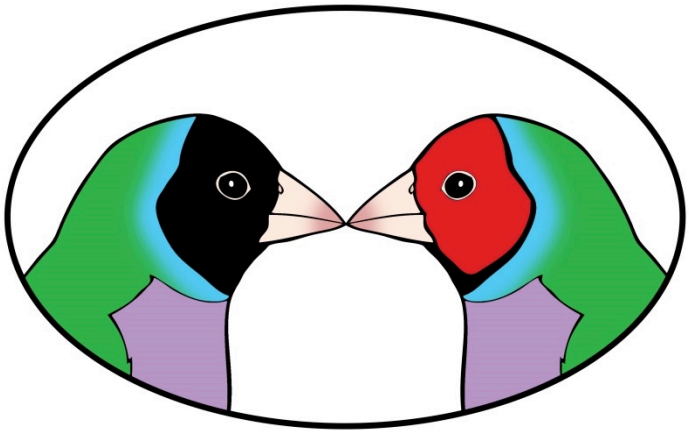
Colourful conservation: genetic incompatibility and conservation genetics in the wild Gouldian finch (*Erythrura gouldiae*)



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Declaration

I do declare that this thesis constitutes my original work, and has not been submitted or accepted for the award of a higher degree at another institution. No persons have contributed to this work that have not been given credit, and all the work of previous scholars influencing this work are cited in the appropriate manner.

The protocols to collect blood samples from Gouldian finches used in this thesis were approved by ethics committees at Macquarie University (AEC2007/037, AEC 2007/038, AEC2010/053), Australian Wildlife Conservancy (CAEC/6/2005,AEC 2007/43, AEC 2010/35), and the University of Wollongong (AE06/25).

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Peri Bolton

17th February 2017

“It’s easy to think that as a result of the extinction of the dodo, we are now sadder and wiser, but there’s a lot of evidence to suggest that we are merely sadder and better informed.”

— Douglas Adams, Last Chance to See

Dedication

I dedicate this thesis to my mother Judy Bolton, my father Matthew Bolton, and my dear friend Michaela Flanigan. Without your intellectual influence I would not have embarked on this foolhardy PhD journey.

Thanks.

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General Abstract

The distinct behavioural and physiological strategies associated with colour morphs can influence population dynamics, where antagonistic interactions and hybrid incompatibilities may be detrimental. Therefore, the covariation between colour and other traits will be relevant to species conservation, but the negative implications have never been explored. Molecular genetic tools allow the measurement of selection on functional genotypes, and place it in the wider context of effective population size (N_e) and gene flow. The Gouldian finch (*Erythrura gouldiae*) is an Australian threatened species, and sympatric head colour morphs associated with distinct strategies. Experiments on domesticated Gouldian finches have shown that interbreeding between morphs leads to significant offspring mortality, which may be detrimental to population recovery. This species has been embroiled in recent controversy regarding its conservation status as there are no robust estimates of population size or movement capacity across its vast and remote range. In this thesis, I use population genetic techniques using microsatellites, mitochondrial DNA and SNPs to quantify N_e and gene flow across the contemporary range, and a novel allele-specific test for head-colour to determine the extent of incompatibility in the wild. Guided by predictions derived from the experiments on domesticated birds, I do not find any evidence of incompatibility, or strategies to avoid it. Furthermore, population genetic evidence suggests the contemporary range supports a single genetically cohesive population, and suggests individuals are able to move vast distances (>700km). The contemporary N_e is likely large, but there is still uncertainty in these estimates due to the dearth of fundamental knowledge of this species' life-history. Together, this work suggests that incompatibility is unlikely to be a threatening process in the wild, and has identified key knowledge gaps that currently hinder effective conservation management of the Gouldian finch.

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Firstly, I would like to acknowledge the first nations peoples of Australia, on whose lands these works were conducted

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List of Original Publications

Paper I, Chapter Two: **Bolton, P.E**., Rollins, L.A., Griffith, S.C. 2015. The danger within: the role of genetic, behavioural, and ecological factors in population persistence of colour polymorphic species. Molecular Ecology. 24: 2907-15.
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Paper II, Chapter Two: **Bolton, P.E**., Rollins, L.A., Griffith.S.C. 2016. Colour polymorphism is likely to be disadvantageous to some populations and species due to genetic architecture and morph interactions. Molecular Ecology, 25: 2713-2718
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Paper III, Chapter Three: **Bolton, P.E**., Rollins, L.A., Brazill-Boast, J., Kim, K-W, Burke, T., Griffith, S.C. 2017. The colour of paternity: extra-pair paternity in the wild Gouldian finch does not appear to be driven by genetic incompatibility between morphs. Journal of Evolutionary Biology. 30:174-190
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List of Conference Presentations

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Bolton, P.E., Cardilini, A.P., Brazill-Boast, J., Legge, S., Maute, K.L., Kim, K-W., Rollins, L.A., Griffith, S.C. 2015 A colourful genomic landscape: patterns of gene flow in an Australian colour polymorphic finch. European Society for Evolutionary Biology (ESEB), Lausanne, Switzerland (poster)

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Bolton, P.E., Brazill-Boast, J., Rollins, L.A., Griffith, S.C. 2014**.** The colour of paternity: patterns of parentage in a polymorphic finch. Australasian Society for the Study of Animal Behaviour (ASSAB), Katoomba, Australia (talk)

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List of Contributors

What co-authors contributed to each section of the thesis, and my own percent contribution in brackets. Where PEB= Peri E Bolton, SCG=Simon C Griffith, LAR=Lee Ann Rollins, JBB=James Brazill-Boast, KWK=Kang-Wook Kim, KLM=Kimberley L Maute, SL=Sarah Legge, TB=Terry Burke, JAC=Jennalee A Clarke, AJW=Andrea J West, APAC=Adam PA Cardilini, JJA=Jeremy J Austin.

	Introduction	**Chapter Two**	**Chapter Three**	**Chapter Four**	**Chapter Five**	**Chapter Six**	**Discussion**
Conception and Planning	PEB (100%)	PEB(70%), SCG, LAR	PEB (80%), SCG, LAR	PEB (90%), KWK, SCG	PEB (90%), LAR, SCG	PEB (80%), LAR, SCG JJA	PEB (100%)
Field Data Collection	-		JBB, SCG	JBB, SCG, KLM, SL	JBB, SCG, KLM, SL	JBB, SCG, KLM, SL	-
Laboratory Data Collection	-		PEB (70%), SCG, LAR, KWK	PEB (80%), KWK	PEB (90%),AJW, JAC, LAR	PEB (80%), JJA	-
Data Analysis	-		PEB (90%), JBB, SCG, LAR	PEB (100%)	PEB (90%), LAR, APAC	PEB (90%), LAR, JJA	-
Interpretation and Writing	PEB (97%), SCG	PEB(70%), SCG, LAR	PEB (80%), SCG, LAR	PEB (90%), SCG	PEB (80%), LAR, SCG	PEB (90%), SCG, LAR, JJA	PEB (97%), SCG
Review and Editing	PEB, SCG	PEB,SCG, LAR	PEB, SCG, LAR, JBB, KWK, TB	PEB, SCG	PEB, LAR, SCG, JBB, KL, SL, APAC, JAC, AJW	PEB, SCG, LAR, JJA	PEB, SCG

Chapter One

Conservation knight marches for the painted damsel

Photo: Mike Fidler

Across the globe, human activity is associated with species extinction (Prescott *et al.* 2012). Concomitant with human population growth are the increasing demands on land area and function, and direct exploitation and persecution of wild animals, which may constitute Earth's sixth mass extinction (Vié *et al.* 2009; Ceballos *et al.* 2015; Newbold *et al.* 2015). These factors can directly or indirectly affect animal behaviour and individual movement (Kitchen *et al.* 2000; Lowry *et al.* 2013), population connectivity, and ultimately reduce population size (Lande 1998; Allendorf *et al.* 2008; Frankham *et al.* 2010). One aim of conservation biology is to use an understanding of ecology and biology to prevent the extinction of individual species from the landscape (Soulé 1985). The International Union for Conservation of Nature (IUCN) Red List is currently the global standard for categorizing extinction risk (Mace *et al.* 2008). Red List version 3.1 extinction risk categories (Critically Endangered to Vulnerable) are based on quantitative criteria that reflect the probability of a species extinction (Mace & Lande 1991; Mace *et al.* 2008; Collen *et al.* 2016). The criteria are designed to be applicable across species and are based on theoretical and empirical relationships of population size, decline and geographical extent with extinction probability (Mace & Lande 1991; Mace *et al.* 2008; Collen *et al.* 2016). These criteria form the basis for assessment of conservation status in Australia's conservation legislation the Environmental Protection and Biodiversity Act 1999.

Australia is a hub of endemic flora and fauna, but European colonization in 1788 has seen massive changes to the Australian biota. In just over 200 years, Australia has lost ~40% of its forests (Bradshaw 2012), and the extinction of 54 animal (EPBC Act List of Threatened Fauna), and 36 plant species (EPBC Act List of Threatened Flora). Although the fraction of extinct Australian endemic birds is much lower than in mammals (Woinarski *et al.* 2015), the many Australian bird species are declining (Brooke *et al.* 2008; Szabo *et al.* 2010, 2012). Bird and mammal species have been declining in abundance and geographic extent in one of Australia's most untouched, and biogeographically complex landscapes – the monsoon tropics (Franklin 1999; Woinarski *et al.* 2001; Bowman *et al.* 2010). Declines are largely associated

with the effects of introduced grasses, cattle grazing and changed fire regimes (Franklin 1999; Woinarski *et al.* 2001; Franklin *et al.* 2005; Kutt & Woinarski 2007). This thesis holds the story of one monsoon tropical Australian threatened bird species, the Gouldian finch (*Erythrura gouldiae*), and my continuing mission to quantify its population size, connectivity and the extent of genetic incompatibility between colour morphs.

The knight: conservation genetics

The discipline of conservation genetics, broadly, deals with the use of population genetic theory and methodologies to address conservation relevant questions. These include the identification and delineation of species, populations, and the characterization and management of small, fragmented populations (Allendorf & Luikart 2007; Frankham *et al.* 2010). Small populations sizes can have deleterious effects on population viability through reduction in genetic diversity and inbreeding, which will be explored below.

The development of molecular genetic and genomic technologies has allowed an increase in precision in the measurement of genetic diversity in individuals and populations (Allendorf 2016). Patterns of genetic diversity, such as number of alleles and heterozygosity/gene-diversity, are ultimately what inform estimates of population size and population connectivity. Indeed, species with small populations have repeatedly been shown to harbour lower levels of genetic diversity than other taxa (Garner *et al.* 2005; Jackson *et al.* 2013; Pinsky & Palumbi 2014; Willoughby *et al.* 2015), and temporal genetic sampling has revealed declines in genetic diversity in line with declines in overall (census, N_c) population size (Weber *et al.* 2000; Hauser *et al.* 2002; Bristol *et al.* 2013). Ultimately, genetic diversity is not determined by N_c, but the effective population size (N_e), for which there are numerous estimators for use on genetic data (Wang *et al.* 2016). The effective population size represents the size of an idealised population that would show the same amount of genetic drift and inbreeding as the population being measured (Crow & Kimura 1970). The assumptions of the idealised (Wright-Fisher) population is no immigration, constant size, with random mating and non-

overlapping generations, are almost never a biological reality, as such N_e is often smaller than N_c (Frankham 1995; Palstra & Ruzzante 2008; Palstra & Fraser 2012).

Small N_e will increase the risk of inbreeding, which can be quantified using genetic techniques by measuring the fraction of homozygotes in the population. Related individuals share many more alleles than any two random individuals drawn from the population, but small population sizes will increase the likelihood of related individuals eventually interbreeding, and increase the level of homozygosity in genes that are identical-by-descent (Keller & Waller 2002). Increases the homozygosity of deleterious alleles causes inbreeding depression, which has been widely shown in captive and wild population to reduce lifetime fecundity and survival, thereby influencing extinction risk (Keller & Waller 2002; Spielman *et al.* 2004; O'Grady *et al.* 2006). As a consequence, captive breeding programs are often managed to minimise the effects of inbreeding (Frankham *et al.* 2010). Further, stochasticity in small populations can lead to the fixation and changes in frequency of alleles, which limits the long-term survival probability of a species by reducing the genetic variation available to natural selection, and can further enhance the effects of inbreeding depression by changing the frequency of deleterious recessives (Frankham 2005). The deleterious effects of small N_e have been used to set the rule-of-thumb of a minimum N_e of 50 individuals to prevent inbreeding depression, and 500 individual to prevent long-term loss of genetic diversity (Franklin 1980), but values of 100/1000 may be more realistic (Frankham *et al.* 2014). However, the legitimacy of this generalization across taxa, demography and threat-processes has been controversial (Reed *et al.* 2003; Brook *et al.* 2006; Jamieson & Allendorf 2012). The maintenance of genetic diversity (and to a lesser extent inbreeding) is arguably irrelevant for the timescales of pending extinction, and not practically viable as a target in current conservation programs (Jamieson & Allendorf 2012; Rosenfeld 2014).

Population fragmentation is primarily driven by habitat losses, and occurs when individuals migrating between habitat patches decreases, ultimately altering the patterns of genetic diversity across the landscape. Fragmentation into small, isolated populations can be

deleterious by reducing population growth rates (dispersers move into unsuitable habitat and subsequently die, or reducing the contribution of incoming migrants), and enhancing the risk of demographic stochasticity, inbreeding and genetic drift (Johnson *et al.* 1992; Reed 2004). Management actions may produce corridors of suitable habitats, or manually introduce migrants to boost population growth rates and reduce the effects of inbreeding depression (e.g. genetic rescue) (Weeks *et al.* 2011; Frankham 2015). On the other hand, the genetic distinctiveness of populations is valued to preserve evolutionary history and accumulated adaptive differences (Moritz 1994; Crandall *et al.* 2000), and to prevent fitness declines owing to outbreeding depression (Frankham *et al.* 2011). For example, molecular work identified genetic differences within the subspecies of Australian corroboree frogs (*Pseudophryne corroboree* and *P. pengilleyi*) (Morgan *et al.* 2008), and have been incorporated into government captive management and recovery plans (OEH NSW 2012).

Population ecology and population genetics are intimately entwined. Life-history traits influence the relationship between N_e and N_c (Palstra & Fraser 2012; Waples *et al.* 2013). Migration influences both population growth rates and genetic diversity although the scale of influence is different (Lowe & Allendorf 2010), and population growth rates can be influenced by inbreeding depression (Keller & Waller 2002). Genetic methods are useful in the conservationist's toolbox to address certain questions, and discern fundamental species biology that would otherwise be intractable to study in the wild (e.g. paternity). However, although genetics is part of the toolbox, conservation of species is a holistic approach that considers behaviour, reproductive biology, demography, nutrition, and human values and economic realities (Soulé 1985).

The damsel: The Gouldian finch

The Gouldian finch (Estrildidae: *Erythrura gouldiae* Gould 1844, synonym: *Chloebia*) is an Australian grassfinch allied with Australasian parrot finches (Van der Meij *et al.* 2005; Arnaiz-Villena *et al.* 2009). This finch has distinctive multi-coloured plumage, where females

have the same colours but duller than males (Figure 1.1a, Figure 1.2). Both sexes display a colour polymorphism for head-colour: black, red or yellow. It was formerly distributed across the monsoon tropical savannas of Australia, where it occupies *Eucalyptus* dominated woodlands (Figure 1.1b). In the early 20th Century it was anecdotally common across the range (Smedley 1904; Barnard 1914), reportedly occurring in flocks of thousands with long-tailed and masked finches (*Poephila acuticauda* and *P. personata*) (Heuman 1926). Comparison of historical and contemporary observer and museum records (1977-1997) indicates that that Gouldian finch numbers declined up to 50%, and their spatial extent by 40% (Franklin 1999). Catch reports from finch trappers in Western Australia indicate that Gouldian finches catches declined by up to 87% from 1972-1981, after which Gouldian finch trapping was banned in 1982 and all finches in 1986 (Tidemann 1996; Franklin *et al.* 1999). Broadly, anecdotal reports and these observer records indicate the worst declines commenced in Queensland in the 1950s, and spread westward to the Kimberley by the late 1970's (O'Malley 2006). Figure 1.1c shows the change in density of records from Atlas of Living Australia from before and after 1986, when all finch trapping was banned (Franklin *et al.* 1999).

The Gouldian finch declines may be an interplay of their relatively specialist niche and the intensification of European land-use in the region (Tidemann 1996; Franklin 1999; Franklin *et al.* 2005). The decline was more severe in Queensland, the state with earlier and more intensive European land use (Tidemann *et al.* 1993; Franklin 1999; Franklin *et al.* 2005). Its granivorous diet is relatively specialist compared with sympatric grassfinches, and is particularly dependent on the seeds of *Sorghum* spp. in the breeding season commencing in the late wet-season (February-July) (Tidemann *et al.* 1999; Dostine & Franklin 2002). The

a)

b)

c)

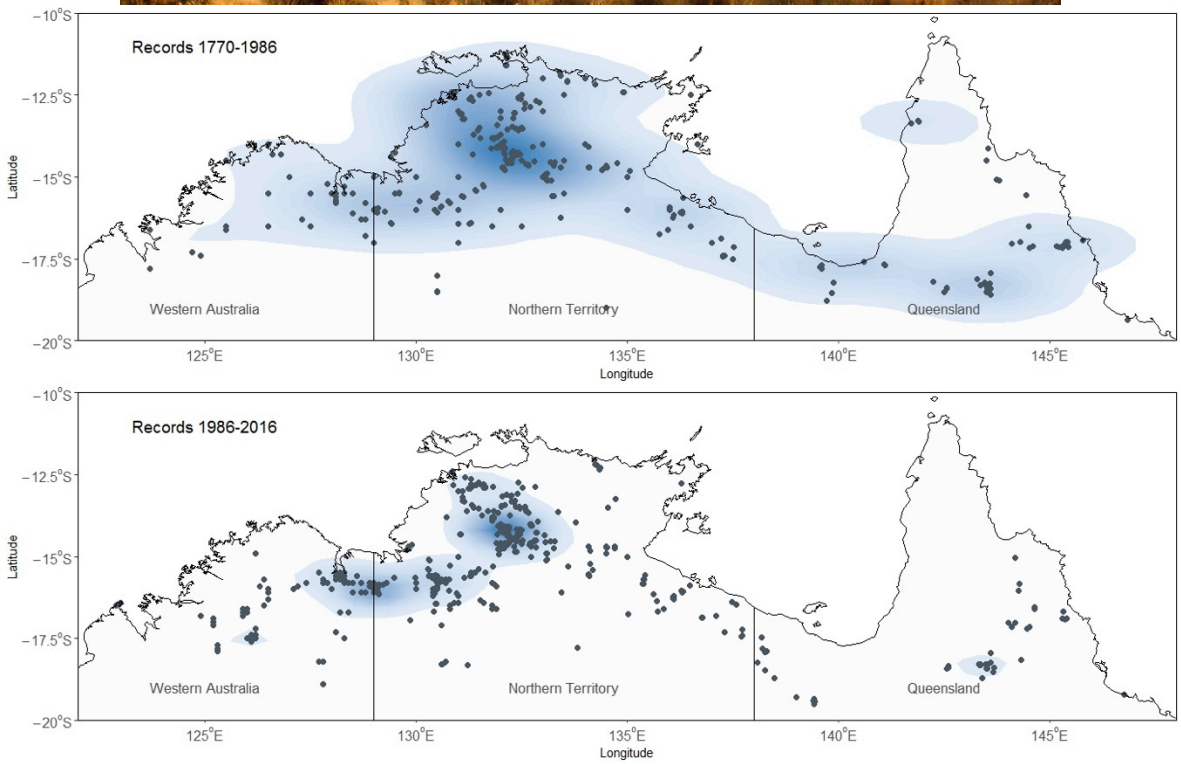

Figure 1.1: a) Wild Gouldian finches drinking at a waterhole in the Northern Territory. Photo features (1) red and (3) black head-colour morph adults and (2) un-coloured juveniles. Photo by Dylan Meyer. Panel b) shows typical Gouldian finch breeding habitat. Photo taken by Mike Fidler. Panel c) uses records from the Atlas of Living Australia to illustrate the decline in the Gouldian finch. Although observations of Gouldian finches persist across the range, the heat map clearly shows a reduction in the density of those observations. Note that this is only for illustrative purposes, for a full quantitative analysis see (Franklin 1999).

availability of key grass species is highly dependent on fire frequency, intensity and extent (Scott *et al.* 2009), where Gouldian finches appear to favour a mosaic of low intensity fires, based on movements, nest site selection, and stress responses (Dostine *et al.* 2001; Legge *et al.* 2015; Weier *et al.* 2016). The Gouldian finch is an obligate cavity nester, and like its diet, it is specialist in the attributes of the cavities occupied (Tidemann *et al.* 1992; Brazill-Boast *et al.* 2010), and suffer reduced fledging success from direct competition with the long-tailed finch (Brazill-Boast *et al.* 2011b).

The Gouldian finch was internationally recognised as a threatened species in 1988, but was down-listed in 2012 from 'Endangered' to 'Near Threatened' by the IUCN (Birdlife International 2013). This was on the basis of bird watcher reports and waterhole surveys in the prior decade that found no evidence for a further decline, with some local population estimates of 1000+ birds (Garnett *et al.* 2011). However, these population estimates are unreliable because a) more than 80% of these flocks are juveniles, and do not represent the breeding population (Woinarski & Tidemann 1992; Garnett *et al.* 2011), and b) individual birds or entire flocks may be counted multiple times over space and time. Very little is known about the movement capacity of these birds, and they have long been suggested to be highly mobile or migratory (Berney 1902; Smedley 1904; Garnett *et al.* 2011), indeed their wing shape reflects that of a highly dispersive bird (Woinarski & Tidemann 1992).

