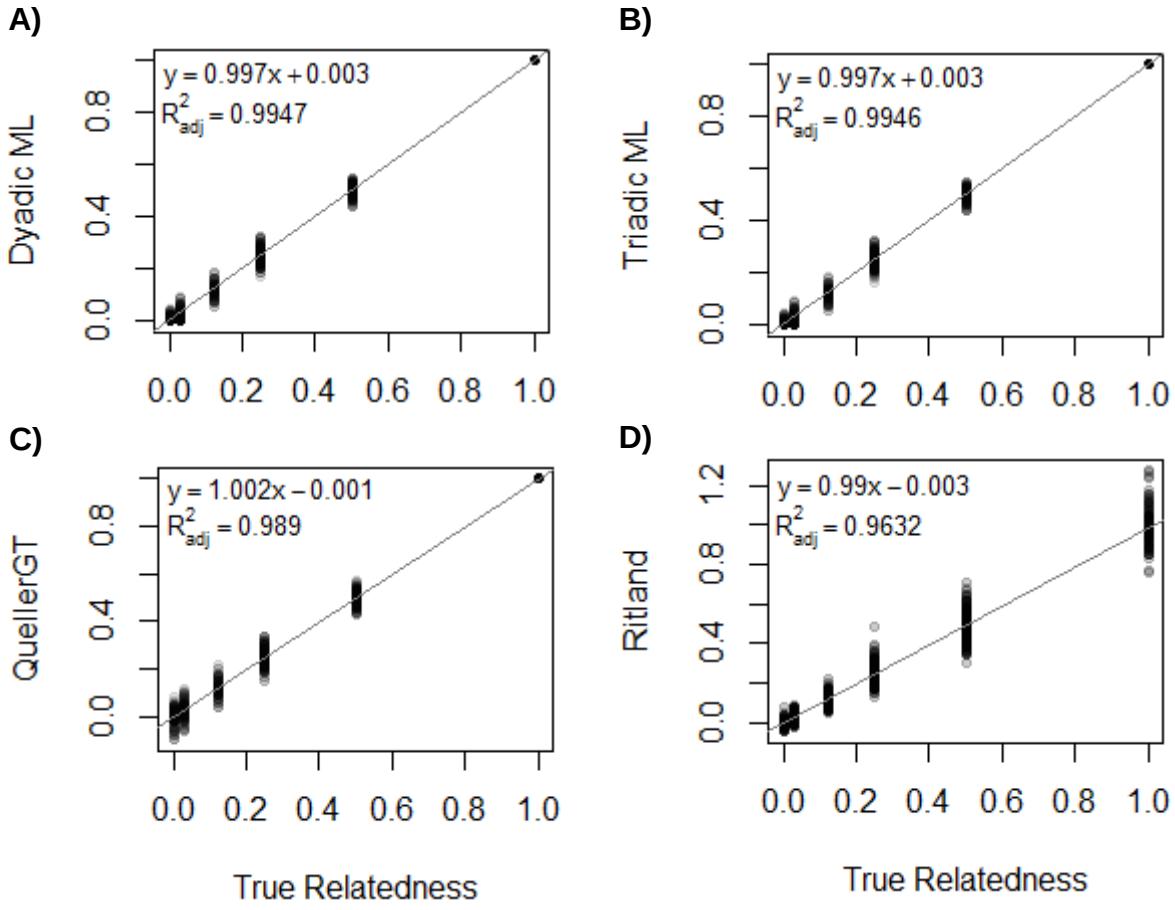

Figure D7: Correlation between true relatedness and estimates obtained for simulated dyads using 2 likelihood estimators (A = Milligan (2003); B = Wang (2007)) and 2 moment estimators (C = Queller and Goodnight (1989); D = Ritland (1996)) in COANCESTRY.

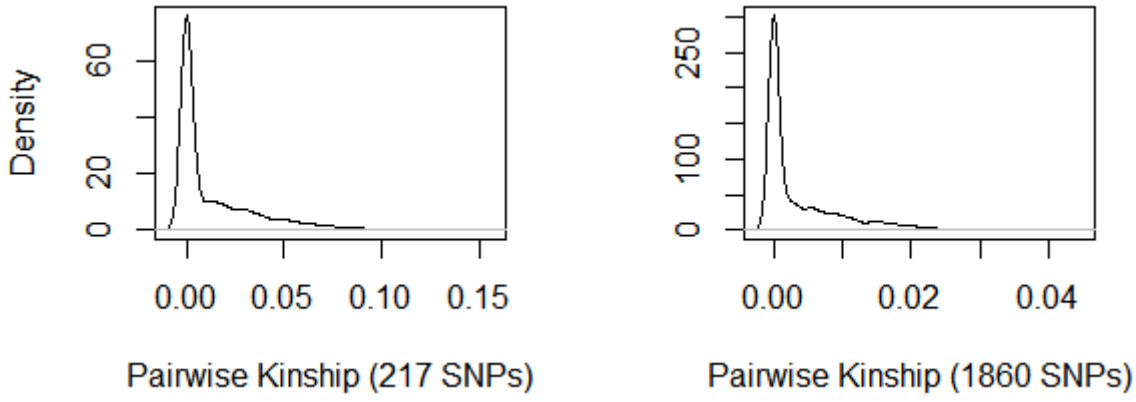

Figure D8. Distribution of kinship scores between all pairs of individuals for 217 LD-pruned SNPs vs 1860 SNPs, calculated using the maximum likelihood estimation of relatedness (MLE) (Milligan 2003).