However, there is no direct evidence of large-scale movements in the Gouldian finch. The maximum distance between banding recaptures is 20km at the Australian Wildlife Conservancy Mornington Sanctuary (Legge *et al.* 2015), and radio tracking suggests birds make daily movements of 3-10km (Woinarski & Tidemann 1992; Tidemann 1993). After the breeding season, birds seem to move away from banding sites with low recapture rates between years (Woinarski & Tidemann 1992; Tidemann *et al.* 1999; Brazill-Boast *et al.* 2013), which suggests highly dispersive behaviours or high mortality rates (or both). This pattern may be partially explained as a seasonal movement, as bird densities in the Yinberrie Hills change between uplands and lowlands (10km apart) in the dry and wet seasons,

respectively (Dostine *et al.* 2001). However, over the Dostine *et al.* (2001) study only four birds were recaptured, no more than 1.5 years between recaptures, so many birds were still lost from the study. Until banding studies expand the area surveyed and account for inter-annual variation in water availability (which may affect congregation densities at waterholes), the question of how far Gouldian finches travel may never be solved.

Population genetic methods can detect the scale on which gene-flow occurs, and be used to infer the effective population size of the contemporary population. An early electrophoretic study of Myoglobin introns found no evidence of population structure (Heslewood *et al.* 1998), but a single locus is rarely sufficient to elucidate gene flow between regions (Slatkin & Barton 1989). Although Heslewood *et al.* (1998) had large samples from three localities in the western part of the range, birds from Queensland were very poorly represented. Unpublished thesis work from six microsatellites and mitochondrial control region on birds from a single site in each of Western Australia and Northern territory again indicated birds were a single randomly-mating population (Esparza-Salas 2007). However, there were only two contemporary population samples (Esparza-Salas 2007), and unsampled populations can have the effect of inflating migration estimates (Slatkin 2005). Further, if genetic differentiation is recent, or subtle, the marker power employed by Esparza-Salas (2007) was insufficient to detect it (Lloyd *et al.* 2013). There was also no evidence of differentiation from mitochondrial control region sequences in 43 natural history specimens, sampled more extensively across the range (Esparza-Salas 2007). Again the representation of birds from Queensland in this study was insufficient to draw robust conclusion about genetic structure in the historical population (Esparza-Salas 2007). Furthermore, these samples were not utilised to explicitly compare genetic diversity and effective population size (N_e) across the period of population decline in the Gouldian finch (Esparza-Salas 2007). This was a missed opportunity to unearth another independent estimate of contemporary population size (via N_e), and the degree of decline and recovery in the Gouldian finch. While the N_e to N_c ratio varies between species (Palstra & Fraser 2012; Waples *et al.* 2013), the Gouldian finch is

expected to have a much reduced effective population size owing to sex ratio bias (Brazill-Boast *et al.* 2013), high juvenile mortality rates (Woinarski & Tidemann 1992), and genetic incompatibility that occurs when colour morphs interbreed (Pryke & Griffith 2009a)..

Colour polymorphism as a threatening process

Colour polymorphism is commonly defined as highly heritable, discrete differences in colour that are maintained in the same population (Ford 1945; Huxley 1955). Colour polymorphism can be linked to variations in microclimate or habitat, or to predator-prey dynamics through apostatic selection (Bond 2007). Colouration can also be important for social and sexual signaling within a species, and colour polymorphisms are often associated with different social and sexual strategies (Roulin 2004; Wellenreuther *et al.* 2014). Indeed, this complex association of colour with all aspects of life means that many colour polymorphic species have a suite of other traits that are tightly associated with colour, and share underlying genetic mechanisms (Sinervo & Svensson 2002; McKinnon & Pierotti 2010). One of the distinctive features of the Gouldian finch is its head-colour polymorphism, and work on domesticated birds to discern some of the mechanisms maintaining colour polymorphism have revealed that the colour polymorphism itself may be a threatening process.

In the Gouldian finch, red head colouration is dominant to black and is determined by a single Mendelian locus on the sex chromosomes (Southern 1945), where females are the heterogametic sex. See Figure 1.2 for a diagram of inheritance patterns and genotype notations used throughout this thesis. In the wild, the red and black head colour morphs occur at stable frequencies across the range (Franklin & Dostine 2000; Gilby *et al.* 2009). However, because of uneven allele frequencies and sex-linked inheritance each sex shows a slightly different head colour frequency, for example 19.8% of females at Yinberrie Hills were red-headed, while 31.7% of males caught were red (Franklin & Dostine 2000). The yellow-headed morph is determined by homozygosity at an autosomal locus that is related to

carotenoid deposition (Murray 1963), but it occurs at very low frequencies (~1%) in the wild (Franklin & Dostine 2000; Gilby *et al.* 2009), and will not be discussed further in this thesis.

A potential threatening process to the Gouldian finch in the wild is the genetic incompatibility between head-colour morphs which was found in experiments on domesticated birds (Pryke & Griffith 2009a). This experiment force-bred all combinations of male and female head-colour genotypes (as determined by pedigree), and cross-fostered entire broods between treatments (Pryke & Griffith 2009a). Offspring from mixed-morph parents (e.g. red female and black male) suffered reduced survival but not fertility, where mortality of female offspring (~44%) was greater than that of sons (~30%) (Pryke & Griffith 2009a). This same pattern was also found when red females bred with heterozygote red males (Pryke & Griffith 2009a). This may be an example of a Dobzhanksy-Muller incompatibility, where two separated populations fix different allelic variants at one or more functional loci, and novel combinations of these loci in hybrids reduces offspring fitness (Dobzhansky 1936; Orr 1995). Haldane's rule predicts the heterogametic sex in hybrid offspring will suffer greater mortality or sterility (Haldane 1922), and may occur when Dobzhansky-Muller incompatibilities are sex-linked and at least partially recessive , meaning the heterogametic sex has no other chromosome to 'cover up' these deleterious loci (Orr 1997). Currently, the exact molecular mechanisms underpinning incompatibility in the Gouldian finch are unknown, but the consequences of incompatibility in the wild will be similar to those of outbreeding depression.

Like inbreeding depression, outbreeding depression results in offspring dysfunction, but are the progeny of individuals with very different genomes. As discussed in the previous section, the cumulative effects of offspring mortality resulting from inbreeding (or outbreeding) depression can reduce population growth rates (Edmands 1999, 2007; Keller & Waller 2002). Maximising population growth rates is one of the central goals of conservation and restoration of endangered species. Gouldian finches are socially monogamous breeders, and in the wild between 15-20% of pairs are mixed morph (Pryke & Griffith 2007), and additional pairs will be between red females and heterozygote red males (depending on the

frequency of heterozygote males in the population). Therefore, a minimum of 15% of pairs in the population may experience reduced fitness, in a species that may already have very high juvenile mortality rates (Woinarski & Tidemann 1992).

A series of follow-up papers found that incompatible pairs were able to ameliorate some individual fitness losses by optimizing their life-history trade-offs through differential investment through reduction in clutch size, and offspring provisioning rates (Pryke & Griffith 2009b, 2010); adjustment of offspring sex ratio towards male offspring (Pryke & Griffith 2009b); gaining extra-pair paternity from a compatible male (Pryke *et al.* 2010); and an assortative mate preference (Pryke & Griffith 2007; Pryke 2010). These kinds of behaviours are common consequences of reinforcement that can occur between hybridising species (Veen *et al.* 2001; Servedio & Noor 2003; Griffith 2010). However, these ameliorations do not fully remove the effects of incompatibility (Pryke & Griffith 2009b, 2010; Pryke *et al.* 2010), and will still lower net population growth rates. Indeed, the reinforcement in sympatric speciation models have predicted extinction in some scenarios (Liou & Price 1994; Doorn *et al.* 1998).Therefore, it is imperative that the extent of incompatibility be assessed in the wild, and is addressed in this thesis.

These experiments on domesticated birds revealed other behavioural and physiological traits associated with head-colour, and the consequences of their interactions may also be detrimental to population growth. Red birds of both sexes are aggressive and socially dominant to black birds (Pryke & Griffith 2006; Pryke 2007), and head-colours exhibit distinct personalities (Williams *et al.* 2012; Mettke-Hofmann 2012). In the wild, nest site quality confers higher reproductive success, and red males have higher success in acquiring and defending these territories (Brazill-Boast *et al.* 2011a, 2013). In experimentally competitive environments, with high proportions of red birds, red-headed birds exhibit higher levels of stress hormones and testosterone (Pryke *et al.* 2007), and reduce offspring provisioning (Pryke & Griffith 2009c). The frequency of red-birds in the wild may be maintained by this negative-frequency dependent selection on their aggressive behaviours

(Pryke *et al.* 2007). Anything that alters the frequency of red and black birds (such as drift, or selection for another trait correlated with colour) in the wild may alter these competitive dynamics. This will influence net population growth rates via frequency dependent behavioural effects and by altering the frequency of incompatible head-colour pairings. Indeed, theoretical modelling accounting for different behaviours and incompatibility between morphs has demonstrated parameter space where the combination of these effects can result in small population sizes, and even extinction of the entire population (Kokko *et al.* 2014). These effects are strongest when the number of suitable breeding habitat patches are low, therefore the maintenance of appropriate habitat may be of additional salience to conservation efforts (Kokko *et al.* 2014).

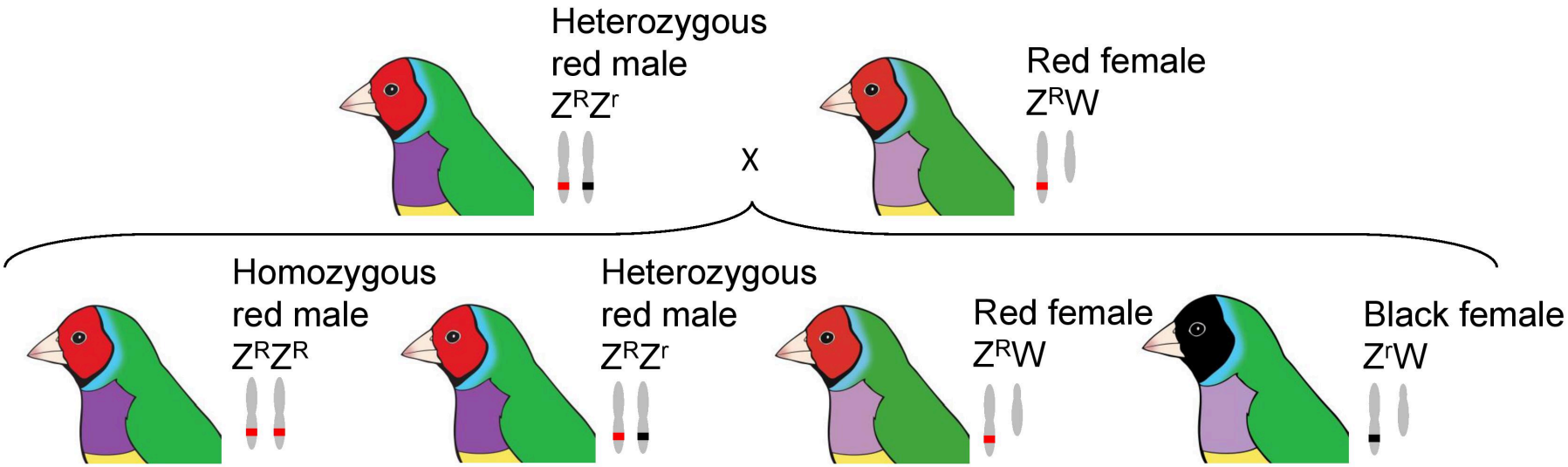

Figure 1.2: A cross between a heterozygous red male and a red female (hemizygous) and their predicted offspring. Each individual shows the genotype notation used, and a diagram of the sex-chromosomes for that genotype

Aims and Thesis Outline

There are two major categories of aims in this thesis, one pertaining to assessment of genetic incompatibility between morphs, and the other to using population genetic methods to assess population size and gene-flow. The different components of each aim are described as specific questions, and how each chapter answers these questions is described.

Aim One: Assess colour polymorphism as a threatening process

Question 1.1: Can colour polymorphism be a threatening process? Some literature suggests that colour polymorphisms will enhance population fitness (Forsman *et al.* 2008), but as outlined in the introduction, the evidence from the Gouldian finch suggests that it can have negative effects on population fitness. Chapter Two (Paper I) aims to broadly assess some of the features of colour polymorphic species, and presents new ideas as to how colour polymorphism may not be universally beneficial as once thought. This stimulated debate with proponents of colour polymorphism, and the position in Paper I is further defended in a reply (Paper II).

Question 1.2: To what extent is incompatibility occurring in the wild Gouldian finch? If incompatibility is occurring, the work on domesticated birds allows us to make predictions about what sorts of patterns we expect to observe in the wild. Previous work in the wild Gouldian finch found that there was no statistically significant effect of incompatible pairings on offspring fledging success (Brazill-Boast *et al.* 2013). The development of a PCR test for head-colour genotype (Kim 2011) has allowed me to conduct further work to assess selection at the genotype level in the Gouldian finch.

In Chapter Three (Paper III), I assess whether there is selection on incompatible pairs by investigating patterns of amelioration through extra-pair paternity with compatible males, offspring sex-ratio adjustment, and patterns of assortative mating. Incompatibility may also manifest through genotype specific mortality at the nestling stage, so I investigated deviation from Mendelian expectation of head-colour genotypes in Gouldian finch offspring.

In Chapter Four, I further explored additional ways incompatibility would be manifest in the wild population. Firstly, I explored whether there was evidence of mortality after fledging, by comparing changes in genotype frequencies across age classes. Secondly, I examined whether incompatible parents, or their offspring, showed sub-lethal effects of incompatibility through reduction in body condition.

Aim Two: Use genetic techniques to assess the 'genetic health' of the population

Question 2.1: What are the contemporary patterns of genetic diversity and gene flow in the Gouldian finch? I use three types of molecular marker to address this aim in Chapter Five. This chapter uses a dataset of the most comprehensively sampled across the contemporary range of the Gouldian finch, and includes the use of thousands of SNP markers to address whether there is subtle genetic differentiation between regions.

Question 2.2: Has the population genetic diversity and gene flow declined over the 20th Century, in line with observed population declines? This is addressed in Chapter Six, where over a hundred museum specimens sampled from across the range prior to the decline were genotyped, and compared with contemporary patterns.

Question 2.3: What is the effective population size of the contemporary Gouldian finch population(s)? The effective population size is assessed using multiple genetic estimators, on a comprehensively sampled dataset with a large number of markers in Chapter Six. The contemporary effective population size is compared with patterns of decline in genetic diversity over the 20th Century (Question 2.2).

Please note that because each chapter in this thesis is a publication (or intended to be) the citation of figures recommences every chapter, and appendices are referenced according journal specific protocols.

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Chapter Two

The danger within

The role of genetic, behavioural, and ecological factors in population persistence of colour polymorphic species

Colour polymorphic *Ctenophorus pictus*. Photo: C.R Friesen

Chapter Two Vignette

In the Gouldian finch, the conflicts between morphs and genetic incompatibility are potentially an additional threatening process in the wild. There was a swathe of literature describing why colour polymorphism was beneficial to population fitness (Forsman *et al.* 2008; Wennersten & Forsman 2012), yet the processes responsible for vulnerability in the Gouldian finch have been acknowledged as potential contributors extinction outside of the colour polymorphism literature (e.g. in speciation, and behavioural ecology (van Doorn *et al.* 1998; Kokko & Brooks 2003)). While not an exhaustive review of either literature, this paper attempts to bridge the gap between them, and challenges the paradigm that colour polymorphisms are universally beneficial to population fitness.

The initial paper prompted a reply from the dominant voice in the literature for colour polymorphism and population fitness (Forsman 2016). We further clarified our position by attempting to provide a framework based on the mechanisms involved in the origin and maintenance with which to describe polymorphisms and how that relates to reduction in population fitness (Paper II in this chapter). This debate has garnered further attention this year, another paper suggests that a false dichotomy has been drawn between colour polymorphic species and "other" species in the literature, and calls for more integration of the colour polymorphism literature with wider evolutionary biology and population genetics (Svensson 2017).

This chapter forms two publications:

I: Bolton, PE, Rollins, LA, Griffith, SC (2015) The danger within: the role of genetic, behavioural, and ecological factors in population persistence of colour polymorphic species. *Molecular Ecology*, **24,** 2907-15.

II: Bolton, PE, Rollins, LA, Griffith.SC (2016) Colour polymorphism is likely to be disadvantageous to some populations and species due to genetic architecture and morph interactions. *Molecular Ecology*, **25**, 2713-2718

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NEWS AND VIEWS

OPINION

The danger within: the role of genetic, behavioural and ecological factors in population persistence of colour polymorphic species

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Polymorphic species have been the focus of important work in evolutionary biology. It has been suggested that colour polymorphic species have specific evolutionary and population dynamics that enable them to persist through environmental changes better than less variable species. We suggest that recent empirical and theoretical work indicates that polymorphic species may be more vulnerable to extinction than previously thought. This vulnerability arises because these species often have a number of correlated sexual, behavioural, life history and ecological traits, which can have a simple genetic underpinning. When exacerbated by environmental change, these alternate strategies can lead to conflict between morphs at the genomic and population levels, which can directly or indirectly affect population and evolutionary dynamics. In this perspective, we identify a number of ways in which the nature of the correlated traits, their underpinning genetic architecture, and the inevitable interactions between colour morphs can result in a reduction in population fitness. The principles illustrated here apply to all kinds of discrete polymorphism (e.g. behavioural syndromes), but we focus primarily on colour polymorphism because they are well studied. We urge further empirical investigation of the genetic architecture and interactions in polymorphic species to elucidate the impact on population fitness.

Keywords: behavioural strategies, conflict, geographic variation, incompatibility, population ecology, sympatry

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Introduction

Polymorphic species exhibit heritable discrete or qualitative phenotypic variants within the same population, with little variance determined by the environment (Ford 1945; Huxley 1955). Polymorphisms are often correlated with a number of characteristics and may form resource polymorphisms or behavioural syndromes (Smith & Skulason 1996; Dochtermann & Dingemanse 2013). Polymorphism has long been studied as discrete variants of colour, many of which also exhibit correlated traits with a simple genetic basis (McKinnon & Pierotti 2010; Roulin & Ducrest 2013; Wellenreuther *et al.* 2014). Previous conceptual models have suggested that the occurrence of multiple morphs (e.g. colour) will decrease extinction risk and promote population persistence (Forsman *et al.* 2008; Wennersten & Forsman 2012). These studies argue that polymorphic species have comparably larger population, niche and range sizes, 'pre-adapted' ecotypes, and niche partitioning that reduces intraspecific competition (Forsman *et al.* 2008; Wennersten & Forsman 2012). We argue that the interactions between morphs and the genetic architecture of sympatric polymorphism are not beneficial to population persistence relative to monomorphic species. With recent advances in genomic technologies, there has been renewed interest in using colour polymorphic species as models for evolutionary processes. We believe that polymorphic species have a number of properties (outlined in recent reviews on colour polymorphism; McLean & Stuart-Fox 2014; Wellenreuther *et al.* 2014) that could lead to a unique response to environmental changes in contrast to monomorphic species, and they may be more vulnerable to environmental changes.

Largely focused on colour polymorphism, experimental and comparative studies have been inconsistent in the definition of polymorphism, encapsulating the following: large-scale geographic variation, with morphs being distributed allopatrically; species that are primarily polymorphic in ecological traits; or couch polymorphism vaguely as 'genotypic diversity' (Fowlie & Krüger 2003; Reusch *et al.* 2005; Forsman & Åberg 2008a,b; Krüger & Radford 2008; Forsman & Hagman 2009; Caesar *et al.* 2010; Agashe & Bolnick 2012). Although there is some overlap between these definitions, true polymorphic species (Ford 1945; Huxley 1955) are generally distinct from species that have clinal or allopatric variation in phenotype, where there is limited overlap and interaction between morphs (see Figure 1 for graphical representation). Indeed, there are different evolutionary processes underlying these forms of variation: geographic variation is driven by large-scale environmental variation and/or restriction of gene flow, while sympatric polymorphism is maintained at a local scale owing to frequency-dependent or balancing selection, often as a result of sexual or social interactions (Roulin 2004; McLean & Stuart-Fox 2014; Wellenreuther *et al.* 2014). Conceptually, we agree with the idea that polymorphisms

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along ecological axes (e.g. trophic or camouflage) can have positive benefits to population fitness (Forsman *et al.* 2008; Wennersten & Forsman 2012). However, we believe properties unique to sympatric polymorphic species have not been thoroughly considered in relation to population dynamics and extinction risk, and a clearer distinction should be made between geographic polymorphisms and sympatric or 'true' polymorphic species. Recent studies have demonstrated genetic incompatibilities between colour morphs and polymorphic populations (Pryke & Griffith 2009a; Corl *et al.* 2012), and agonistic interactions between morphs (Kokko *et al.* 2014) that may adversely threaten the continued persistence of both morphs over time. Thus, we outline an argument for how the strong social or sexual interactions between morphs in polymorphic species may have negative consequences for parameters of population 'fitness', and the sum of the demographic and genetic effects which predict population persistence (Reed 2005; Willi & Hoffmann 2009).

Few empirical studies have explicitly considered true colour polymorphic species in relation to extinction risk and ecological niche breadth, with mixed results. One comparative study in Australian frog taxa found extinction risk was not consistent across families with sympatric polymorphism (Forsman & Hagman 2009). An experimental study examining the polymorphic pygmy grasshopper (*Tetrix subulata*) is ambiguous: only an intermediate number of morphs improved population survival in high densities, and treatments where individuals were more related reduced the antagonism and enhanced the survival (Caesar *et al.* 2010). This suggests that there can be a negative effect of antagonistic interactions between morphs on survivorship. A recent study on two viper species with geographic variation in polymorphism (where some populations have sympatric polymorphism) demonstrates that polymorphic populations have a narrower niche than their monomorphic counterparts (Broennimann *et al.* 2014). This is contrary to the prediction that polymorphic species should have a broader niche (Forsman *et al.* 2008; Wennersten & Forsman 2012). Further, after morphs are lost from populations of the side-blotched lizards (*Uta stansburiana*), there is a strong effect of character release on the remaining morphs and is indicative that competition is maintaining polymorphism (West-Eberhard 1986; Corl *et al.* 2010). To our knowledge, these five studies represent the entire body of literature on the empirical effects of sympatric polymorphism on population persistence and suggest that sympatric polymorphism warrants further attention. The colour polymorphism literature has been concentrated on a variety of different species, but in none of these do we fully understand the nature of the underlying genetic architecture and the morph specific trait correlations, or how they relate to population persistence (Table 1). These knowledge gaps hinder our ability to predict how populations will behave in the present and future.

We believe that the beneficial effects of polymorphism described in Forsman *et al.* (2008) are less likely to apply in sympatric, classically defined polymorphic systems. When multiple morphs co-occur, they tend to be linked to alternate life history or social strategies (reviewed for colour polymorphism in McLean & Stuart-Fox 2014; Wellenreuther *et al.* 2014), and it is the consequence of this that

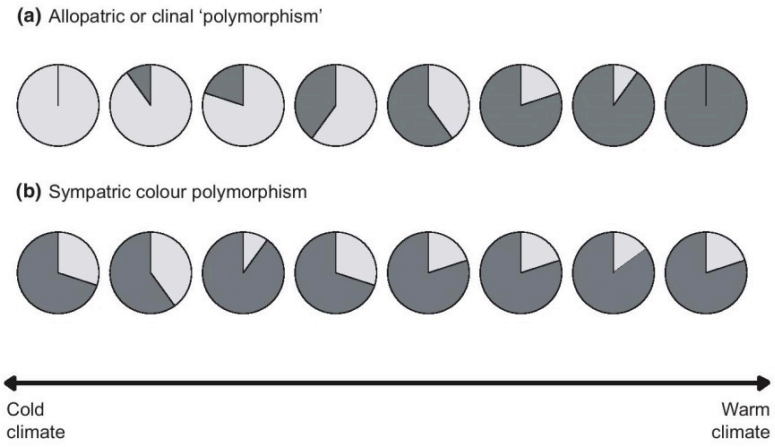

Fig. 1 The difference between the types of polymorphism commonly described with respect to a hypothetical geographic difference in climate. (a) clinal or allopatric polymorphic species will vary according to ecology or geography, potentially with some region of overlap between colours. However, the majority of the species consists of populations which are consistently monomorphic. These might be the result of a direct relationship between colour and the environment (e.g. through camouflage or thermoregulation) such as in the Bananaquit (*Coereba flaveola*) (Theron *et al.* 2001), or because they have evolved as random differences in geographically isolated populations. (b) True polymorphic species show both morphs co-occurring across the entire range, as the primary driver of polymorphism here is behavioural interactions (e.g. rock–paper–scissors games; Sinervo & Lively 1996). These are not mutually exclusive categories, and some sympatric polymorphisms will vary geographically (as shown in b) as a result of some correlation with environmental properties or stochasticity (see McLean & Stuart-Fox 2014).

Table 1 A compilation of commonly studied colour polymorphic species, summarizing current understanding of correlated traits that could affect population fitness

Species	Genetics	Behaviour	Life history	Ecology	Population persistence	Selected References
Brown-lipped snail (*Cepaea nemoralis*)	Y*	N	N	Y	N	Cain & Sheppard (1950), Silvertown *et al.* (2011), Richards *et al.* (2013) Rosin *et al.* (2013)
Common lizard (*Lacerta vivipara*)	N†	Y	Y	~	N	Vercken *et al.* (2007), Cote *et al.* (2008), Lepetz *et al.* (2009), San-Jose *et al.* (2014)
Forktail damselfly spp. (e.g. *Ischnura elegans)*	Y	Y	Y	~	N	Sánchez-Guillén *et al.* (2005), Abbott & Svensson 2007; Hammers & van Gossum 2008; Gosden & Svensson (2009)
Gouldian finch (*Erythrura gouldiae)*	Y	Y	Y	N	Y‡	Southern (1945), Pryke & Griffith (2006, 2009a) Brazill-Boast *et al.* (2013), Kokko *et al.* (2014)
Lake Victoria cichlid spp. (e.g. *Neochromis omnicaeruleus*)	Y	Y	~	Y	N	Seehausen *et al.* (1999), Dijkstra *et al.* (2009), Magalhaes *et al.* (2010), Henning *et al.* (2014)
Midas cichlid spp. (e.g. *Amphilophus xiloaensis*)	Y	Y	N	~	N	Barlow *et al.* (1990), Elmer *et al.* (2009), Henning *et al.* (2010), Kusche & Meyer (2014),
Pygmy grasshopper (*Tetrix subulata*)	N	~	~	Y	Y	Forsman (2000) Caesar & Forsman (2008), Karlsson *et al.* (2009)
Side-blotched lizard (*Uta stansburiana*)	N†	Y	Y	~	Y‡	Sinervo & Lively (1996), Sinervo & Clobert (2003), Lancaster *et al.* (2008) Micheletti *et al.* (2012)
Soay sheep (*Ovis aries*)	Y*	N	Y	~	N	Moorcroft *et al.* (1996), Gratten *et al.* (2007, 2012)
White-throated sparrow (*Zonotrichia albicollis*)	Y*	Y	~	~	N	Lowther (1961), Thorneycroft (1966), Thomas *et al.* (2008), Brown *et al.* (2014)

*Locus responsible has been identified.
†Indicates conflicting evidence regarding the genetic architecture of polymorphism.
‡Modelling studies only.
Y indicates good understanding; N indicates poor or no understanding; ~ Indicates that trait type has been mentioned in relation to colour, but not the focus of any one study, or that there is information on these traits but not how they pertain to specific morphs. *Category definitions:* Genetics refers to evidence of simple genetic inheritance (e.g. Mendelian); Behaviour refers to sexual and social strategies and their interactions; Life history refers to development and reproductive investment; Ecology refers to diet, ecophysiological tolerances; microhabitat preferences and predator–prey interactions (e.g. aposematic coloration); Population persistence refers to experimental or modelling studies that explicitly consider these factors and their relationship with population persistence.

we wish to discuss. We focus specifically on colour polymorphisms due to the large literature on genetic architecture and evolutionary processes relating to those, but these principles apply to other forms of discrete polymorphism (e.g. behavioural syndromes: Pruitt *et al.* 2008), as such we draw examples from other kinds of polymorphism where available. In this perspective, we identify ways in which the social and sexual interactions between discrete morphs, and their genetic architectures, can have negative effects on population and evolutionary dynamics. It is important to understand the nature of morph interactions, particularly against a context of rapidly changing environments. Morph ratios can respond rapidly to environmental change enabled by habitat or thermoregulatory correlations (Forsman *et al.* 2008; Ożgo & Schilthuizen 2012; McLean & Stuart-Fox 2014; Roulin 2014). However, morphs may track environmental variability irrespective of the main driver of polymorphism; for example, there is climate-related geographic variability in damselfly (*Ischnura elegans*) morphs, but the polymorphism itself is maintained by agonistic sexual interactions (Hammers & van Gossum 2008; Gosden *et al.* 2011). There are two potential negative consequences of trait correlation in the face of environmental change which we discuss first: (i) a shift in the equilibrium morph ratio, which can alter population processes through interactions and correlated traits, and (ii) trait correlations may constrain adaptation if selection on multiple traits is in opposing directions. We continue by outlining how the genetic architecture of polymorphism itself may also facilitate the evolution of genetic

incompatibilities, which can have additional population-level costs.

Ecological and evolutionary consequences of trait correlation: consequences of morph ratio variation

Colour polymorphisms are often associated with traits that may affect population dynamics. Trait correlations may have direct effects on population dynamics through variation in life history parameters, such as clutch size in *k*- and *r*-selected female side-blotched lizards (Sinervo *et al.* 2000), or parental investment in Gouldian finches (*Erythrura gouldiae*; Pryke & Griffith 2009b, 2010) and barn owls (*Tyto alba*; Almasi *et al.* 2008). If a morph with a lower reproductive rate is favoured by a changing climate (e.g. on account of its thermal properties), the population is subject to the demographic problems that are associated with low growth rates, and could cause a population collapse (Gilpin & Soule 1986). In addition, colour may be associated with dispersal traits (Van den Brink *et al.* 2012; Vercken *et al.* 2012), and thus, morph ratio variation may significantly alter population connectivity and local extinction rates (Gyllenberg *et al.* 2002; Hanski & Saccheri 2006). Further, morph ratio or morph composition of a population will change the relative costs and benefits of specific morph strategies (Bastiaans *et al.* 2013), which will affect intermorph interactions.

Intermorph conflicts may arise through direct means (e.g. heightened aggression), or indirect means (e.g. resource monopolization). Such interactions enable a mismatch between what is good for the individual and what is good for the persistence of the population (Kokko & Brooks 2003; Rankin 2005, 2007). Conflicts and interactions have been demonstrated to affect population dynamics through territorial conflicts between individuals in monomorphic species (López-Sepulcre *et al.* 2009, 2010) and the interplay between sex ratio skew and sexual conflict (Clutton-Brock *et al.* 2002; Milner-Gulland *et al.* 2003). We predict these effects to be important in colour polymorphic species too, because colour morphs are often associated with particular social or sexual strategies, or competitive abilities (through aggression or reproductive investment). Indeed, theoretical models suggest that there is a limited number of scenarios in which morphs can stably coexist (Alonzo & Calsbeek 2010; Kokko *et al.* 2014).

Recent theoretical work (Kokko *et al.* 2014) has demonstrated how trait correlations, interactions and density dependence between morphs interfere with population persistence in the endangered Gouldian finch, a cavity nesting species with specific nest-site requirements (Brazill-Boast *et al.* 2010). Socially dominant red males have higher reproductive success than black males because they acquire superior nest sites (Brazill-Boast *et al.* 2013). At the landscape scale, suitable nest cavities are an increasingly limited resource (Brazill-Boast *et al.* 2011) due to a changing fire regime in this region (Yates *et al.* 2008). Intuitively, we might predict an increase in the frequency of the better competitor (reds), as resource competition intensifies. However, in a highly competitive social context (high proportion of reds in a population, or elevated competition for a scarce resource), red males elevate the level of circulating testosterone and consequently suffer reduced immunocompetence (Pryke *et al.* 2007), and investment in parental care (Pryke & Griffith 2009a,b), leading to a reduction in their fitness. There are theoretical scenarios under which populations become monomorphic for the red morph, but because reds invest less parental care, population growth rates are much smaller. Thus, in different modelled scenarios of habitat suitability and assortative mating, the Gouldian finch morph ratio responds dramatically, between coexistence, single-morph extinction and simultaneous morph extinction (Kokko *et al.* 2014).

When colour polymorphism is associated with mating system, variation in morph ratios will lead to systemic mate limitation (Wellenreuther *et al.* 2014). Mate limitation can affect individual fitness, when individuals either completely forego reproduction or choose inappropriate partners (Møller & Legendre 2001). Morph ratio variation has been demonstrated to have population-level effects in heterostylous common lungwort (*Pulmonaria officinalis*): populations with skewed morph ratios have shown lower levels of female fecundity, seedling recruitment and lower levels of genetic diversity (Brys *et al.* 2008; Meeus *et al.* 2012). These effects will be particularly salient in socially monogamous species (e.g. birds) (Legendre *et al.* 1999), as partnership reduces the pool of available mates and constrains the choice of subsequent choosers (Griffith *et al.* 2011). In these situations, individuals are exposed to the fitness costs of genetic (see 'The evolution of genetic incompatibility') and behavioural incompatibilities. Behavioural compatibility between partners can affect fitness through the ability to coordinate reproductive activity and parental care (Spoon *et al.* 2006; Schuett *et al.* 2011; Mariette & Griffith 2012). Examples in polymorphic species include the lack of coordination between assortative pairs in white-throated sparrow (*Zonotrichia albicollis*) (Houtman & Falls 1994; Tuttle 2003; Horton *et al.*, 2013), and disassortative pairs in the Gouldian finch (Pryke & Griffith 2010). Although differential allocation and polyandry provides a mechanism to offset some of these fitness costs (Sheldon 2000; Tregenza & Wedell 2000; Griffith & Immler 2009), these mechanisms can be costly to population fitness (Anthony & Blumstein 2000; Castro *et al.* 2004; Holman & Kokko 2013).

Ecological and evolutionary consequences of trait correlation: constraint of adaptation

The response of polymorphic populations to climate change or competitive release (through morph loss) will depend on the genetic architecture of the trait. The genetic architecture of trait correlations may constrain or facilitate adaptation (Wilkinson *et al.* 1990). For example, in many polymorphic species, there are correlations between the polymorphic trait and other traits through close linkage, changes to regulatory genes or chromosomal inversions

(McKinnon & Pierotti 2010; Wellenreuther *et al.* 2014). Such genetic architectures may constrain adaptation of a population, if the direction of selection is not on the same axis as the trait correlation (Walsh & Blows 2009; Chevin 2013; Dochtermann & Dingemanse 2013). For example, in the Soay sheep (*Ovis aries*) on the island of St Kilda, there are distinct dark and light morphs controlled by a single locus *TYRP1* (Gratten *et al.* 2007). The dark morph is associated with a larger body size (Clutton-Brock *et al.* 1997), which tends to confer a fitness advantage through increased reproductive success and offspring viability (e.g. Milner *et al.* 1999). However, body size remains constant (Wilson *et al.* 2006) and dark sheep are becoming less common (Gratten *et al.* 2008), due to close linkage of the *TYRP1* locus to a locus that antagonistically reduces lifetime fitness in the homozygous condition (Gratten *et al.* 2008). Conversely, the 'character release' seen in the side-blotched lizard (Corl *et al.* 2010) is enabled by genetically unlinked traits (Sinervo & Clobert 2003), which can respond more quickly to a selective change. However, as they share the same genome, trait correlations within and between morphs may prevent optimal divergence of phenotypes in a manner similar to sexual and eusocial insect intercaste genetic conflicts (Chapman *et al.* 2003; Holman *et al.* 2013; Pennell & Morrow 2013).

The evolution of genetic incompatibility

Although colour polymorphism may promote prezygotic isolation (Gray & McKinnon 2007; Wellenreuther *et al.* 2014), the nature of selection and genetic architecture may promote the evolution of genetic incompatibility. The evolution of genetic incompatibility has been well described in the speciation literature (Seehausen *et al.* 2014), and there are an increasing number of examples of incompatibilities within species (Corbett-Detig *et al.* 2013; Kradolfer *et al.* 2013), but this was only very recently linked with colour polymorphism in a general sense (McLean & Stuart-Fox 2014). Disruptive selection has been implicated in the evolution of genetic incompatibilities (Seehausen *et al.* 2014) and is an important driver of polymorphism and associated genetic architecture (Rueffler *et al.* 2006). Indeed, genetic incompatibility is more likely to arise in situations where loci are involved in pleiotropic or epistatic interactions and are of large effect, similar to the genetic architectures seen in many polymorphic species (McKinnon & Pierotti 2010; Seehausen *et al.* 2014; Wellenreuther *et al.* 2014). Incompatibilities are especially likely to arise in polymorphisms that are due to chromosomal inversions, because inversions suppress recombination and allow genetic divergence between homologous chromosomes (Rieseberg 2001; Stathos & Fishman 2014). In addition, genetic conflict between morphs may also promote the evolution of incompatibility (Chapman *et al.* 2003; Crespi & Nosil 2013; Rice 2013). Although there are few current examples of incompatibilities in polymorphic species (Pryke & Griffith 2009a,b; Corl *et al.* 2012; Scarpino *et al.* 2013), we believe that future research will discover they are widespread because genetic architectures and selective regimes that characterize polymorphic species also facilitate the evolution of incompatibilities. These genetic incompatibilities are also important to population fitness, particularly when morph ratios vary and mate choice is increasingly constrained.

Summary and future directions

In this perspective, we have outlined how characteristics of true polymorphism (Ford 1945; Huxley 1955) can lead to a reduction in population fitness, contrary to predictions based on wider definitions of polymorphism (Forsman *et al.* 2008; Wennersten & Forsman 2012). True polymorphic species differ from clinally distributed species in the evolutionary dynamics that drive their coexistence, and the genetic architecture that underpins discrete trait correlations. Using predominantly examples from the colour polymorphism literature, we have demonstrated the opportunity for direct and indirect negative effects on population fitness when morphs are sympatric and frequently interacting, and even more so when there are genetic incompatibilities. These examples show the fundamental biology of trait correlations and morph interactions in sympatry can have effects on other forms of polymorphism as well. The relationship between the unique characteristics of true polymorphic species and population fitness remains to be tested thoroughly, and future work should strive to clearly delineate geographic variation from true sympatric polymorphism. To this end, we provide examples of colour polymorphic species that are amenable to study in the wild and in the laboratory (Table 1). The key message from Table 1 is that there is no single species for which we have a comprehensive understanding of the behavioural, ecological and genetic processes that could interact to adversely affect population persistence, even if attempts have been made to test their relationship with population persistence. In addition to assessing the linkage of colour to sexual traits (Wellenreuther *et al.* 2014), it is worth assaying whether other traits (e.g. life history) are correlated with colour, and how they relate to morph interactions and population dynamics (such as negative-frequency dependence). We suggest that these gaps be filled over the course of addressing the specific research questions we highlight below.

Throughout the text, we have suggested that environmental change and morph ratio variation will expose many of these hidden dangers to population fitness. In the wild, the conflicts or incompatibilities that exist between morphs in stable equilibria may be resolved through reasonably cryptic behaviour or physiology (e.g. sperm competition; see Pryke *et al.* 2010), or through spatial distributions (e.g. *Ctenophorus pictus*; Olsson *et al.* 2007). As such, we suggest that researchers should experimentally manipulate morph ratios and measure the following: (i) the inter- and intragenerational responses of individual morphs to elucidate how morph ratio variation impacts individual fitness, and the ability of individual morphs to respond through

adaptive or plastic mechanisms (e.g. Corl *et al*. 2010; Bastiaans *et al*. 2013); and (ii) parameters that affect overall population fitness such as demographic growth rates, genetic structuring and effective population size. By varying population size and environmental parameters that have ancillary relationship to the polymorphism (e.g. temperature), we can discern the extent to which morph strategy loss is predictable and what conditions promote morph loss (Corl *et al*. 2010; Kokko *et al*. 2014).

We also suggest that there are also many unresolved questions concerning the genetic architecture of sympatrically polymorphic species and that the nature of the architecture of such species is likely to determine the nature of genetic and behavioural interactions between morphs. In addition to discerning the genetic architecture of colour variation in polymorphic species (Roulin & Ducrest 2013; Wellenreuther *et al*. 2014), there needs to be more focus on the architecture and the nature of correlated physiological and behavioural traits to identify how these correlations enable or constrain adaptation, and whether some architectures are more likely to evolve genetic incompatibilities. Adaptive constraint can be tested in the framework we discussed above, by measuring morph responses to environmental and morph ratio change. Genetic incompatibilities between morphs might be demonstrated either through controlled mating trials (e.g. Pryke & Griffith 2009a,b), or through comparative genomic approaches by similarity to other incompatibility genes (Payseur & Hoekstra 2005; Tang & Presgraves 2009). We should utilize genomic techniques to isolate simple genotype tests for colour and its associated traits, and these will permit the better quantification of spatial and temporal selection in wild populations at the level of genotype (Gratten *et al*. 2007, 2010).

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NEWS AND VIEWS

REPLY

Colour polymorphism is likely to be disadvantageous to some populations and species due to genetic architecture and morph interactions

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Polymorphism describes two or more distinct, genetically determined, phenotypes that co-occur in the same population, where the rarest morph is maintained at a frequency above the mutation rate (Ford 1945; Huxley 1955). In a recent opinion piece, we explored a new idea regarding the role of genetic architectures and morph interactions in colour polymorphisms and how this can negatively affect population performance (Bolton *et al.* 2015). In this issue of Molecular Ecology, Forsman (2016) thoroughly discusses the current evidence for polymorphisms enhancing population performance and critiques the validity of the definitions of polymorphism we use in our original paper. We respond by clarifying that the negative consequences of polymorphisms that we discussed are likely to be most pertinent in species that have a particular set of characteristics, such as strong sexual or social interactions between morphs and discrete genetic architectures. Although it was not our intention to redefine polymorphism, we do believe that there should be further discussion about refining or characterizing balanced polymorphisms with respect to the degree of morph sympatry, discreteness of traits and their underlying genetic architecture, and the types of selection that drive and maintain the variation. The latter describes whether polymorphism is primarily maintained by external factors such as predation pressure or internal factors such as interactions with members of the same species. The contribution of Forsman (2016) is useful to this discussion, and we hope that our exchange of opinions will inspire new empirical and theoretical ideas on the origin and maintenance of colour polymorphisms.

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In our recent paper (Bolton *et al.* 2015), we explained how colour polymorphism may be deleterious to a species when (i) the polymorphism is sustained by social or sexual interactions and (ii) when the distinct phenotypes of a polymorphic species are underpinned by discrete genetic architectures. In his comment on our opinion paper, Forsman (2016) thoroughly reviews the literature that demonstrates the effects of polymorphism on various measures of population performance, in line with earlier work in that area (Forsman *et al.* 2008), and challenges our suggestion that it might be useful to consider some new definitions of polymorphism. It was not our intention to put forward a generalizable theory of the effect of polymorphism on population persistence nor to write an exhaustive review of that area. Therefore, the additional studies raised by Forsman (2016) and the main conclusions that he reiterated of the papers we cited, do not change the substance of our argument. Here, we respond to Forsman (2016) by further exploring the interpretation of key papers on this subject and then clarify our position by discussing the definitions and underlying mechanisms of polymorphism by citing an example from a very recently published study that demonstrates precisely the kind of polymorphism we believe to be most 'at risk'. We explained that a range of species are currently described by the broad term 'polymorphic', which could be further refined by considering the different processes that sustain polymorphism and the genetic architectures that characterize it (Bolton *et al.* 2015). We believe that these underlying processes may have different consequences for population persistence. We focused our study on those species in which there are discrete, genetically determined phenotypes that co-occur and interact across the majority of their range, and we highlighted some candidate species that might share some or all of these key characteristics. In particular, we explored the mechanisms that may negatively influence population persistence through correlation of colour with social and sexual traits.