Table D2: Effective population size bias-adjustment pipeline for the whole sampling region (n = 66), inland (n = 30) and coastal regions (n = 36), based on a P_{crit} of 0.02 and 0.05.

	SE Australia (n = 66)		**Inland (n = 30)**		**Coastal (n = 36)**	
	0.05	*0.02*	*0.05*	*0.02*	*0.05*	*0.02*
$\boldsymbol{Raw\widehat{N}_b}$	3191.4	7592.3	2401.3	4105	2313.3	2913.8
JK	1113.2, ∞	1443.6, ∞	470.6; ∞	563.4; ∞	457.7; ∞	490.9; ∞
P	1978.4, 8162.8	3760.5, ∞	1175.9, ∞	1631.4, ∞	1249.6, 14918.5	1610.6, 14918.5
$\boldsymbol{\widehat{N}_{b(Adj2)}}$	3710.3	8826.7	2791.7	4772.4	2689.4	3387.6
JK	1294.2, ∞	1678.3, ∞	547.1, ∞	655.0, ∞	532.1, ∞	570.7, ∞
P	2300.1, 9490.0	4371.9, ∞	1367.1, ∞	1896.6, ∞	1452.8, 17344.08	1872.5, 17344.1
$\boldsymbol{\widehat{N}_{e(Adj2)}}$	3001.0	7139.3	2258.0	3860.1	2175.3	2740.0
JK	1046.8, ∞	1357.5, ∞	442.5, ∞	529.8, ∞	430.4, ∞	461.6, ∞
P	1860.4, 7675.8	3536.1, ∞	1105.8, ∞	1534.0, ∞	1175.1, 14028.5	1514.5, 14028.5
$\boldsymbol{\widehat{N}_{e(Adj3)}}$ **(*Chr*)**	3448.3	8203.5	2594.6	4435.5	2499.6	3148.4
JK	1202.8, ∞	1559.9, ∞	508.5, ∞	608.8, ∞	494.6, ∞	530.4, ∞
P	2137.7, 8820.0	4063.2, ∞	1270.6, ∞	1762.7, ∞	1350.3, 16119.7	1740.3, 16119.7
$\boldsymbol{\widehat{N}_{e(Adj4)}}$ **(*cM*)**	3675.6	8744.2	2765.6	4727.9	2664.3	3356.0
JK	1282.1, ∞	1662.7, ∞	542.0, ∞	648.9, ∞	527.2, ∞	565.4, ∞
P	2278.6, 9401.3	4331.0, ∞	1354.4, ∞	1878.8, ∞	1439.3, 17182.1	1855.0, 17182.1

APPENDIX E. ETHICS APPROVAL

MACQUARIE University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2017/051-3 **Date of Expiry:** 30 September 2018

Full Approval Duration: *01 December 2017 to 30 September 2018*

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:
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0452 180 342

Associate Investigators:
Kaytlyn Skye Davis 0430 409 948

In case of emergency, please contact:
the Principal Investigator / Others named above
or **Animal Welfare Officer:** 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **Finding water in the face of uncertainty: Australian white ibis connectivity in a changing landscape**

Purpose: **4 - Research: Human or Animal Biology**

Aims:
1. **Produce a large genetic dataset from DNA extracted from feathers and sequenced using a next generation sequencing technique for 90 individuals from both inland and urban white ibis populations**
2. **Estimate genetic relatedness between populations to estimate levels of gene flow and connectivity between inland and urban populations of white ibis in south-eastern Australia and examine how movement and interbreeding between populations may be affected by the temporal stability of wetlands**
3. **Estimate genetic diversity, population size and effective number of breeders to assess population health and long-term survival.**

Surgical Procedures category: 1 - Observation Involving Minor Interference

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Weight/Sex	Total	Supplier/Source
20 - Native Wild Birds	Australian white ibis - *Threskiornis moluccus*	Adults/Any/Any	250	Feathers collected from breeding grounds (nests, ground or plucked from deceased birds)
			250	

Location of research:

Location	***Address***
Narran Lake Nature Reserve	Brewarrina, NSW
Macquarie Marshes Nature Reserve	Warren, NSW
Gwydir Wetlands State Conservation Area	Moree, NSW
Centennial Park	Moore Park, NSW
Lake Gillawarna	Georges Hall, NSW
Barmah National Park	Barmah, VIC
Braeside Park	Braeside, VIC
Jells Park	Wheelers Hill, VIC
Shepherds Bush	Wantirna South, VIC
Troups Creek and River Gum Creek Reserve	Hampton Park, VIC
Jawbone Flora and Fauna Reserve	Williamstown, VIC
Heatherton Road	Melbourne, VIC
Black Swamp Wetlands	Cleveland, QLD
Brisbane City Botanic Gardens	Brisbane, QLD
Vintage Lakes	Tweed Heads, NSW
Centennary Lakes Park (& adjacent wetlands)	Caboolture, QLD

Amendments approved by the AEC since initial approval:

1. Amendment #1 - *Amend Number of Animals to allow the collection of a maximum of 250 Australian White Ibis feather samples and additional locations of research (Executive approved, Ratified by AEC 15 February 2018).*

AEC Reference No.: 2017/051-2 **Date of Expiry:** 30 September 2018

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

A/Prof. Nathan Hart (Chair, Animal Ethics Committee) **Approval Date:** 15 February 2018