In his comment, Forsman (2016) explores how important empirical works have demonstrated that polymorphism enhances population performance (Forsman & Hagman 2009; Caesar *et al.* 2010; Forsman *et al.* 2012; Wennersten *et al.* 2012). However, closer examination of these studies suggests that these results are not decisively in favour of Forsman's argument, and we clarify below how the results of these studies can be explained under the paradigm we presented (Bolton *et al.* 2015).

With regard to the comparative study of Australian frog species (Forsman & Hagman 2009), the main conclusions were that when frog families were pooled, there was a significant effect of variable coloration on lower extinction risk. However, the phylogenetically independent contrasts failed to find any effect of variable coloration on the three measures of population performance (range size, habitat diversity and conservation status) (Forsman & Hagman 2009). Furthermore, it is unclear whether pooling the families provides a biologically meaningful or general conclusion, as strong results from single families could bias the outcome and the pooling may be masking important biological differences between the nature of polymorphisms in different Australian frog families. Indeed, the authors comment on this in their discussion (*sic* Forsman & Hagman 2009: p1541):

> We do not know why variable coloration was not associated with lower endangerment in Myobatrachidae [when controlling for range size]. Most Myobatrachidae are stout bodied and burrowing or terrestrial and none are primarily arboreal, whereas Hylidae and Microhylidae generally are more slender bodied and include several arboreal and semiarboreal forms. These differences in ecology may somehow affect the relative importance of coloration and associated traits.

It is precisely this aspect that we addressed in our opinion piece; differences in the importance or function of coloration may have different downstream population consequences (Bolton *et al.* 2015). With specific regard to these families, we suggest that coloration in Myobatrachids might be more important with regard to sexual and social signalling, whereas coloration might be more important for trophic interactions (e.g. camouflage) in the Hylids and Microhylids.

The studies on population performance in the Pygmy Grasshopper *Tetrix subulata* also have alternative interpretations. In the wild, there was a positive effect of morph diversity on establishment success (Forsman *et al.* 2012), but in a 'seminatural' experiment, there was a strong increase in the variance of establishment success with increasing morph diversity (Wennersten *et al.* 2012; figure 1). While the overall trend in these experiments is in favour of the Forsman *et al.* (2008) model, the strongly increasing variation in establishment success with diversity requires further explanation (Wennersten *et al.* 2012) and is compatible with the ideas presented in Bolton *et al.* (2015). Further, Forsman (2016) critiques our interpretation of the survivorship experiments conducted in Caesar *et al.* (2010) and explains that the decrease in survival with increasing morph diversity is due to the statistical over-representation of inferior morphs, and not antagonism. This is actually one element of the argument we presented in Bolton *et al.* (2015), whereby random variation in morph ratio might also affect population fitness when those morphs have different reproductive strategies. Like the *K*- and *r*-selected female reproductive strategies in the side-blotched lizards (*Uta stansburia*; Sinervo *et al.* 2000), there are similar trade-offs in the reproductive strategies of pygmy grasshopper morphs (Forsman 1999, 2001). Therefore, if an over-representation of morphs can affect population fitness in this experimental context, it can equally affect population fitness after the random sampling that occurs during natural founder events and population bottlenecks. Further, high-diversity treatments were not well replicated in these experiments, so it is difficult to disentangle the effects of relatedness and diversity on survival and antagonistic interactions, particularly when behavioural interactions were only measured with regard to relatedness (Caesar *et al.* 2010).

Forsman (2016) highlights the population benefits of polymorphism in *Ischnura* damselflies (Takahashi *et al.* 2014a), whose female polymorphisms arose from sexual conflict (Svensson *et al.* 2005; Gosden & Svensson 2007; Takahashi & Watanabe 2010), in challenging the ideas we presented (Bolton *et al.* 2015). He specifically challenges our assertion that polymorphisms arising from sexual or social interactions might be particularly vulnerable to negative population consequences (Forsman 2016). Indeed, Takahashi *et al.* (2014a) find that when morph ratios are at parity, female fecundity and population fitness are improved, because the deleterious effect of male harassment and sexual conflict is ameliorated. We appreciate that the full resolution of conflicts will have positive effects on population fitness (Takahashi *et al.* 2014a; Forsman 2016), but we also explain in our original article that variation in morph ratios may lead to conflicts that are not properly resolved, and morph interactions may be innately antagonistic (Bolton *et al.* 2015). Indeed, in the experimental treatments in *Ischnura elegans*, it was found that morph ratios that deviated strongly from parity resulted in significantly reduced female fecundity and other measures of population fitness (Takahashi *et al.* 2014a; figure 3). Moreover, morph ratios in *I. senegalensis* naturally vary across a latitudinal gradient owing to correlated thermal properties of morphs (Takahashi *et al.* 2011, 2014b), which demonstrates selection on correlated traits may influence morph ratio that in-turn influences population fitness (Bolton *et al.* 2015). We feel that the study by Takahashi *et al.* (2014a) begs the question: how do female and population fitness in this population compare to a population with no female polymorphism or in a population with no/less sexual antagonism? In our opinion piece, we aimed to highlight that polymorphic species, like the *Ischnura* damselflies, are more complex owing to such interactions, and that the relationship to population persistence and extinction risk may not be as clear as previously suggested (Forsman *et al.* 2008).

As we suggested in our original paper, costs (and benefits) of polymorphism should be measured explicitly at the both the individual and population level, within a framework that explicitly considers correlated traits and genetic architectures (gene number and their interaction). The sexual conflict literature provides a wealth of theoretical and empirical studies that provide a conceptual framework that can be borrowed to examine the interactions of morphs

and the role of genetic architecture in shaping individual and population fitness, and adaptive evolution (Rice 1996; Rankin & Arnqvist 2008; Bonduriansky & Chenoweth 2009). Indeed, these studies demonstrate that the links between genetic architecture of a trait, individual and population level fitness may be complicated. For example, degree of sexual dimorphism (as a resolution of intralocus sexual conflict in optimal development time) was positively correlated with measures of population fitness in laboratory seed beetles, yet the diverged male phenotype has direct negative fitness effects on females through reduced lifetime fecundity (Maklakov *et al.* 2005; Arnqvist & Tuda 2010). Therefore, although net population productivity can be positively affected, morphism can have individual costs that may become important under different environmental conditions, high density or morph ratio deviation. This is only relevant when morphs interact frequently, and these interactions form the basis for the evolution and maintenance of the polymorphism itself.

Forsman (2016) suggests that our definition of polymorphism ignores the dynamic nature of polymorphisms and states that polymorphic species will vary in characteristics (such as morph ratio) across the geographic range in accordance with environmental variables. We agree that polymorphisms are extremely dynamic and will often vary across the geographic range, which may include monomorphic and polymorphic populations (Ford 1945; Huxley 1955; McLean & Stuart-Fox 2014; Forsman 2016). However, we believe within the dynamism of polymorphic species, there are operational 'axes' that characterize the underlying biology and are relevant to the relationship with population persistence as we describe here and in Bolton *et al.* (2015). These axes, described below, reflect both biological patterns and mechanisms, and polymorphic species ought to lie somewhere within this hypothetical space.

1 *The origin and maintenance of balanced polymorphism*: There should be an important distinction between polymorphisms that are primarily driven by sexual or social interactions and those driven primarily by ecological causes and the types of selection that maintain their sympatry. These will influence the kinds of interactions that morphs have, and the kinds of traits that become correlated with morphs. We clarify that we consider ecological causes to be primarily external, such as predation, interspecific competition, and thermal and microhabitat niche separation. Further, these 'ecological' or externally driven polymorphisms can be maintained by balancing selection (e.g. apostatic selection), and also maintained in sympatry by fine-scale selection mosaics, or contact zones/migration between locally adapted populations. However, other polymorphisms are found to exhibit complex social and sexual interactions between morphs [e.g. rock–paper–scissors game in the side-blotched lizards (*Uta stansburiana*) (Sinervo & Lively 1996)] that contribute significantly to the maintenance of that polymorphism. Spatial gradients and selection mosaics are likely to be less relevant in the maintenance of these polymorphisms. However, we appreciate that other traits that are ecologically relevant can also be correlated with colour morph, such as thermal tolerance in *Ischnura* (Takahashi *et al.* 2011, 2014b).

The origin and maintenance mechanisms of morphs will relate to the type of interactions morphs engage in and ultimately their opportunity to interact. The distinction between these as drivers for polymorphism is not mutually exclusive, but there is value in considering which processes are more important for the evolution and maintenance of a polymorphism, and the effects on downstream consequences for population fitness.

2 *Degree of sympatry or opportunity to interact*: The degree to which morphs overlap reflects the opportunity to interact and thus how relevant any negative consequences of polymorphism are to populations and species. The degree of sympatry should also consider the underlying population structure: are morphs in sympatry because they are being fed by migration from monomorphic populations? Recent contact between previously isolated or locally adapted populations can also be deleterious to individual and population fitness through extrinsic or intrinsic isolating mechanisms, but this is thoroughly dealt with in the speciation literature (Barton 1980; Coyne & Orr 2004).

3 *Discreteness of morphs and underlying genetic architecture*: Within organisms commonly considered polymorphic, there is variation in the degree to which morphs and their correlated traits are discrete in the underlying genetic architecture. Recent work has highlighted the complexities of the underlying genetic architectures whereby suites of traits are co-expressed by tight physical linkage in the genome, inversions or through epistatic interactions (McKinnon & Pierotti 2010; Wellenreuther *et al.* 2014). Genetic architectures that underlie polymorphisms are more likely to be discrete in strongly interacting and sympatric morphs. Divergent and negative frequency-dependent selection on multiple traits will tend to favour the evolution of simple genetic architectures which can maintain balanced polymorphisms (Sinervo & Svensson 2002; Kopp & Hermisson 2006). When selection is focused on social or sexual traits, it is the combination of these types of traits with their genetic architecture that make such polymorphisms worthy of separate attention. Increased accessibility of genomic techniques to studies of non-model systems will result in the characterization of these genetic architectures in many different polymorphic species.

We stress that that this is not intended to be a formal model, but a useful guide for how we should think about the variation within polymorphic species. The types of species we think most harbour 'the danger within' are those that are driven primarily by social/sexual interactions, morphs are largely sympatric, and have discrete traits and genetic architectures. An example of a species that does not fit comfortably at either of the extreme ends of any of our criteria and highlights the complexity within

polymorphisms is the barn owl (*Tyto alba*). The barn owl has heritable, but not phenotypically discrete, morphs for brown and black coloration (phaeomelanins and eumelanins) that are clinally distributed along a north–south axis (Roulin 2003; Roulin & Dijkstra 2003) and are maintained by selection in the face of extensive gene flow because coloration covaries with ecological niche (diet and habitat choice) (Roulin 2004; Antoniazza *et al.* 2010; Dreiss *et al.* 2012). In addition, coloration is related to sex-specific life history strategies and fitness traits (e.g. offspring quality, nest attendance), influencing an independent cline in female spottiness (Roulin *et al.* 2000, 2001; Roulin 2003; Roulin & Altwegg 2007). Sex-specific selection for plumage traits influences sex ratios at the nest (Roulin *et al.* 2010) and is involved in nonrandom mating and parental coordination (Roulin 1999). This species' polymorphism is maintained by both internal and external factors, and its coloration traits are not discrete. Yet they display some of the 'danger' traits we discussed previously and would be interesting to investigate the relative costs and benefits of these traits on the individual and population scale (Bolton *et al.* 2015).

The recent discovery of inversions underlying the incredible morphological and behavioural polymorphism in the lek-breeding Ruff (*Philomachus pugnax*) provides a timely opportunity to illustrate the 'extreme' kind of polymorphism we described previously: those that are underlain by discrete genetic architectures and sustained by behavioural interactions (Küpper *et al.* 2015; Lamichhaney *et al.* 2015). In the ruff, each of three male morphs morph has distinct plumage, sexual, behavioural, immune and hormonal profiles (as summarized in figure 1 of Küpper *et al.* 2015). The morphs are determined by uninverted and two genetically dominant nested inversions on chromosome 11, spanning hundreds of protein coding genes (Küpper *et al.* 2015; Lamichhaney *et al.* 2015). This polymorphism is not without fitness costs – in both sexes inversion homozygotes are lethal, inversion heterozygotes have lower survivorship than noninverted haplotypes, and females with the female mimic genotype may be infertile (Lank *et al.* 2013; Küpper *et al.* 2015). To compensate for these disadvantages, inversion genotype males must have relatively higher reproductive success to maintain the inversion haplotype frequencies (Küpper *et al.* 2015; Lamichhaney *et al.* 2015). Indeed, these males have much larger testes than territory holding males (uninverted homozygote genotype), which allows them to gain a large share of paternity through sperm competition (Küpper *et al.* 2015). Therefore, in the ruff, there is discrete genetic architecture determining the morph phenotypes, and morphs are sustained by sexual interactions and opportunities for intralocus conflict between morphs and between sexes. Incidentally, there have been local trends of decline in the Ruff (Jaatinen *et al.* 2010; Birdlife International 2012). Although recent population declines are largely attributed to increasing anthropogenic pressures on wetlands, it remains unknown whether the polymorphic ruff is more vulnerable than other shorebirds sharing similar habitat requirements.

We believe that there is a good opportunity, and an expanding methodological repertoire to embrace the molecular complexity underlying a variety of phenomena that are grouped together under the term polymorphism, defined over 50 years ago. It was not our intention to obscure or redefine the notion of polymorphism in our opinion piece (Bolton *et al.* 2015), but we did wish to highlight some of the complexity surrounding the current usage of this term and start a conversation about how this might be amended. We agree with Forsman (2016) that the definitions provided by Huxley (1955) and Ford (1945) are useful and encompass much important dynamism. Similar to the criticism in our original paper (Bolton *et al.* 2015), Huxley himself (1955) described that there was imprecision in the use of the word 'polymorphism' with respect to genetic underpinning, seasonality and geographic variation and suggested the use of *morphism* instead. Further, the original definition specifies that morphs should coexist in a single interbreeding population (Huxley 1955), which means that polymorphism can be geographically restricted (i.e. some population monomorphic and some polymorphic) or occur within multiple populations. When a species only has a few isolated polymorphic populations, whether potential costs and benefits apply to an entire species will depend on gene flow and local selection (Forsman *et al.* 2008; Bolton *et al.* 2015). Therefore, consideration of the scale of polymorphism is important.

We anticipate more genomic work in the vein of the recent work (Küpper *et al.* 2015; Lamichhaney *et al.* 2015), which will provide increasing insight into the mechanisms through which polymorphic species evolve and are maintained. As such, we think it important to consider how the term 'polymorphic' is being used to define different phenomena on the basis of some of the criteria we described here and previously (Bolton *et al.* 2015). We agree that the beginnings of a definition put forward by us would be difficult to put into practice (Forsman 2016), but we think that we should start working towards dividing polymorphic species into different categories on the basis of their underlying genetic architecture, trait correlation (complexity and kinds of traits) and degree of sympatry. We strongly believe that the newly elucidated nature of the ruff polymorphism (Küpper *et al.* 2015; Lamichhaney *et al.* 2015) is very different from polymorphisms, for example, maintained primarily by camouflage or apostatic selection, including the classic peppered moth (*Biston betularia*) and the brown-lipped Snail (*Cepaea nemoralis*) (Cain & Sheppard 1954; Cook *et al.* 2012). Although discrete genetic architectures will underlie many discrete traits driven by some form of divergent/negative frequency-dependent selection (Sinervo & Svensson 2002; Kopp & Hermisson 2006), what trait axes these affect (e.g. ecological or sexual) and how they interact will be most important for determining population performance (Kokko & Brooks 2003; Rankin *et al.* 2011; Holman & Kokko 2013).

The purpose of Bolton *et al.* (2015) was to start further discussion on this interesting topic, and we appreciate that Forsman (2016) has engaged in this conversation. We

completely agree that when polymorphism (and other forms of variation) lies primarily on ecological axes (e.g. camouflage) that there will be benefits to population performance as has been extensively shown (Forsman *et al.* 2008; Agashe & Bolnick 2010; Agashe *et al.* 2011; Forsman 2014; Forsman & Wennersten 2015). We did not intend to dismiss the validity of this previous work, but simply to highlight that there is more to explore with respect to how the types of traits, and their genetic architectures might differentially contribute to population performance, and explored some of the potential mechanisms. We hope that future work finds value in both of these contributions and will hopefully engage some exciting new work.

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Chapter Three

The colour of paternity

Extra-pair paternity in the wild Gouldian finch does not appear to be driven by genetic incompatibility between morphs

Photo: Sarah Pryke

Chapter Three Vignette

After a general exploration of how the processes underlying colour polymorphism may be a threatening process, I now dive into characterising the incompatibility between morphs in the wild Gouldian finch. Ascertaining the extent of incompatibility is a key aim in my thesis, as it offspring mortality and hence may slow population growth rates. This chapter forms part of a larger body of work using observational evidence from this species to assess the extent of incompatibility. Using key predictions derived from the studies on domesticated bird, this paper explores whether Gouldian finch parents strategically ameliorate incompatibility. This is the first published utilisation of a novel allele specific test for head-colour in the Gouldian finch, developed by colleagues (and co-authors) at Sheffield. This allows us to directly measure selection on head colour in offspring, and determine which head-colours (or head colour alleles) are siring more extra-pair offspring.

The analyses conducted on wild Gouldian finches in this chapter found no evidence for strategic amelioration of incompatibility, nor selection against particular offspring head-colour genotypes. Given there were such strong effects of incompatibility in the domesticated experiments, this begged the question: are the domesticated birds different genetically? Indeed, if incompatibility were occurring we would expect there to be some low-level genetic differentiation between morphs. A key finding was that domesticated birds show evidence of genetic differentiation between morphs, but not in the wild. Therefore, maybe the incompatibility was intensified as part of the domestication process.

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The colour of paternity: extra-pair paternity in the wild Gouldian finch does not appear to be driven by genetic incompatibility between morphs

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domestication;
Estrildidae;
mate choice;
polyandry;
post-zygotic isolation.

Abstract

In socially monogamous species, individuals can use extra-pair paternity and offspring sex allocation as adaptive strategies to ameliorate costs of genetic incompatibility with their partner. Previous studies on domesticated Gouldian finches (*Erythrura gouldiae*) demonstrated a genetic incompatibility between head colour morphs, the effects of which are more severe in female offspring. Domesticated females use differential sex allocation, and extra-pair paternity with males of compatible head colour, to reduce fitness costs associated with incompatibility in mixed-morph pairings. However, laboratory studies are an oversimplification of the complex ecological factors experienced in the wild and may only reflect the biology of a domesticated species. This study aimed to examine the patterns of parentage and sex ratio bias with respect to colour pairing combinations in a wild population of the Gouldian finch. We utilized a novel PCR assay that allowed us to genotype the morph of offspring before the morph phenotype develops and to explore bias in morph paternity and selection at the nest. Contrary to previous findings in the laboratory, we found no effect of pairing combinations on patterns of extra-pair paternity, offspring sex ratio or selection on morphs in nestlings. In the wild, the effect of morph incompatibility is likely much smaller, or absent, than was observed in the domesticated birds. Furthermore, the previously studied domesticated population is genetically differentiated from the wild population, consistent with the effects of domestication. It is possible that the domestication process fostered the emergence (or enhancement) of incompatibility between colour morphs previously demonstrated in the laboratory.

Introduction

Given that mating and raising offspring is costly, there is a trade-off between future and current reproductive events, and the two parents will be selected to maximize their own fitness and modulate their investment in a reproductive event according to their own condition and the perceived reproductive value of their mate (Trivers & Willard, 1973; Burley, 1986, 1988). For example, mates that have low reproductive value could be closely related, therefore increasing the risk of inbreeding depression (Szulkin *et al.*, 2013). In socially monogamous species, the common goal of raising offspring together is tempered by constraints on optimal choice of partner (Griffith *et al.*, 2011), and individuals will use different strategies to maximize their fitness in these situations, including modifying parental effort,

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offspring sex ratio and through extra-pair paternity. For example, female zebra finches (*Taeniopygia guttata*) will modulate egg size and incubation effort in relation to partner quality (Rutstein *et al.*, 2004; Gorman *et al.*, 2005; Bolund *et al.*, 2009), and female collared flycatchers (*Ficedula albicollis*) will produce male-biased broods when paired with high-quality males (Bowers *et al.*, 2013). Partners may have low reproductive value because they are genetically incompatible, such that offspring are inviable or infertile (Tregenza & Wedell, 2000; Griffith, 2010; Presgraves, 2010). For example, female collared flycatchers (*F. albicollis*) paired with pied flycatchers (*F. hypoleuca*) use extra-pair paternity with conspecific males to reduce the number of infertile hybrid offspring produced in these heterospecific social pairings (Veen *et al.*, 2001).

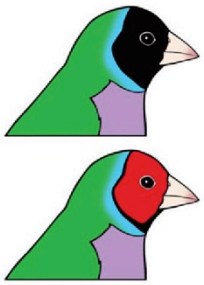
In colour polymorphic species, mate choice options are often discrete, and when colour is tightly correlated with a number of other traits, there can be fitness detriments to partnering with the wrong colour morph (McKinnon & Pierotti, 2010; Griffith *et al.*, 2011; Bolton *et al.*, 2015). For example, colour polymorphism in the white-throated sparrow (*Zonotrichia albicollis*) is controlled by an inversion polymorphism and pairings are almost always disassortative (Thorneycroft, 1966; Houtman & Falls, 1994). Assortative white-throated sparrow pairs would lack necessary parental coordination to raise offspring successfully and risk production of inversion homozygotes that may suffer reduced fitness relative to inversion heterozygotes (Tuttle, 2003; Horton *et al.*, 2013). Moreover, if selection for colour morph differs between the sexes, then parents of a particular morph may bias their offspring sex ratio to match that selection. For example, large spotted barn owls (*Tyto alba*) produce female-biased brood sex ratios, whereas small-spotted parents have male-biased broods (Roulin *et al.*, 2010). Furthermore, because many colour polymorphic species are undergoing strong divergent correlational selection and mate assortatively, this may facilitate the evolution of genetic incompatibilities between sympatric colour morphs, although examples are rare (Sinervo & Svensson, 2002; Roulin & Bize, 2006; Seehausen *et al.*, 2014; Bolton *et al.*, 2015). In cases where colour morphs are genetically incompatible, there should be selection for strategies to avoid the costs of incompatibility.

The Gouldian finch (*Erythrura gouldiae*) is a colour polymorphic bird that has behavioural and physiological traits correlated with sex-linked Mendelian red and black head colour morphs, and exhibits a genetic incompatibility between morphs that strongly affects mortality of female offspring (Southern, 1945; Pryke & Griffith, 2006, 2009b; Pryke *et al.*, 2007) (Box 1). This is unusual, because until relatively recently genetic incompatibility is generally thought to be proportional to the degree of genetic divergence between allopatric populations (Presgraves, 2010). However, in the Gouldian finch, head colour morphs are sympatric across the entire distribution at stable frequencies (Gilby *et al.*, 2009). In accordance with strong selection against offspring from mixed-morph parents, mate choice experiments on domestic Gouldian finches have demonstrated assortative mate preference for head colour (Fox *et al.*, 2002; Pryke & Griffith, 2007; Templeton *et al.*, 2012). To ameliorate some of the costs of incompatibility, females in mixed-morph pairs invest less in rearing offspring and offspring primary sex ratios favour the sex (males) less affected by incompatibilities (Pryke & Griffith, 2009a, 2010). Further, when experimentally given the opportunity for extra-pair copulations, a single copulation with a compatible male was able to sire a large proportion of the clutch, consistent with a post-copulatory cryptic female choice mechanism (Pryke *et al.*, 2010). All of the previous work (cited above) was conducted on domesticated Gouldian finches, and no previous work has examined the extent to which incompatibility and related selection occurs in the wild. Although controlled laboratory studies can tease apart possible factors guiding animal behaviour, they may not be completely relevant to the complex ecological contexts in the wild (Healey *et al.*, 2007), nor do they account for any behavioural and physiological differences that accrue as a result of the domestication process (Burns *et al.*, 2009; Christie *et al.*, 2012). Here, we have used the previous work on the domesticated Gouldian finch to guide predictions for patterns in incompatibility avoidance and morph selection in the wild.

Theoretical modelling (based on the parameters of incompatibility identified in domesticated populations) has demonstrated that incompatibility and morph interactions can be detrimental to population fitness and cause extinction of one or both morphs (Kokko *et al.*, 2014), and could be an additional threatening process for this threatened species (Environment Protection and Biodiversity Conservation Act 1999). In the wild, there are more red males than red females (and more black females than black males), because of uneven morph allele frequencies in the wild, and sex-linked inheritance (Box 1a) (Southern, 1945; Franklin & Dostine, 2000). This means that mates of the same head colour are limiting, and 15–20% of individuals are constrained to mate with an individual from a different morph to themselves (Pryke & Griffith, 2007; Griffith *et al.*, 2011). In such mixed pairs, any genetic incompatibilities would be exposed and this would reduce reproductive success. In contrast with findings on offspring survival in the domesticated birds (Pryke & Griffith, 2009b), previous work in the wild has shown that the survival of nestlings from mixed-morph pairs was not significantly lower in comparison with those from same-morph pairs (Brazill-Boast *et al.*, 2013a). The previous study in the wild (Brazill-Boast *et al.*, 2013a), however, did not account for strategic extra-pair

Box 1 (a) Explanation of the alleles and sex linkage of the red/black polymorphism in the Gouldian finch. (b) Explanation of the key predictions used in this paper that are derived from the laboratory studies on domesticated Gouldian finches.

(a)



Black morph, recessive allele

Males: Z^rZ^r

Females: Z^rW

Red morph, dominant allele

Males: Z^RZ^r, Z^RZ^R

Females: Z^RW

(b)

Results from domesticated birds	Prediction in the wild
1. Females in mixed-morph pairs had a significantly higher fraction of offspring sired by a compatible extra-pair male, than females in same-morph pairs (Pryke *et al.*, 2010). This was an average of 56% of offspring per clutch, and 76.5% of all possible offspring	1. In the wild: a).Mixed-morph pairs will have a higher incidence of any extra-pair offspring than same-morph pairs b) Mixed-morph pairs will have a larger fraction of extra-pair offspring than same-morph pairs c) Extra-pair offspring will be sired by compatible males.
2. There is a significantly male biased sex-ratio in offspring of mixed-morph pairs (Pryke & Griffith, 2009a)	2. There will be a male bias in the offspring from mixed-morph pairs in the wild.
3. The offspring of red females paired with heterozygous red males did equally poorly as those from mixed-morph pairs (Pryke & Griffith, 2009b). These females also have male biased offspring, but not as strongly as in mixed pairs.	3. a) Red females paired with heterozygote red males will have similar compensation strategies as black females in mixed-morph pairs. b) Incompatibility effects are likely to be strongest in heterozygous red-headed young, therefore there will be a reduction in heterozygous red offspring.
4. Gouldian finches exhibit mutual mate choice preferences for individuals of the same morph (Fox *et al.*, 2002; Pryke & Griffith, 2007; Pryke, 2010).	4. There will be a pattern of assortative mating with respect to morph in the wild
5. Restriction of gene-flow not tested previously in captivity.	5. There will be genetic differentiation between morphs

paternity or differential sex ratio allocation to ameliorate some of these costs, as shown in domesticated birds (Pryke & Griffith, 2009b; Pryke *et al.*, 2010).

Based on the collection of previous work on domesticated birds in the laboratory, we therefore predicted that compatibility selection will be the primary driver for patterns of extra-pair paternity and offspring sex ratio in the wild (Pryke & Griffith, 2009a,b; Pryke *et al.*, 2010). Like in the domesticated birds, we assumed that females of all morphs and pairing types are equally likely to engage in extra-pair copulations (Pryke *et al.*, 2010) and that females in the wild are able to access at least one extra-pair copulation with a compatible male. This seems plausible as the species is nonterritorial and they forage in small flocks (J. Brazill-Boast & S.C. Griffith, Pers. Obs.), providing ample opportunity for females to find and copulate with an extra-pair partner. We therefore predicted that females in mixed-morph pairs should have a higher proportion of offspring sired by an extra-pair (compatible) male, than females in

same-morph pairs (Box 1b, Prediction 1). Further, offspring from mixed pairs are expected to have a male-biased sex ratio, due to the combined effects of sex allocation and female-biased mortality as demonstrated in the domesticated birds (Box 1b, Prediction 2; Pryke & Griffith, 2009a). The contribution of compatible males to extra-pair offspring is examined through the identification of extra-pair sires through genotyping and by inference from the head colour of extra-pair chicks using the novel PCR assay developed for the *red* head colour gene in Gouldian finches (Kim, 2011). This marker also allowed us to directly test whether there was any selection against heterozygote red males, as inferred from the work on domesticated birds (Box 1b, Prediction 3; Pryke & Griffith, 2009b). To explore mate limitation and adaptive mate choice, we measured the frequency of assortative and disassortative pairs in the population and predicted that there will be more assortative mate pairs than expected under random mating (Box 1b; Prediction 4). Further, if incompatibility is occurring, we predicted there to be a restriction of gene flow between red and black morphs (Box 1b; Prediction 5) and compared levels of genetic differentiation between head colour morphs both in the wild and in a sample of the domesticated population. We discuss how these findings relate to the evolution of genetic incompatibilities in this species and more broadly.

Materials and methods

Study site and nest monitoring

This study was conducted on a population of Gouldian finches, near Wyndham in the eastern Kimberly region, Western Australia (S15°340′, 128°090′). This site comprises 109 Ha of suitable Gouldian finch breeding habitat (see Brazill-Boast *et al.*, 2011 for specific details). The study site was supplemented with nest boxes that resemble the natural cavities preferred by Gouldian finches to facilitate the study by providing access to the nest chamber (Brazill-Boast *et al.*, 2010, 2013b). The data were collected from February to August, which encompasses the breeding season of the Gouldian finch, in consecutive years (2008–2009). In the study area, all nest boxes and natural hollows were marked and checked for nest initiation every 7–10 days. After initiation, the nest was checked every 2–3 days to record brood size and hatching date. Once hatched, nestlings were monitored every 2–4 days, and if they were in nest boxes, nestlings were blood-sampled and banded between the age of 14 days and fledging, which occurred after day 18. On first capture, all adults were given a unique combination of plastic colour bands in addition to a metal band supplied by the Australian Bird and Bat Banding Scheme, and their head colour was recorded. Blood samples were taken from the brachial vein (< 50 μL) and stored in 95% ethanol. Putative parentage (or social parentage, the pair that raises the offspring) was assigned by either: (1) capture of an adult entering an active nest using a hand net or (2) direct observations of a colour-banded adult visiting a nest to feed offspring. In addition, every 1–2 weeks, birds congregating around waterholes were mist-netted and blood-sampled, to gain a broader sample of the adult population, including individuals that were deemed to be nonbreeding (although it is possible that they were breeding in undetected nests either inside or outside the study area).

Only nestlings from nest boxes were bled, as offspring in natural hollows were inaccessible. In total, blood samples were collected from 51 putative families, including 257 offspring sampled at day 14. In total, we also had 252 adult blood samples from the population, which included parents sampled at nests and also adults caught in the local vicinity of the breeding areas. For more details on the total sample of natural hollows and nest boxes, see Appendix S1.

The above protocols were approved by the animal ethics committees at Macquarie University (AEC2007/037 & AEC 2007/038) and followed all Australian legislation.

Molecular methods and parentage assignment

Blood samples were extracted using a Qiagen PureGene Kit. We amplified 10 variable microsatellite loci described previously (Pryke *et al.*, 2010). In addition to these markers, we genotyped all adults and offspring in the sample for two consecutive SNPs that segregate almost perfectly with head colour phenotype (Kim, 2011). These SNPs (locus *Ego172*) were developed into a simple allele-specific PCR test, where alleles are differentiated by labelled dyes and a 3-bp size difference (Kim, 2011). This assay was included into the above microsatellite multiplexes. In our sample of 252 adults, only a single adult's genotype at this colour marker conflicted with the observed phenotype at the time of capture and blood sampling (see Appendix S1). All offspring were sex-typed at the *CHD* locus using primers 2550F and 2718R (Fridolfsson & Ellegren, 1999). The details for the protocols we used are in Appendix S2.

For each year in our data set, genetic data were used to assign parentage to offspring using a likelihood approach in CERVUS 3.0.6 (Marshall *et al.*, 1998; Lemons *et al.*, 2014). All families had at least one putative social parent identified at the nest through behavioural observations, and 47 of 51 families had both social parents identified. Allele frequencies were estimated using the default settings, and parentage was simulated using 100 000 offspring and assuming 80% of the adult population was sampled. We assigned mothers to all offspring in the data set, followed by fathers after the exclusion of maternal alleles. Parents were assigned on the basis of the highest log-likelihood

ratio score (LOD). The CERVUS assigned mother was compared with the field observation of the social mother to verify the results and to identify causes for mismatches (allelic dropout or different parents). Allelic dropout was inferred by a manual check when a chick mismatched its parents at a locus where either the respective parent or offspring was homozygous, but matched both parents at all other loci. We subsequently assigned paternity having accounted for the maternal alleles using the known mothers, based on the consensus mother derived from manual, automated and field observations of maternity. Rates of null alleles were calculated using CERVUS (Appendix S2).

Parents were assigned based on a conservative set of rules based on number of mismatches and allelic dropout. Parents were only assigned if they either matched at all loci, or mismatched at a maximum of two loci that could be readily explained by allelic dropout and backed up by a high LOD score. No more than two mismatches attributable to dropout were tolerated. Instances of extra-pair paternity were defined when offspring mismatched the social father at two or more loci (of ten). If an offspring mismatched both social parents at two or more loci (not attributable to dropout), then intraspecific brood parasitism (IBP) was inferred. For the identified extra-pair offspring, potential fathers from the population pool were only assigned when there were zero mismatches at all loci typed, or if there was just one mismatch readily attributable to allelic dropout. As can be seen in the results, the bimodal distribution of mismatches indicates that all offspring were assigned unambiguously.

Across genetically sampled nests, there were four families with only one social parent identified at the nest, and the missing parent was not identified with parentage analysis. The genotypes of the missing parents were reconstructed from the offspring genotypes, but to be conservative we excluded three (of four) families where reconstructed genotypes included more than three alleles because it was not possible to distinguish between multiple paternity and allelic dropout without the second parent as a reference.

The final genetic data set comprised 57 broods from 48 families (comprising 257 day 14 offspring), with a total of 92 sampled parents and an additional pool of 154 adults captured in the study area. Of the sampled families, six pairs were sampled over two breeding attempts, and two were sampled over three attempts.

Determinants of extra-pair paternity

Although the primary focus of this study was to explore morph- and incompatibility-related drivers of extra-pair paternity, other factors such as the opportunity for extra-pair copulations can be important in explaining variation in paternity (Griffith *et al.*, 2002). Breeding density and synchrony were explored in relation to patterns of extra-pair paternity as described in Appendix S3. Additional insight into spatial constraints on extra-pair paternity was explored by characterizing the distances females travelled to attain extra-pair copulations, based on the nestlings that were confidently assigned to an individual extra-pair father. For these nestlings, we tabulated whether that father was observed nesting at the same time, and if so, how far his nest was from the focal female (the mother).

We focused on genetic incompatibility in mixed-morph pairs as a primary driver of extra-pair paternity patterns and compared our results directly with those from the previous captive experiments (Pryke *et al.*, 2010). We included only the first observed clutch in these analyses because (1) it was more directly comparable with the captive experiments and (2) to remove pseudoreplication, as there were not enough replicate clutches to run nested analyses. There was one nest included in the analysis where the social mother as not observed in the field and was not identified by the parentage analysis, but the head colour assay allowed us to reconstruct her head colour genotype based on the genotype of the social father and their offspring. We also repeated the analyses described below with respect to intraspecific brood parasitism and further explore our broader findings of intraspecific brood parasitism in relation to other Estrildid finches (Appendix S3).

We evaluated whether females in mixed-morph pairs were more likely to have any offspring from an extra-pair father (Prediction 1a). To do this, we coded whether any given family had at least one instance of extra-pair paternity. We compared the number of families with extra-pair paternity in mixed-morph (total families = 12, red female (Z^RW) × black male (Z^rZ^r) = 4; black female (Z^rW) × red male (Z^RZ^r) = 8) and same-morph pairs (total families = 36; black female × black male = 34; red female × red male = 2). Throughout this paper, females will be referred to first when describing pairing combinations. We tested the difference in number of families with any extra-pair offspring in mixed- and same-morph pairs using a Fisher's exact test.

To test whether the results in the wild conform to expectations from the captive females, we extracted the occurrence of extra-pair paternity across all families in the experimental data set from Fig. 1 in Pryke *et al.* (2010) and tested whether there was a difference in the occurrence of extra-pair paternity (see Appendix S3 for more details). From the previous study, we present only the level of extra-pair paternity in mixed pairs in the adaptive treatment ($n = 10$) and the pure pairs in the neutral treatment ($n = 10$). We use this subset of data throughout as a comparison with wild birds and it will hereafter be referred to as 'domesticated'.

In our wild sample, all red females in same-morph pairs were with heterozygous red males, and this

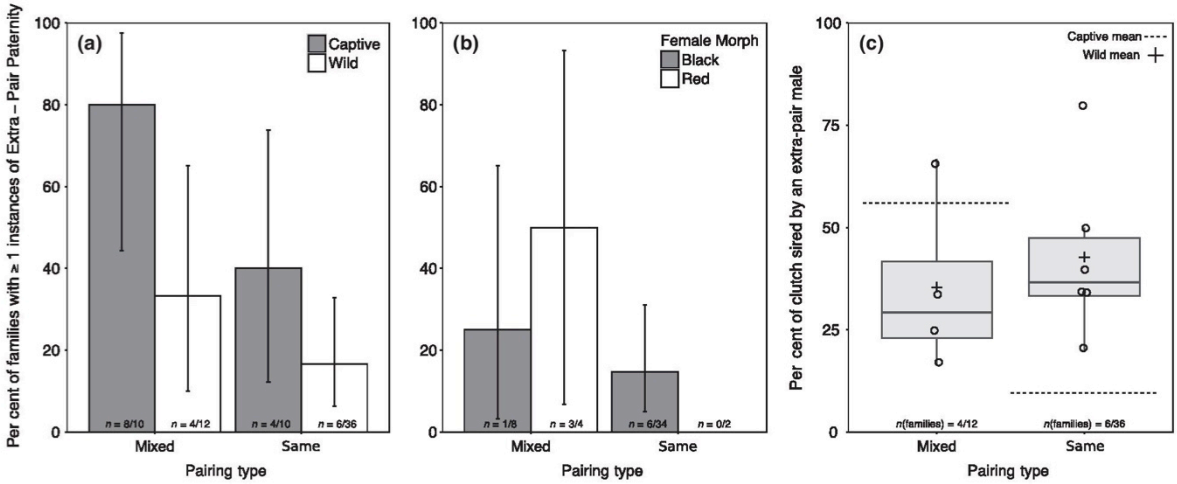


Fig. 1 (a) The per cent of all families that had at least 1 instance (extra-pair offspring ≥ 1) of extra-pair paternity by pairing type from the data set pooled across years (Fisher's exact test, one-sided *P*-value = 0.24). Number of families is above the category on the *x*-axis. Black bars represent results from experiments on domesticated birds where mixed pairs are in the adaptive context and same-morph pairs in the neutral context (Pryke *et al.*, 2010; Fig. 1); white bars are the results from wild birds. The error bars represent 95% binomial confidence intervals. (b) Per cent of wild families with ≥ 1 extra-pair offspring according to female head colour and pairing class. Black bars represent black females, and white bars are red females. There was no significant difference between the number of black-headed females engaging in extra-pair paternity across pairing contexts (Fisher's exact test, two-sided *P*-value = 0.17). Sample sizes for each category are above the *x*-axis. (c) The per cent of wild offspring that were sired by an extra-pair male, from those nests with extra-pair paternity (Mann–Whitney *U*-test: $W = 8$, $P = 0.45$). Dashed lines represent the average per cent of clutch in each context sired by an extra-pair male (in adaptive and neutral context for mixed- and same-morph pairs) from experiments on domesticated birds (Pryke *et al.*, 2010: Fig. S1), and the cross represents the mean per cent in the wild data set.

pairing type was previously found to be incompatible in the domesticated birds (Pryke & Griffith, 2009b). To test Prediction 3a, we tested for differences in the number of black females with any instance of extra-pair paternity using a Fisher's exact test (same morph, $n = 34$; mixed morph, $n = 8$).

We compared whether mixed-morph pairs had more extra-pair offspring (Prediction 1b). We tested this by comparing the proportion of extra-pair offspring in mixed pair broods with same-morph broods using a Mann–Whitney *U*-test. To control for differences in clutch size, we also compared mixed- and same-morph pairs using a binomial logistic regression with a logit link. From nests with any instance of extra-pair paternity, we tallied the total extra-pair offspring and compared pairing types with a Fisher's exact test and compared these values against the number of offspring observed in the Pryke *et al.* (2010) domesticated data set.

We also conducted power analyses on the Fisher's exact tests about whether we could reject a false null hypothesis of no difference between mixed- and same-morph pairs in the wild. We ran three different tests: (1) power when the effect size is as observed in the wild, (2) the sample size required to reject Ho with the observed effect size and (3) power to reject Ho from the effect size observed in the domesticated birds (subset as above). All analyses were conducted in R using the '*pwr*' package and considered 0.8 to be adequate power (Champely, 2015). All effect sizes reported are Cohen's *h*, as a measure of distance between two probabilities.

Offspring morph

Fathers of extra-pair offspring should be a compatible morph with the mother (Prediction 1c). When not all extra-pair offspring are assigned fathers, genotyping offspring with the head colour marker (*Ego172*) allows us to infer patterns of paternity by expectations from Mendelian inheritance and known allele frequencies in the parental generation. See Appendix S4 for details about how expected genotype proportions were calculated. For this, we pooled all extra-pair offspring across all broods (not just the first brood) by maternal morph. We then used a binomial exact test to compare whether extra-pair offspring genotype frequency deviated from expectation. We tested against two scenarios: whether offspring genotype frequency matched that expected under random paternity (no female preference or morph-specific fertilization) or under assortative morph preference. These were tested against expectations using a goodness-of-fit binomial exact test, or a multinomial exact ('*XNomial*' package) test in R (Engels, 2015).

In domesticated birds, red females paired with heterozygote red males showed evidence of incompatibility; therefore, heterozygote red offspring should be selected against (Prediction 3b). In our data from the wild, we tested selection through deviation from Mendelian expectations (based on their parents' genotypes) in each pairing type for all within-pair offspring of the first observed clutch ($n = 190$). Unfortunately, the PCR assay for head colour was not available when the original (captive) experiments were conducted, so we are not able to make quantitative predictions about the degree of deviation from Mendelian expectations from genotype-specific survival or allele-specific fertilization. For each pairing type, offspring genotype frequencies were compared to Mendelian expectations using a goodness-of-fit binomial exact test, or a multinomial exact ('*XNomial*' package) test in R (Engels, 2015).

Offspring sex ratio

If incompatibility is occurring in the wild as observed in domesticated birds (Pryke & Griffith, 2009b), along with adaptive sex ratio allocation (Pryke & Griffith, 2009a), then there should be a significantly male-biased sex ratio in the broods of mixed-morph pairs (Prediction 2). To account for variation in clutch size, we used a binomial logistic regression with a logit link, with offspring sex as the response and pairing type as the predictor. We tested pairing type as the two-factor mixed vs. same morph, and as four factors for each individual pairing type. The latter approach was used because, in captivity, red females in 'assortative' pairs with heterozygote males also showed (albeit weaker) male-biased sex ratios (Pryke & Griffith, 2009a) (Box 1b; Finding 3).

Assortative pairing

In the domesticated Gouldian finch, birds showed assortative mate preferences and therefore avoided the costs of incompatibility by pairing with same-morph partners, and we therefore predict an assortative social mating pattern in the Wyndham population (Prediction 4). We assessed the extent to which birds were pairing assortatively with respect to head colour in the Wyndham population. We compared unique breeding pairs Wyndham (pairs; $n = 59$), against two hypotheses: a) random mating, calculated by random union of morph genotypes, and b) 'perfect assortative mating'. Perfect assortment was defined as the situation where all individuals will always mate assortatively. As the frequency of head colours differs in males and females in the population, any strict assortative mate preference means that surplus red males are inevitably forced to breed with black females or forego reproduction (Southern, 1945; Griffith *et al.*, 2011). We compared the expected frequencies of mixed- and same-morph pairs in these scenarios against the observed social pairings in Wyndham using a binomial exact test, where red males (regardless of underlying genotype) paired with red females were considered 'same morph'. We also assessed whether there was any difference in head colour frequency between the individuals that were observed to breed and the wider population using a Fisher's exact test by comparing breeders with non-breeders.

Population genetics of morph phenotypes in domesticated and wild populations

If incompatibility is occurring, then we would expect to see associated genetic differentiation between red and black morphs (Prediction 5). We examined genetic differentiation in red and black wild birds and in a cohort of domesticated birds used in previous studies (Pryke *et al.*, 2010). We also examined genetic differentiation between domesticated and wild populations. These domesticated birds were selected as wild-type birds (those without avicultural colour mutations) from the broader population of domesticated Gouldian finches held by aviculturists in Australia and had been held by us for no more than four generations at the time that the key previous studies were conducted (Pryke & Griffith, 2009b; Pryke *et al.*, 2010). Little information is available on how many generations this domesticated population has been maintained in captivity, but it was difficult to breed Gouldian finches in captivity reliably prior to the 1980s (Evans & Fidler, 2005). Thus, we estimate the captive population to be around 30 years old (~ 30 generations). Although it has been illegal to trap wild Gouldian finches since 1987, there are still anecdotal reports of wild finches being taken into captivity, and thus the degree of isolation from the wild populations is difficult to ascertain.

To explore genetic changes since domestication, we genotyped 48 adult birds (16 red and 32 black) from the domesticated population that had been studied previously (Pryke *et al.*, 2010) and compared them with the wild birds. We genotyped these individuals at the same ten microsatellite loci using the laboratory protocols described above. We created a random sample of individuals from the wild population to match the data set of domesticated birds. We compared levels of genetic diversity between red and black morphs and between wild and domesticated populations, and calculated the rarefied allelic richness in FSTAT 2.9.3.2 (Goudet, 1995), and heterozygosity, deviation from Hardy–Weinberg equilibrium and inbreeding coefficients in ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010). Linkage disequilibrium between markers was assessed using GENEPOP v4.2 (Raymond & Rousset, 1995).

We examined population structure between red and black morphs in both wild and domesticated populations, and between wild and domesticated populations, using AMOVA implemented in ARLEQUIN (10 000

permutations) (Excoffier & Lischer, 2010). We also ran the AMOVA on the full data set of wild birds, comprising 47 red and 197 black birds. Groupings for the AMOVA were devised based on the provenance of the birds (wild or domesticated, $n = 48$ each), and the head colour morph observed of the individual bird (red ($n = 16$) or black ($n = 32$)), and analyses were run separately for each comparison (red vs. black, wild vs. domesticated).

To explore whether there was genetic structure that did not fall into our *a priori* population groups, we ran a genetic clustering analysis using STRUCTURE v2.3.4 with domesticated/wild or red/black in the captive population as *a prior* (LOCPRIOR) (Pritchard *et al.*, 2000; Hubisz *et al.*, 2009). The LOCPRIOR model sets sampling provenance as *a prior* in the analysis to be considered as potentially informative to population structure and will aid in the detection of weak population structure according to sampling provenance, but will not bias the result if there is not structure according to these localities (Hubisz *et al.*, 2009). We used the default admixture model and simulated genetic clusters (K) 1–10 with ten iterations per value of K. We chose a maximum K of ten to ensure that we captured any clusters additional to those in our *a priori* groups. For each value of k, the burn-in was 100 000 with a final MCMC length of 1×10^6. A STRUCTURE analysis has previously been conducted on the red/black comparison in wild birds (Kim, 2011), so we do not present those results here. We compared the likelihoods (LnP(D)) and ΔK of assignment for clusters in each comparison using the output in Structure Harvester v0.6.94 (Pritchard *et al.*, 2000; Evanno *et al.*, 2005; Earl & VonHoldt, 2012). Q-plots that graphically depict the probability an individual belongs to a particular genetic cluster were generated using CLUMPP v1.1.2 and DISTRUCT v1.1 (Rosenberg, 2004; Jakobsson & Rosenberg, 2007). Because the Evanno method (2005) cannot distinguish between $k = 1$ and 2, a single genetic cluster was inferred when Q-plots were equally admixed and an $\text{LnP(D)}_{\max} = 1$.

Results

Parentage analysis

Across years, CERVUS was able to correctly predict the social mother 94.5% of the time at 95% confidence levels. The combined nonexclusion probability for these data sets for the first parent in 2008 and 2009 was 0.0008 and 0.0009, and 0.00002 for the second parent (see Table S3 for characteristics of the individual loci used). Of 257 offspring, 212 were identified as the offspring of the social parents identified in the field. Of these, 67 offspring showed a mismatch with their putative parents, but in all cases CERVUS assigned parentage to the social parents observed in the field. All these mismatches could be readily attributed to allelic dropout, as dropouts tended to run in families and matched the estimated rates of null alleles for each locus (Table S3). Of the nestlings that mismatched their putative father, 24 nestlings mismatched at one locus, and three nestlings mismatched at two; and 24 nestlings mismatched their putative mothers at one locus. Four nestlings mismatched both their parents at one locus, and twelve mismatched either maternal or paternal alleles at two loci.

There were 20 nestlings assigned as extra-pair offspring and these mismatched their social father at three or more loci (mean of 5.25 loci). Only one pair that renested was observed to have extra-pair paternity in both broods (Family 33). Five nests had multiple instances of extra-pair paternity within a clutch and, of these, three were from multiple extra-pair fathers (based on manually reconstructed genotypes). We were able to assign four (of 20) extra-pair offspring to fathers in our adult population pool, and three of these fathers matched the offspring at all loci (i.e. 10 of 10). One extra-pair father mismatched at one locus, but this could be attributed to allelic dropout. Of the observed first clutches with extra-pair paternity ($n = 10$) and IBP ($n = 9$), three of these contained both IBP and extra-pair offspring. Overall, the level of extra-pair paternity was found to be 22.8% of broods (13 of 57), representing 8.6% of offspring produced by the social mother (20 of 232).

There were eleven nestlings that mismatched both their social parents at more than one locus each, plus mismatched a mixture of maternal and paternal alleles at more than two other loci. These were considered to be the result of either intraspecific brood parasitism (IBP) or nest takeovers (where a new pair takeover a nest containing eggs laid by the female of the usurped pair) that were observed in the field (Brazill-Boast *et al.*, 2013a). IBP offspring mismatched the social mother at a mean of 4.5 loci and mismatched the social father at a mean of 6.1 loci. There was no evidence of pseudo-IBP or quasiparasitism, where the social father but not mother matches the offspring (Griffith *et al.*, 2004). Most observations of IBP were a single offspring per clutch, and no families had more than one clutch with any IBP offspring. Further, one family in each year had two IBP offspring; in each, less than four alleles were reconstructed and may represent offspring from the same parents. Two IBP offspring had both parents identified with no mismatches, and in one offspring the father was identified with no mismatches. Overall, the level of IBP was found to be 15.8% broods (9 of 57) representing 4.5% all offspring (11 of 243) (Table 1). See Appendix S3 for further exploration of the IBP data.

Determinants of extra-pair paternity

There was no evidence for any effect of either spatial constraints or breeding density on the incidence of

Table 1 Summary of the extra-pair paternity/offspring (EPP/O) and intraspecific brood parasitism (IBP) data in the Gouldian finch from 2008 to 2009 breeding season.

	Number families	Number Broods	Number WPO	Number EPO	EPO assigned fathers	Broods with EPP (%)	Offspring EP (%)	Average % Brood EP (± SE)*	Number IBPO	Broods with IBP (%)	Offspring IBP (%)
Overall	48	57	212	20	4	22.8	8.6	39.8 (± 6.4)	11	15.8	4.53
2008	26	27	108	7	0	18.5	6.1	30.3 (± 6.3)	7	22.2	5.74
2009	22	30	104	13	4	26.7	11.1	49.0 (± 10.0)	4	10.0	3.31
Mixed morph	12	17	51	7	1	35.3	12.1	35.4 (± 11.0)	4	23.5	6.45
$Z^RW \times Z^rZ^r$	4	6	14	5	1	66.7	26.3	36.1 (± 15.5)	2	33.3	9.52
$Z^rW \times Z^RZ^r$	8	11	37	2	0	18.2	5.1	33.3 (± 0)	2	18.2	4.88
Same morph	36	40	161	13	3	17.5	7.5	42.8 (± 8.5)	7	12.5	3.87
$Z^rW \times Z^rZ^r$	34	38	153	13	3	18.4	7.8	42.8 (± 8.5)	7	13.2	4.05
$Z^RW \times Z^RZ^r$	2	2	8	0	-	0	0	0	0	0.0	0.00

*Average calculated from only those broods with extra-pair paternity.

extra-pair paternity, and extra-pair sires were identified breeding themselves and nesting up to 4.28 km away from the nest in which they gained paternity (other males were 0.33 and 0.54 km away). There was no evidence that the number of pairs breeding or that time in the breeding season influenced patterns of extra-pair paternity. For more information, see Appendix S3.

Across pairing types, we compared the instances where females engaged in extra-pair paternity (≥ 1 extra-pair offspring). There was no difference between pairing types in the incidence of extra-pair paternity (4/12 vs. 6/36, Fisher's exact test, two-sided *P*-value = 0.24, Fig. 1a). However, the power to reject the null at the effect size observed in the wild was low ($h = 0.39$, power = 0.22), and we would have required a sample of 65 mixed pairs and 195 same-morph pairs to confidently conclude there was no difference. A sample size of 260 pairs represents about 20% of the estimated total adult population in the entire species (Garnett *et al.*, 2011). In the subset of birds from the domesticated study, there was no significant difference in whether a family had any instance of extra-pair paternity between mixed-morph pairs and same-morph pairs, although the effect size and power were much larger (8/10 vs. 4/10, Fisher's exact test $P = 0.17$, $h = 0.84$, power = 0.76, Appendix S3 (Pryke *et al.*, 2010a)). However, if the same biological effect size ($h = 0.84$) was present in the wild, we would have had much greater power to discriminate differences in extra-pair paternity between morphs (power = 0.72). Mixed pairs in the domesticated birds had a significantly higher incidence of extra-pair paternity than mixed pairs in the wild (two-tailed Fisher's exact test, $P = 0.043$, $h = 0.98$, power = 0.63).

We had insufficient power to determine whether black females in different pairing contexts had significant differences in extra-pair paternity to test Prediction 3a (1/8 vs. 6/34, Fisher's exact test, two-sided *P*-value = 1, $h = 0.14$, power = 0.066; Fig. 1b).

In the wild, there was no significant difference between mixed- and same-morph pairs in the proportion of the brood sired by an extra-pair male same morph (Mann–Whitney *U*-test: $W = 8$, $P = 0.45$), nor was there a difference when accounting for brood size (GLM: $\chi^2 = 0.073$, d.f. = 1 $P = 0.79$) (Fig. 1c; Prediction 1b). From those nests with extra-pair paternity, the number of extra-pair offspring in each pairing type did not differ (5/16 vs. 12/28 Fisher's exact test, two-sided *P*-value = 0.53, $h = 0.24$, power = 0.09). In mixed pairs, 31.3% of the offspring were sired by an extra-pair male, which is significantly less than the observed 75.0% of offspring observed in the domestic population (Fisher's exact test, two-sided $P = 0.002$, $h = 0.91$, power = 0.88). Further, total extra-pair offspring in same-morph pairs was higher (42.9%), but not significantly different from what was observed in the domesticated population (24.0%) (Fisher's exact test, two-sided $P = 0.245$, $h = 0.40$, power = 0.31) (Pryke *et al.*, 2010). In the domesticated birds, the biological effect size was four times greater (and in the opposite direction) than we observed in the wild, and for the total number of extra-pair offspring in pairing types, this effect size would have given ample power with our sample sizes ($h = 1.07$, power = 0.92).

Offspring morph

In the wild, we tested for an effect of morph on the paternity patterns of extra-pair offspring using the genotypes of the extra-pair offspring (Fig. 2; Prediction 1c). For the offspring of red females, goodness-of fit-tests were unable to reject that offspring were fertilized according to random mating, or according to a preference for red males, but our power was very low due to small sample size (extra-pair offspring from red female $n = 5$). From the genotypes of the extra-pair offspring we can conclude that none of their fathers were homozygote red males. There was no evidence of red

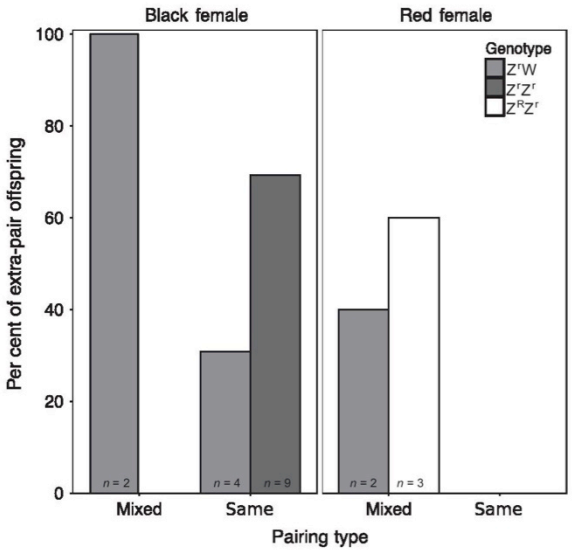


Fig. 2 The proportion of extra-pair offspring by morph genotype in each pairing type. Bar colours represent offspring morph genotype. The panes correspond to the maternal morph and bars correspond to offspring head colour genotype. Sample size of chicks is above the *x*-axis.

alleles in the extra-pair offspring of black morph mothers, and the three assigned extra-pair offspring from black morph mothers showed the father was also a black morph. Interestingly, the single remaining assigned offspring was from a red female in a mixed pair, but the father who was assigned was also a black morph male (matched 9/9 loci, LOD = 11.36, nest 4.28 km away).

We found little evidence that morph frequencies deviated from Mendelian expectations in WP offspring (Table 2; Prediction 3b), but power was low due to small sample sizes (sample sizes in Table 2). Contrary to our prediction, there was no significant reduction in heterozygote male offspring from red female and heterozygote red male parents (Table 2). There was deviation from Mendelian expectations in offspring of same-morph black pairs ($Z^rW \times Z^rZ^r$), where black morph females were more numerous than expected by chance (binomial test, obs = 0.61, exp = 0.5, $P = 0.011$).

Offspring sex ratio

There was no effect of same-morph vs. mixed-morph pairing types on brood sex ratio (GLM: $\chi^2 = 1.52$, d.f. = 1, $P = 0.29$), nor when considering all four pairing types observed (GLM: $\chi^2 = 2.65$ d.f. = 3 $P = 0.45$) (Fig. 3), therefore brood sex ratios were not distinguishable from parity.

Assortative pairing

In Wyndham, observed social pairs did not differ significantly from the pattern expected by random mating with respect to head colour when the adult morph frequencies were accounted for (16 mixed, 43 same morph: binomial test: expected = 0.30, $P = 0.67$, $h = 0.07$, power = 0.081), but were significantly different from estimates of perfect assortment (binomial test: expected = 0.14, $P = 0.0076$, $h = 0.32$, power = 0.71). There was no difference in the head colour of females or males represented in the breeding vs. the wider population (Fisher's exact test: $P = 1.0$, $P = 0.844$ for females and males, respectively).

Population genetics of morph phenotypes in domesticated and wild populations

None of the markers used to explore population structure in the wild and captive populations were consistently out of Hardy–Weinberg equilibrium, and allelic richness and heterozygosity estimates for each locus are presented in Table S4. Two pairs of loci were in linkage disequilibrium in the domesticated population after Bonferroni correction (Table S5). One locus from each pair was excluded and the analyses were run on eight loci. The summary rarefied allelic richness and

Table 2 The observed and expected frequency of offspring genotypes in different pairing types. *P*-values are the outputs from binomial tests or multinomial tests (more than two cases), and *w* is the effect size.

Maternal genotype	Paternal genotype	Number families	Number offspring	Expected/ Observed	Offspring genotype					*P*	*h*	Power
					Z^rW	Z^RW	Z^rZ^r	Z^RZ^r	Z^RZ^R			
Z^rW	Z^rZ^r	34	141	Expected	0.5	–	0.5	–	–	0.01	0.22	0.75
				Observed	86	–	55	–	–			
Z^rW	Z^RZ^r	8	31	Expected	0.25	0.25	0.25	0.25	–	0.55	0.26	0.20
				Observed	6	8	6	11				
Z^RW	Z^rZ^r			Expected	0.5	–	–	0.5	–	0.55	0.27	0.15
		4	11	Observed	7			4	–			
Z^RW	Z^RZ^r			Expected	0.25	0.25	–	0.25	0.25	0.48	0.62	0.25
		2	7	Observed	3	3		0	2			

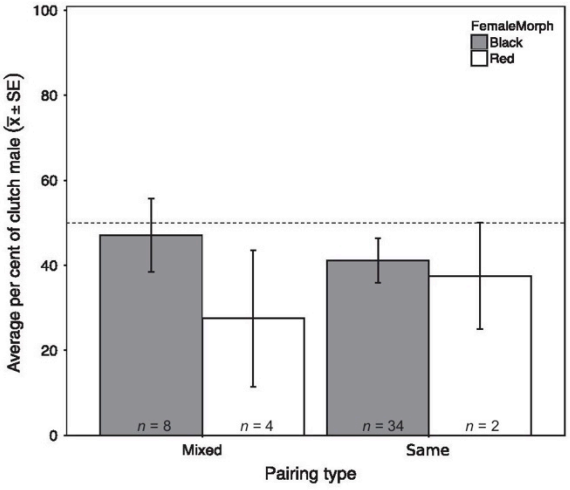


Fig. 3 Average sex ratio per clutch according to pairing type, where bar coloration represents the maternal morph. Number of clutches are above the *x*-axis.

heterozygosity estimates for reds and blacks in the domesticated and wild population are presented in Table 3. There was a significant reduction in allelic richness in the domesticated population compared with the wild population (paired two-tailed *t*-test, $t = -2.823$, d.f. = 9, $P = 0.02$), but no significant difference between expected heterozygosity (two-tailed paired *t*-test, $t = -1.95$, d.f. = 9, $P = 0.08$) and observed heterozygosity ($t = -1.81$, d.f. = 9, $P = 0.10$).

In the domesticated population, red and black were slightly, but significantly differentiated from each other (AMOVA $F_{ST} = 0.041$, $P = 0.00079$), but there was no evidence of differentiation between morphs in the wild in either the reduced data set (AMOVA $F_{ST} = -0.0038$, $P = 1$) or the full data set (AMOVA $F_{ST} = 0.0040$, $P = 0.31$). In the domesticated population, the clustering analysis indicated a $\Delta K_{max} = 2$, and LnP(D)max was also $K = 2$ (Fig. 4a). There was slight but highly significant differentiation between the wild and domesticated populations ($F_{ST} = 0.016$, $P = 0.0015$). The clustering analyses including the wild and domesticated populations indicated that $\Delta K_{max} = 2$ LnP(D)max = 4. We compared the Q-plots for $K = 4$ (Fig. S5) and $K = 2$ (Fig. 4b), and it showed that the two additional clusters in $K = 4$ were restricted entirely to the domesticated population, which is consistent with hierarchical structure and with the result observed in Fig. 4a.

Discussion

The observed rate of extra-pair paternity in the Gouldian finch (22.8% of broods, 8.6% of offspring) is relatively low and less than the mean frequency found across socially monogamous bird species (11% of offspring; Griffith *et al.*, 2002). These estimates of extra-pair paternity are not different from those observed in another Australian Estrildid finch, the long-tailed finch (*Poephila acuticauda*) (12.8%) (Rooij *et al.*, 2016), but are different from the frequency observed in the zebra finch (*Taeniopygia guttata*) (2%) (Birkhead *et al.*, 1990; Griffith *et al.*, 2010). Intraspecific brood parasitism observed in the Gouldian finch is within the range observed in these same Estrildid finches (0.51–10% of offspring) (Appendix S3). In the wild population, females appeared not to be constrained by nesting synchrony or density in their ability to gain extra-pair paternity (Appendix S3), and some offspring were sired by extra-pair males breeding up to 4.2 km away which is within the normal daily movement previously observed in the Gouldian finch (Woinarski & Tidemann, 1992; K. Maute Pers Comm). This is in contrast to highly territorial bird species where distance to copulate and overall breeding synchrony can influence patterns of extra-pair paternity (e.g. Canal *et al.*, 2012; Garcia-Navas *et al.*, 2014).

We found little evidence to suggest that patterns of extra-pair paternity across Gouldian finch morph pairings were related to amelioration of genetic incompatibility, as predicted by work on domesticated birds (Pryke & Griffith, 2009b; Pryke *et al.*, 2010). Relatively small sample sizes, particularly of the key mixed-morph pairs, reduced the power of our tests. However, the biological effect size seen in earlier studies of

Table 3 Summary of genetic diversity indices using eight loci for red and black domesticated birds and a sample size-matched subset of the wild birds. *N* is the number of individuals in each category; N_{Ar} is the rarefied allelic richness; H_o is the observed heterozygosity ± standard deviation; H_E is the expected heterozygosity ± standard deviation; F_{IS} is the inbreeding coefficient, none of which were statistically significant.

Population	*N*	N_{Ar}	H_o	H_E	F_{IS}
Domesticated	48	9.75 (± 5.80)	0.72 (± 0.14)	0.75 (± 0.14)	0.04
Red	16	6.26 (± 3.78)	0.69 (± 0.15)	0.74 (± 0.13)	0.06
Black	32	7.50 (± 4.10)	0.73 (± 0.14)	0.73 (± 0.16)	−0.01
Wild	48	11.86 (± 7.66)	0.75 (± 0.16)	0.77 (± 0.15)	0.01
Red	16	8.76 (± 5.43)	0.74 (± 0.20)	0.74 (± 0.20)	0.01
Black	32	8.60 (± 4.91)	0.75 (± 0.14)	0.77 (± 0.16)	0.01

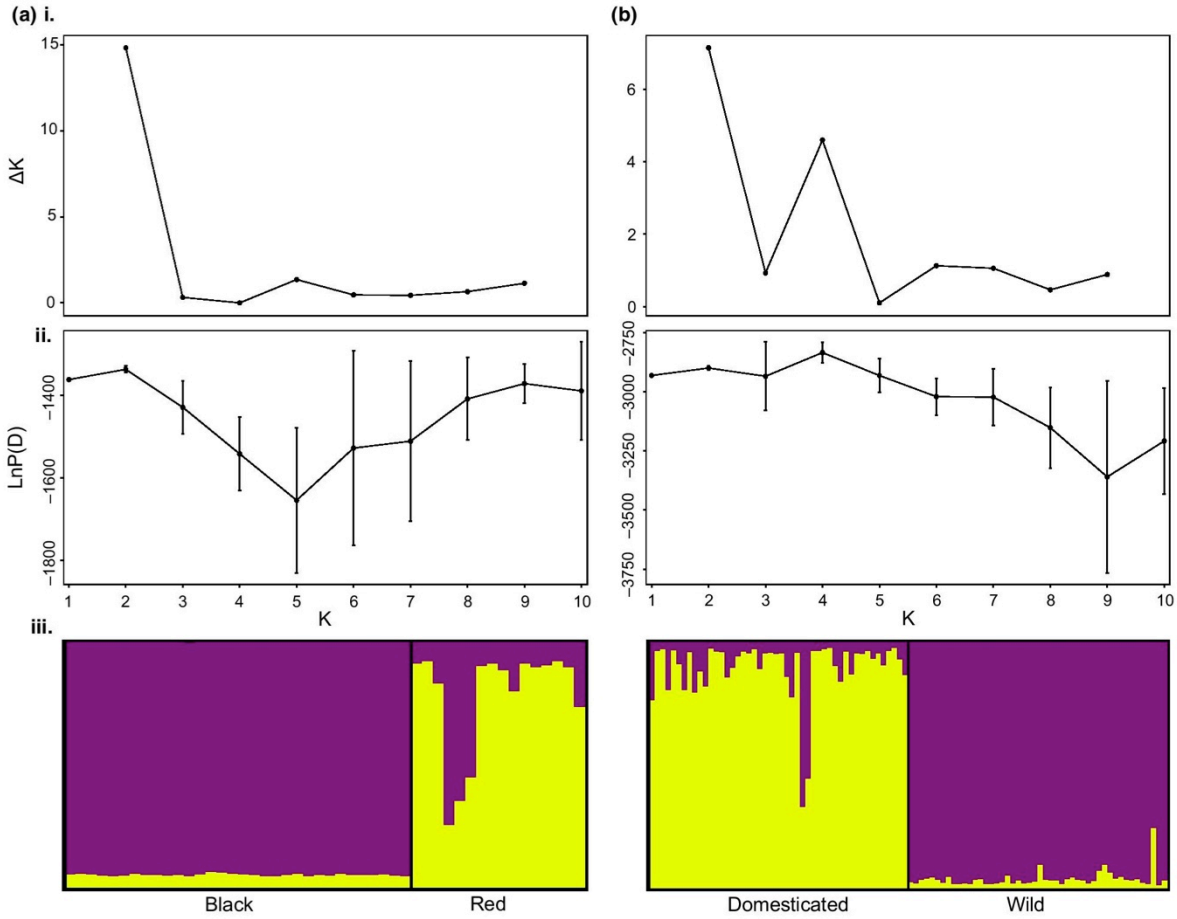


Fig. 4 Results of Bayesian genetic clustering analyses using STRUCTURE. (a) Analysis including domesticated red and black birds: (i)ΔK plot of the most likely number of genetic clusters in the sample where $\Delta K_{max} = 2$, (ii) log posterior probabilities (LnP(D)) for the number of clusters in the data set, where $K = 2$ is the most likely cluster; (iii) Q-plot for $K = 2$. (b) Analysis including domesticated and wild birds: (i) ΔK plot showing the most likely number of clusters is 2 (ΔK_{max}), (ii) log posterior probabilities (LnP(D)) for the number of clusters in the data set, where $K = 4$ is the most likely cluster;(iii) Q-plot for $K = 2$, showing distinct clusters for captive and wild populations. Q-plot for $K = 4$ is shown in Fig. S5.

domesticated birds was very strong and such a strong effect should have been apparent even with our sample size. Further, to achieve a sample size necessary to test the effect sizes observed in this study, we would have to sample approximately 20% of the adult population, which is estimated to be 2500 individuals (Garnett *et al.*, 2011).Across pairing types in the wild, we consistently observed fewer incidences of extra-pair paternity and an equivalent number of extra-pair offspring to domesticated females in pure pairs. Furthermore, there was no evidence of bias in the head colour of extra-pair or within-pair offspring despite the prediction that paternity would be biased towards compatible males and show a reduction in heterozygous red offspring. Another prediction for incompatibility based on the domesticated population was that there would be a male bias in the brood sex ratio produced by females in mixed-morph pairs, given sex allocation and stronger female-specific mortality costs of genetic incompatibility (Pryke & Griffith, 2009a,b). However, we found there was no significant difference in day 14 offspring sex ratio between pairing types. Together with previous observations of no difference in offspring survival in the wild, we suggest that the genetic incompatibility previously observed in the domesticated population is weaker, or absent, in the wild (Pryke & Griffith, 2009b; Brazill-Boast *et al.*, 2013a).

In further contrast with findings in the domesticated birds, we found no evidence of assortative mating by

head colour morph. This contrasts with an earlier report of assortative mating in a population at Mornington, in the central Kimberley, WA (~ 300 km away) (Pryke & Griffith, 2007). However, in their study, Pryke & Griffith (2007) did not survey a wider sample of adults to sample the total pool of possible mates as we have done in this study. In addition, pairs in the earlier study were possibly pseudo-replicated (only 12 of 59 adults were uniquely identified by bands), as it was assumed that each water hole (where families were identified) had a unique complement of individuals (Pryke & Griffith, 2007). We know now from our subsequent work at Wyndham that the same individuals (identified uniquely by leg bands) will be sighted at different waterholes on different days (JBB, SCG Pers Obs), and the mobility of adults is also supported by the distance over which extra-pair paternity is gained. However, observed patterns of mating in the wild may not represent true mate preferences. For example, the choice of social partners may be constrained by inherent competitive interactions between head colour morphs, as seen in white-throated sparrows (Houtman & Falls, 1994). Competitive interactions are likely relevant to Gouldian finch mate choice, because Brazill-Boast *et al.* (2013a) found that red-headed males secured the best quality nesting cavities – an important and limited resource (Brazill-Boast *et al.*, 2010, 2011). Indeed, female pied flycatchers (*Ficedula hypoleuca*) choose partner according to resource quality (Alatalo *et al.*, 1986), so female Gouldian finches might choose partners on resource quality, or trade-off between head colour and resource quality. Even if there are constraints on the 'ideal' mate choice, these constraints should be alleviated or completely removed by selection if the cost of mating with a suboptimal partner is high enough (Brooks & Griffith, 2010). Our results suggest that mating with a partner of a different head colour in the wild may not be as costly to reproductive success as in the domesticated population (Pryke & Griffith, 2009b).

In the wild, the selection on head colour polymorphism and mate choice may be different from domesticated birds. This has been demonstrated in other systems, such as the colour polymorphic painted dragon (*Ctenophorus pictus*). Under experimental conditions, colour morphs exhibited alternative reproductive strategies, but these were not realized in the wild due to the constraints of habitat structure (Olsson *et al.*, 2007). In the zebra finch, the frequency of extra-pair paternity was significantly higher in multiple domesticated birds than in wild populations (Griffith *et al.*, 2010; Forstmeier *et al.*, 2011). Furthermore, wild female zebra finches show different mate choice preferences to their domesticated counterparts (Rutstein *et al.*, 2007). Therefore, these differences in polyandry and mating patterns in the Gouldian finch may be the result of the domestication process itself or may reflect the more complex factors faced in the wild that were removed from the experiment, such as a constraint on extra-pair copulation through mate guarding (Komdeur *et al.*, 1999; Pryke *et al.*, 2010). We have presented results from the experiments on domesticated birds as a guide to the effect size expected between mixed- and same-morph pairs in the wild, but we fully appreciate that differences in experimental design make these studies difficult to compare directly. Therefore, although we cannot conclude unequivocally that there was no difference in incompatibility amelioration strategies between pairing types, we can conclude the effect was not as strong as observed in the domesticated birds.

The final key prediction is that incompatibility will be associated with a restriction of gene flow between the morphs. Accordingly, we find evidence of genetic differentiation between morphs in captivity, where the effects of incompatibility have been demonstrated (Pryke & Griffith, 2009b). In contrast, previous work found there was no evidence of genetic differentiation between morphs in the wild (Kim, 2011), which further strengthens the evidence for weak or absent incompatibility derived from the paternity and sex allocation data. Although we took steps to minimize the inclusion of highly related individuals, the presence of family groups may overestimate population structure (Rodríguez-Ramilo & Wang, 2012). Indeed, we found some evidence that there was substructuring beyond head colour morphs in our STRUCTURE analysis, which may represent family lineages from the original captive sources. Although the prediction is that incompatibility will be associated with genetic differentiation, the differentiation observed here is probably not caused by incompatibility, *per se*. It is more likely that the incompatibility arose due to differentiation between morphs, in small and isolated domesticated populations. Indeed, Gouldian finch breeders are often interested in establishing exaggerated traits for competitive avicultural shows, and their general management practices include the maintenance of same-morph red and black families to enhance plumage colour traits (Evans & Fidler, 2005; Hoffman *et al.*, 2014). If domesticated Gouldian finch morphs have consistently bred separately, then there is potential to exaggerate morph-specific traits and the associated agonistic interactions (Rice & Holland, 1997; Hesketh *et al.*, 2013; Pennell & Morrow, 2013). Experiments on *Drosophila* have shown that when females are not allowed to co-evolve with male sexual strategies, subsequent female generations will rapidly experience reduced fitness in response to matings (Rice, 1996). This, in combination with artificial selection, and perhaps bottlenecks, may generate the conditions necessary to establish incompatibilities between head colour morphs. Our analyses of microsatellite variation demonstrated some of the expected genetic differences between the populations of domesticated and wild Gouldian finches. We found that there was a significant reduction in allelic richness (but

not heterozygosity) in the domesticated population, which is expected to accompany a reduction in effective population size. Bottlenecks and artificial selection that accompany domestication can have profound effects on the genome (e.g. Montague *et al.*, 2014) and can alter genetic variation and the strength and direction of trait correlations (Bryant & Meffert, 1988; Haudry *et al.*, 2007). Therefore, we propose that the population history of the domesticated Gouldian finch may have driven the genetic incompatibility observed in the earlier studies (although this remains to be further examined in detail).

Our evidence from patterns of extra-pair paternity, sex ratio allocation, assortative mating and population structure in the wild is inconsistent with predictions about intermorph incompatibility that was observed in domesticated Gouldian finches. The effects seen in the domesticated birds could be a unique result of the domestication process and/or stochastic processes that have resulted from breeding small populations. If this is the case, the domesticated Gouldian finch provides an opportunity to investigate the emergence of genetic incompatibilities and sexual and intermorph conflict. The time is ripe to investigate these questions, as the rapid influx of genomic resources is allowing us to detect signatures of selection and genetic conflict in all manner of organisms (Parsch & Ellegren, 2013). Genomic comparison of the head colour polymorphism in both wild and captive birds will yield insights into the role of sex chromosomes in genetic conflict and speciation (Qvarnström & Bailey, 2009). Thus, comparisons between domesticated and wild Gouldian finches may provide an interesting future model into the evolution of colour polymorphism and genetic incompatibilities.

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Supporting information

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Data deposited at Dryad: doi: 10.5061/dryad.2m2r2

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Appendix S1: Sampling of families at Wyndham

In 2008-2009 there were 144 observations of Gouldian finch nests, of 124 adults observed to breed, 61 pairs were verifiably unique based on band number (59 pairs with genotyped blood samples). A summary of the sampling in this paper is provided in Table S1, where the final sample used to estimate extra-pair paternity was from 48 families. We also sampled an additional 16 eggs across 14 nests, and two clutches of 11 eggs. Because nest takeover rates were high (Brazill-Boast *et al.* 2011b), and sampling of eggs was not even between nests, we only included the 14 day old nestlings for our estimates of extra-pair paternity . The larger dataset of breeding observations was used to analyse the spatial and temporal constraint in extra-pair paternity (Supplement 3). There were more uniquely identified breeding pairs (n=59) than pairs with nestling bloods used in the paternity analysis (n=51). Therefore, for the analysis of assortative mating we used the larger dataset of 59 pairs.

Table S1: Sample sizes for breeding used in this study, where a) describes the overall breeding dataset, and b) describes the sample of nests for which parents and 14 day old offspring were sampled. Divorce was defined bas whether one or both in a pair were observed to renest with a different partner during the same year. Re-pairing was defined as whether an individual had renested with another partner in the following year, and the partner from the previous year was not observed again.

a)	Total observations	Breeding females	Breeding males	Number re-laid	Number divorced	Number re-paired
2008	82	38	37	9	2	-
2009	62	25	24	10	1	5
b)	**Sampled Families**	**Eggs**	**Number re-laid**	**Number nestlings**	**Excluded families**	**Excluded nestlings**
2008	28	11	1	130	2	8
2009	23	16	6	127	1	5

Appendix S2: Laboratory methods and genetic analyses

Sex-typing:

All offspring were sex typed using the primers developed for the CHD locus (Fridolfsson & Ellegren 1999). The PCR reactions were 8uL comprising 1uL template DNA, 3.2 uL of Qiagen Multiplex Mix, 3uL Water and 0.8uL of primers to a final concentration of 0.18uM. Thermal Cycler conditions were 94°C for 15min; 3 cycles of 94°C, 60°C and 72°C for 30sec each; 30 cycles of 94°C, 55°C, 72°C for 30sec each; and a final extension of 72°C for 10min. PCR products were visualised on a 2.5% agarose gel.

Multiplexes:

Microsatellite markers used for this study were as in the captive study (Pryke *et al.* 2010), but were arranged into different multiplexes (Table S1). Although all the same markers were included, two of these did not amplify reliably in the wild and were removed from the final analysis (ten markers). These multiplexes also included the PCR assay for head-colour at locus Ego172. This marker differentiates red or black allele based on the dye label, and a 3bp size difference in the allele specific primers. This makes it readily visualised in an electropherogram alongside other microsatellite markers. There was one individual that conflicted at this locus with the observed phenotype, and was a black female that was genotyped as a red (Kim 2011). Field observations wild females have observed 'black' females but with varying amounts of red flecking through the black mask. We suspect that these birds are genotypically red, and this is the focus of ongoing research. This "mis-typed" female was not observed to breed.

Samples were run at Macrogen Inc. on an ABI 3730 machine using a GS-500 LIZ size standard. Genotypes were scored using GeneMapper 3.7 (Applied Biosystems, Foster City, CA, U.S.A.).

All 257 14 day old offspring, and 246 adults were included in the paternity analysis in CERVUS. Output from CERVUS describing the information content of each locus used is presented in Table S2.

For the analysis of genetic differentiation according to head colour morph in domesticated and wild birds, we analysed used the same marker sets and analysed deviation from Hardy-Weinberg equilibrium in ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010). No locus was consistently out of Hardy-Weinberg equilibrium in our populations after Bonferroni correction for multiple testing (Table S4).

Linkage disequilibrium between markers was assessed using GENEPOP v4.2 (Raymond & Rousset, 1995). After correction for multiple testing no two loci were in linkage disequilibrium (Table S5).

Table S2: Primers and multiplexes used in this study. Final concentrations (uM) are per forward and reverse primer, except the Ego172 locus, which details each of three primers separately.

Marker	Dye	Multiplex	uM	Reference
Pco2	6FAM	1	0.142	(Saito *et al.* 2001)
Pca7	VIC	1	0.532	(Dawson *et al.* 2000)
Cuu4	NED	1	0.212	(Gibbs *et al.* 1998)
Ind41	6FAM	1	0.638	(Forstmeier *et al.* 2007)
Titgata2	PET	2	0.334	(Wang *et al.* 2005)
Ind28	6FAM	2	0.234	(Sefc *et al.* 2001)
Ego172_Rblack	6FAM	2	0.1	(Kim 2011)
Ego172_Rred	VIC	2	0.1	-
Ego172_Fa	n/a	2	0.1	-
Tgu11	VIC	3	0.1	(Forstmeier *et al.* 2007)
Ind37	VIC	3	0.8	(Sefc *et al.* 2001)
BF18	6FAM	3	0.2	(Yodogawa *et al.* 2003)
Ase24	6FAM	3	0.334	(Richardson *et al.* 2000)

Table S3: Characteristics for each locus used in this study, as calculated for all adults and nestlings in those years. Where N_A is the number of alleles, and the final row shows alleles per locus; N is the number of individuals typed at that locus and the final row is total number of typed individuals; H_O and H_E are the observed and expected heterozygosity, final row is the mean; PIC is the polymorphic information content; NE-1P/2P is the non-exclusion probability for the first and second parent, where the final row is the combined non-exclusion probability; Null is the estimate of null alleles

a)					2008		
Locus	k	H_O	H_E	PIC	NE-1P	NE-2P	Null
Ase24	9	0.59	0.70	0.65	0.71	0.54	0.08
BF18	15	0.73	0.77	0.74	0.61	0.43	0.02
Ind37	21	0.88	0.91	0.90	0.31	0.19	0.01
Tgu11	8	0.60	0.58	0.52	0.82	0.68	-0.02
Cuu4	19	0.79	0.84	0.83	0.48	0.31	0.04
Ind28	8	0.73	0.73	0.69	0.68	0.49	-0.01
Ind41	40	0.90	0.95	0.94	0.20	0.11	0.03
Pca7	24	0.90	0.92	0.91	0.28	0.16	0.01
Pco2	5	0.59	0.58	0.51	0.83	0.69	-0.01
Titgata02	20	0.86	0.86	0.84	0.45	0.29	0.00
Mean	16.90	0.76	0.78	0.75	0.0008	0.00002	-
b)					2009		
Locus	k	H_O	H_E	PIC	NE-1P	NE-2P	F
Ase24	7	0.53	0.65	0.60	0.76	0.59	0.11
BF18	11	0.71	0.75	0.72	0.63	0.45	0.03
Ind37	16	0.77	0.90	0.89	0.34	0.21	0.07
Tgu11	6	0.58	0.56	0.51	0.83	0.68	-0.02
Cuu4	14	0.71	0.83	0.80	0.51	0.34	0.08
Ind28	9	0.75	0.75	0.72	0.64	0.46	-0.01
Ind41	32	0.95	0.94	0.94	0.21	0.12	-0.01
Pca7	22	0.95	0.93	0.92	0.26	0.15	-0.01
Pco2	5	0.65	0.61	0.52	0.81	0.68	-0.03
Titgata02	15	0.90	0.85	0.84	0.46	0.29	-0.03
Mean	13.70	0.75	0.78	0.75	0.0009	0.00002	-

Table S4: Diversity of microsatellite loci used for population genetic analysis in the 48 samples in domesticated and 245 wild samples: (a) red and black morphs in the domesticated sample (b), and red and black morphs in the wild sample (c). Where NA is the number of alleles, H_O and H_E are the observed and expected heterozygosity, and P is the p-value for deviation from Hardy-Weinberg equilibrium, were bolded values are significant at 0.05. Only Pca7 in domesticated black birds was out of HWE after Bonferroni correction ($p<0.002$), and was kept in the analysis because it was not consistent across populations.

a)	Domesticated				Wild			
Locus	N_A	H_O	H_E	P	N_A	H_O	H_E	P
Ase24	6	0.69	0.58	0.663	8	0.54	0.67	0.068
BF18	9	0.67	0.79	0.091	12	0.68	0.78	**0.009**
Ind37	14	0.81	0.85	0.107	14	0.87	0.91	0.352
Tgu11	5	0.58	0.61	0.901	5	0.68	0.57	0.412
Cuu4	8	0.71	0.74	**0.010**	14	0.80	0.86	0.646
Ind28	6	0.65	0.68	0.841	8	0.79	0.77	0.197
Ind41	18	0.96	0.92	0.193	26	0.96	0.96	0.870
Pca7	17	0.83	0.92	0.024	19	0.92	0.93	**0.023**
Pco2	3	0.54	0.60	0.051	3	0.56	0.58	0.576
Titgata2	12	0.77	0.87	0.381	13	0.88	0.87	**0.019**
Mean (± SD)	9.8 (± 5.2)	0.72 (± 0.12)	0.76 (± 0.13)		12.2 (± 6.8)	0.77 (± 0.14)	0.79 (± 0.14)	
b) Domesticated	Red				Black			
Locus	N_A	H_O	H_E	P	N_A	H_O	H_E	P
Ase24	3	0.69	0.60	0.745	5	0.69	0.57	0.584
BF18	5	0.56	0.71	0.145	8	0.72	0.81	0.328
Ind37	10	0.75	0.87	0.148	13	0.84	0.83	0.490
Tgu11	3	0.63	0.57	0.495	5	0.56	0.61	0.696
Cuu4	6	0.81	0.67	**0.011**	7	0.66	0.77	**0.041**

Ind28	4	0.60	0.74	0.596	6	0.68	0.64	0.924
Ind41	12	0.94	0.91	0.403	16	0.97	0.90	0.366
Pca7	11	0.88	0.88	0.783	16	0.81	0.94	**0.002**
Pco2	3	0.50	0.67	0.301	3	0.56	0.51	0.630
Titgata2	8	0.69	0.85	0.031	12	0.81	0.86	0.926
Mean (± SD)	6.5 (± 3.5)	0.70 (± 0.14)	0.75 (± 0.12)		9.1 (± 4.8)	0.73 (± 0.13)	0.74 (± 0.15)	
c) Wild		Red				Black		
Locus	N_A	H_O	H_E	P	N_A	H_O	H_E	P
Ase24	5	0.50	0.67	**0.030**	7	0.56	0.66	0.395
BF18	7	0.56	0.71	0.071	11	0.74	0.81	**0.046**
Ind37	11	0.93	0.92	0.421	14	0.84	0.92	0.535
Tgu11	5	0.75	0.62	0.760	4	0.65	0.56	0.776
Cuu4	10	0.80	0.86	0.971	12	0.81	0.87	0.537
Ind28	7	0.75	0.71	0.433	8	0.81	0.80	0.119
Ind41	19	0.93	0.95	0.277	24	0.97	0.95	0.883
Pca7	16	1.00	0.95	0.779	17	0.88	0.93	**0.019**
Pco2	3	0.50	0.62	0.373	3	0.59	0.57	0.829
Titgata2	9	0.88	0.83	0.093	13	0.88	0.89	**0.037**
Mean (± SD)	9.2 (± 5.1)	0.76 (± 0.18)	0.78 (± 0.13)		11.3 (± 6.3)	0.77 (± 0.13)	0.80 (± 0.15)	

Table S5: Results from linkage disequilibrium analysis conducted in GenePop, for 244 wild (a) and 48 domesticated samples (b). Bolded p-values are those significant at $p<0.05$, Cuu4xPca7 and Ind41xTitgata02 were significantly in linkage disequilibrium after Bonferroni correction ($p<0.001$, marked by asterisk*).

a) Wild			b) Domesticated		
Locus 1	Locus 2	P-Value	Locus 1	Locus 2	P-Value
Ase24	BF18	**0.048**	Ase24	BF18	0.629
Ase24	Ind37	0.419	Ase24	Ind37	0.719
BF18	Ind37	0.349	BF18	Ind37	0.519
Ase24	Tgu11	0.236	Ase24	Tgu11	0.218
BF18	Tgu11	0.350	BF18	Tgu11	0.647
Ind37	Tgu11	0.100	Ind37	Tgu11	0.845
Ase24	Cuu4	0.120	Ase24	Cuu4	0.087
BF18	Cuu4	0.549	BF18	Cuu4	0.364
Ind37	Cuu4	0.366	Ind37	Cuu4	0.873
Tgu11	Cuu4	0.815	Tgu11	Cuu4	0.651
Ase24	Ind28	0.380	Ase24	Ind28	0.365
BF18	Ind28	0.569	BF18	Ind28	0.820
Ind37	Ind28	0.095	Ind37	Ind28	0.664
Tgu11	Ind28	0.220	Tgu11	Ind28	0.425
Cuu4	Ind28	0.179	Cuu4	Ind28	**0.005**
Ase24	Ind41	0.411	Ase24	Ind41	0.502
BF18	Ind41	0.268	BF18	Ind41	0.309
Ind37	Ind41	1.000	Ind37	Ind41	**0.013**
Tgu11	Ind41	**0.037**	Tgu11	Ind41	0.706
Cuu4	Ind41	0.450	Cuu4	Ind41	1.000
Ind28	Ind41	0.286	Ind28	Ind41	0.356
Ase24	Pca7	0.369	Ase24	Pca7	0.364
BF18	Pca7	0.745	BF18	Pca7	0.055
Ind37	Pca7	0.227	Ind37	Pca7	0.409
Tgu11	Pca7	0.375	Tgu11	Pca7	0.823
Cuu4	Pca7	0.243	Cuu4	Pca7	**0.000***
Ind28	Pca7	0.515	Ind28	Pca7	0.871
Ind41	Pca7	**0.038**	Ind41	Pca7	0.183
Ase24	Pco2	0.782	Ase24	Pco2	0.514
BF18	Pco2	0.196	BF18	Pco2	0.652
Ind37	Pco2	0.104	Ind37	Pco2	0.289
Tgu11	Pco2	0.040	Tgu11	Pco2	0.732
Cuu4	Pco2	0.269	Cuu4	Pco2	0.459
Ind28	Pco2	0.190	Ind28	Pco2	0.726
Ind41	Pco2	0.730	Ind41	Pco2	**0.004**
Pca7	Pco2	0.441	Pca7	Pco2	0.112
Ase24	Titgata02	0.345	Ase24	Titgata02	0.936
BF18	Titgata02	0.626	BF18	Titgata02	0.291
Ind37	Titgata02	0.923	Ind37	Titgata02	0.277
Tgu11	Titgata02	0.539	Tgu11	Titgata02	0.098

a) Wild			b) Domesticated		
Locus 1	Locus 2	P-Value	Locus 1	Locus 2	P-Value
Cuu4	Titgata02	0.852	Cuu4	Titgata02	0.881
Ind28	Titgata02	0.153	Ind28	Titgata02	0.276
Ind41	Titgata02	0.210	Ind41	Titgata02	**0.000***
Pca7	Titgata02	0.996	Pca7	Titgata02	0.054
Pco2	Titgata02	0.991	Pco2	Titgata02	0.619

Appendix S3: Determinants of Extra-Pair Paternity & Intra-specific brood parasitism

Methods

Spatial and temporal opportunity

Spatial and temporal opportunities for extra-pair copulations can be an important determinant of extra-pair paternity in other bird species (Griffith *et al.* 2002). To examine whether females were spatially or temporally constrained in their choice of extra-pair partners we calculated whether the density and synchrony of active nests affected the rate of extra pair paternity. As such we also examined whether females were constrained by the density and synchrony of males with compatible head-colour. For every nest in the sample, we calculated synchrony as the number of concurrent sexually active nests based on an estimated male and female finch fertile period. This was derived from the total sample of nesting observations, which is larger than those we had genetic samples for (families with genetics; n=51; families with parents but no nestling genetics, n = 29; total nesting attempts, n = 144), but the final pool used for testing extra-pair only includes families where there is genetic data for chicks. In this analysis we focused on each individual breeding observation, and the "fertile period" was considered the window in which sperm from a copulating male could have been included in the focal clutch (and produce our observations of paternity). In females, this period begins eleven days prior to laying the first egg, and is based on viable storage of about ten days in the Zebra finch (Birkhead *et al.* 1989). Female fertile period ended six days after the first egg and allows for fertilization of the sixth egg the morning before it was laid, and by mid-incubation follicles and oviducts regress (L.L Hurley Pers Comm.). The sixth day was chosen based on

the average clutch size in the dataset (5.98), but we chose not to use the focal female's observed clutch size to inform this window because individual clutches may be inflated because of frequent nest take-overs between Gouldian and long-tailed finches (Brazill-Boast *et al.* 2011b), and intra-specific brood parasitism. Outside strictly photo-periodic birds, little is known about the production and viability of sperm across a single reproductive episode, but we assumed that their reproductive capacity would decline when eggs hatched and they had to invest in raising chicks. Therefore, we set the male reproductive period (to derive the pool of synchronous nests) as -15 days prior to first egg, and 20 days after first egg. The caveat for all these calculations is that Australian Estrildid finches are multi-clutch breeders so breeding periods are not as strictly definable as most Northern Hemisphere photoperiodic breeding birds. Therefore, although we define computationally hard-bounds for the male and female reproductive periods in this analysis, in reality they are probably flexible soft bounds. Therefore, the calculated number of reproductively synced birds should be considered a minimum value, and also does not consider reproductively active "floater" males (e.g. Smith 1978).

From the nests that were found to be 'active' at the same time as the focal nest, we calculated a number of spatial variables to measure the spatial opportunity for extra-pair copulations. We calculated the minimum distance a female would have to travel for an extra-pair copulation (the nearest nest), and the average distance between the focal nest and all other 'synchronous' nests. We calculated these same parameters but only including the nests with a male with a compatible head-colour.

We also explored whether extra-pair paternity was related to time in breeding season. This is because Gouldian finch productivity changes throughout a season (Tidemann *et al.* 1999), thus access to mates and appropriate resources to rear chicks will change with time. We compared the incidence of extra-pair paternity in a clutch with nest initiation (day of first egg) time within breeding season, with observations over 123 days. To reduce dimensionality

in this small dataset, we also divided the breeding period into 41-day bins of "early", "middle" and "late" breeding season.

Statistics

There were too few re-nesting attempts within the dataset to reliably run a mixed model to test the effects of synchronicity and density, so in order to retain independent observations necessary for a generalized linear model (GLM), we kept only the first clutch where chicks were bled to retain only independent observations. The effects of synchronicity, density and time of season were tested using logistic regression against the presence/absence of extra-pair paternity as a binary response. The proportion of extra-pair offspring at a given nest (binomial count response) were first run with a binomial model and checked for over-dispersion and goodness of fit using a deviance goodness-of-fit test. If over-dispersed ($\hat{\psi}$>1 and a poor model fit) we corrected for this in a quasi-likelihood glm framework by specifying "quasibinomial" as the model family. This correction for over-dispersion using the quasi-likelihood framework gives a more realistic and estimate of the model coefficient standard errors. Predictor significance was for binary models were assessed formally using likelihood ratio test, and for quasibinomial models using the F-test in the *drop1* function.

By pairing type in captivity:

In captivity Pryke *et al.* (2010) found a striking effect where a large portion of offspring could be sired by an extra-pair compatible male, from just a single copulation. Although they stated there was no difference in the propensity of female morphs to copulate with an extra-pair male in different contexts, they never explicitly tested the binary outcome of whether a female had any extra-pair young with regard to pairing context and experimental treatment (Pryke *et al.* 2010). Because presence of extra-pair paternity was a metric we used in this paper, we explored whether there was an effect at this level in captivity by mining the data presented in Figure 1 of Pryke *et al*. (2010). Because in the wild we cannot measure how many actual copulations there are with extra-pair males, we just included overall number of clutches with any EPO of ALL families in each treatment. Furthermore, in our paper, we only

considered the rates of EPO in mixed pairs in the adaptive treatment (n=10), and the pure pairs in the neutral treatment (n=10) to be relevant comparison, because we assumed that females would not be limited in the number of copulations in the way captive birds were, so females in mixed pairs could gain more adaptive copulations/fertilizations. We compared the number of families with extra-pair paternity in the mixed and pure pairs by Fisher's Exact Test.

Intra-specific Brood Parasitism

We replicated the analyses conducted in the main paper section "Determinants of extra-pair paternity" with the rates of Intra-specific Brood Parasitism (IBP), and predict that mixed morph pairs ought to have higher rates of IBP. This prediction arose because in captivity individuals in incompatible pairs experience higher stress levels and provision their offspring less frequently (Pryke & Griffith 2009, 2010; Griffith *et al.* 2011), therefore they might be less able to defend against intra-specific brood parasites in the wild. Moreover, in the wild, red males were found to occupy higher quality nest sites and experience more interference from conspecifics and sympatric long-tailed finches, but were more capable of defending their nesting resource (Brazill-Boast *et al.* 2011b, 2013). Therefore, we predict there to be more IBP in nests with black morph fathers. So we additionally tested with a Fisher's exact test whether there was an overall morph effect as well as by pairing context.

Results & Discussion:

Temporal and spatial opportunity

There was no effect of either measure of nest synchronicity or density on the presence of extra-pair paternity (Table S6a), nor on the number of extra-pair offspring (Table S6a). All binomial models describing the proportion of extra-pair offspring were overdispersed and were all modelled with quasibinomial models. There was no effect of synchronicity or distance to males of compatible head colour on either measure of extra-pair paternity (Table S6b).

There was no association between the day that a nest was laid and whether it would have an extra-pair offspring (Table S6c), but a weakly negative significant effect on the proportion of extra-pair offspring (Table S6c). This means the odds of extra-pair offspring decreases by 0.96 per day, but corresponds to a very small change in the actual number of extra-pair offspring. Moreover, this result is driven entirely by a single observation of 80% extra-pair offspring early in the season (Family 44: Figure S1), and this effect disappeared completely when removing this observation (β1=-0.020, p=0.165,F=3.3689, p(F)=0.07).

Table S6: results from generalized linear models exploring the relationship between measures of nesting synchrony and density on the rates of extra-pair paternity. When response variables show the proportion of extra-pair offspring (EPO) these are results from quasibinomial, with the corresponding parameter significance using F-tests. Each section of the table (a,b or c) describes the individual models pertaining to particular questions.

a) Synchronicity and density of all breeding males					
Response	Parameter	β0	β1	$\chi 2$	P
≥ 1 instance of EPP	Number synchronous	-1.190	-0.010	0.030	0.863
	log(nearest nest)	-1.209	0.090	0.039	0.884
	Average distance	**-1.528**	0.030	0.053	0.818
Response	Parameter	β0	β1	F	P
Proportion EPO	Number synchronous	**-1.989**	-0.024	0.230	0.634
	log(nearest nest)	-2.166	0.153	0.154	0.697
	Average distance	**-2.187**	-0.094	0.591	0.446
b) Synchronicity and density of compatible morph males					
Response	Parameter	β0	β1	$\chi 2$	P
≥ 1 instance of EPP	Number synchronous	-0.257	-0.123	2.125	0.145
	log(nearest nest)	**-1.053**	0.284	1.340	0.247
	Average distance	**-1.449**	0.010	0.007	0.935
Response	Parameter	β0	β1	F	P
Proportion EPO	Number synchronous	-1.989	-0.115	2.840	0.098
	log(nearest nest)	**-2.101**	0.232	1.288	0.262
	Average distance	**-2.189**	-0.103	0.840	0.364
c) Timing of Breeding					
Response	Parameter	β0	β1	F or $\chi 2$	P
≥ 1 instance of EPP	Initiation	**-0.968**	-0.020	2.315	0.128
Proportion EPO	Initiation	**-1.951**	**-0.030**	7.323	0.009

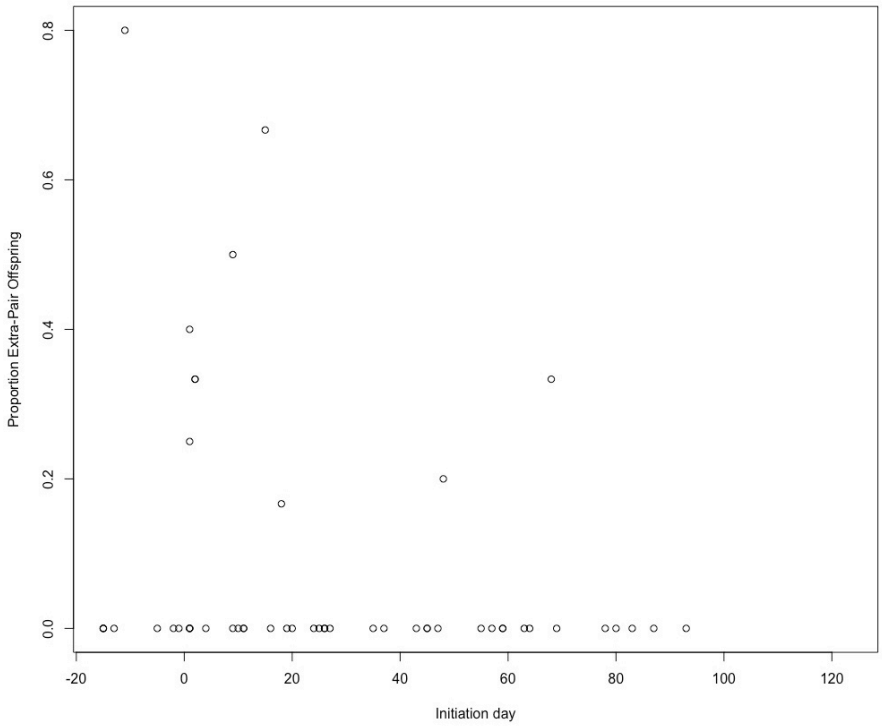

Figure S1: Plot showing the observed proportion of extra-pair offspring per nest according to the day initiated in the season.

The finding that females did not appear to be constrained with respect to either the temporal or spatial opportunity for extra-pair copulations strengthens our conclusion that this incompatibility is absent in the wild. If the same sperm competition mechanisms and selective force were present in the wild, then females should have been able to find and copulate with at least one male of the appropriate head-colour during their fertile period. From our observations of the location and timing of reproduction by both red and black morphs in the local population, females should have ample opportunity to seek extra-pair copulations with males of both head-colours. On the basis of the breeding locations of the identified extra-pair sires, we found that males or females were quite mobile during their fertile period. Furthermore, radio tracking of Gouldian finches has demonstrated that they can move anywhere between three and 17km in a single day (Woinarski & Tidemann 1992; K. Maute Pers. Comm). As a consequence there was always an abundance of males (of different head-colours) nesting at the scale over which some females gained extra-pair paternity. The distances between nests may also not be a constraint because the Gouldian finch is not a

territorial species and there are large congregations of finches at the common waterholes to drink at dawn, which may provide convenient opportunities for extra-pair copulations.

Pairing type in captivity

When considering mixed pairs in only adaptive treatments, and pure pairs in neutral the neutral treatment, mixed pairs had a higher proportion of families with EPP, but this effect was not significant (8/10 vs 4/10, Fisher's exact test p=0.17, w=0.84, power=0.76).

This result is surprising, but might be because females were limited in the number of extra-pair copulations or simply that this metric is not useful and the majority of fitness gains in incompatible pairs come from the outcome of sperm competition.

Intra-specific brood parasitism

As summarised in the main text, we found intra-specific brood parasitism in Gouldian finches at a rate of 15.8% of broods, and 4.5% of offspring. These mismatched both the social parents at an average of half the genotyped loci. Further, the likelihood approach in the latest version of CERVUS takes into account allele frequencies when assigning probable parentage, and has a low rate of mistyping related offspring as unrelated (Lemons *et al.* 2014). Additionally, these offspring were never assigned the social mother or father, even at low LOD scores.

Individuals may choose to lay in conspecific nests through an alternate reproductive strategy, or IBP may simply reflect individuals attempting to make the best-of-a-bad-job in resource limiting situations (Lyon & Eadie 2008). In particular, higher rates of IBP has been correlated with species with high reproductive rates and those that nest in cavities, which are more limited than other nest types (Eadie *et al.* 1988). IBP is taxonomically widespread (Yom-Tov 2001), and percent of broods affected by IBP is commonly less than ten percent (but can be up to 50%) (Arnold & Owens 2002). Ours is the third molecular estimate of IBP in Estrildid finches, which estimates rates in the Gouldian finch are lower than those observed in wild zebra-finches (17-36% of broods, 10.9% of offspring (Birkhead *et al.* 1990; Griffith *et al.* 2010)). Conversely, the sympatric and occasional cavity nesting long-tailed finch had

much lower levels of IBP than the Gouldian finch (2 of 391 offspring (Rooij *et al.* 2016). This conforms to previous correlations between IBP and reproductive rate and cavity nesting (Lyon & Eadie 2008). Gouldian finches are obligate cavity nesters, and may have a more r-selected life-history than long-tailed finches (Tidemann *et al.* 1999; van Rooij & Griffith 2011). Indeed, even within the available cavities, Gouldian finches are have highly specialised requirements for nesting cavities, which may preclude low quality individuals from obtaining suitable nesting sites in this highly competitive environment (Brazill-Boast *et al.* 2010, 2011a; b). Previous work has shown that red morph males are more competitive at obtaining and defending high-quality nest sites (Brazill-Boast *et al.* 2013), so black morph birds might be more likely to parasitise and be parasitized.

There was limited support for our predictions that mixed morph pairs or black morph males will experience higher rates of IBP. There was a greater proportion of nests with IBP in mixed pairs than in pure, but this effect was not significant (4/12 vs 5/36, Fisher's exact test p=0.2, w=0.47, power=0.28). There was a higher proportion of nests with IBP in mixed pairs with a black morph male, but this effect was not statistically significant (Figure S2: two-tailed Fisher's exact test, p=0.15, w=0.78, power=0.31). There was no effect of male morph irrespective of pairing type on the presence of IBP (7/31 vs 2/10, two tailed Fisher's exact test, p=1, w=0.04, pwr=0.05).

There was no effect of pairing type on the percent of clutch that was from IBP (Figure S3, Mann-Whitney Test, W=9, p=0.9), nor in the overall number of offspring (4/17 vs 17/26, Fisher's exact test, p=1). Similarly, there was no effect of male morph irrespective of his partner on the percent IBPO in a clutch (Figure S3: Mann-Whitney Test, W=7.5, p=1), nor within black morph males in different pairing contexts (Mann-Whitney test, W=4.5, p=1).

The final prediction was that black morph individuals were more likely to be parents of IBP offspring. Our results suggest the frequency of genotypes in the offspring do not deviate from the parental generation genotype frequencies (Figure S4: binomial test for frequency of red males: obs=0.18, exp=0.24, p=1).

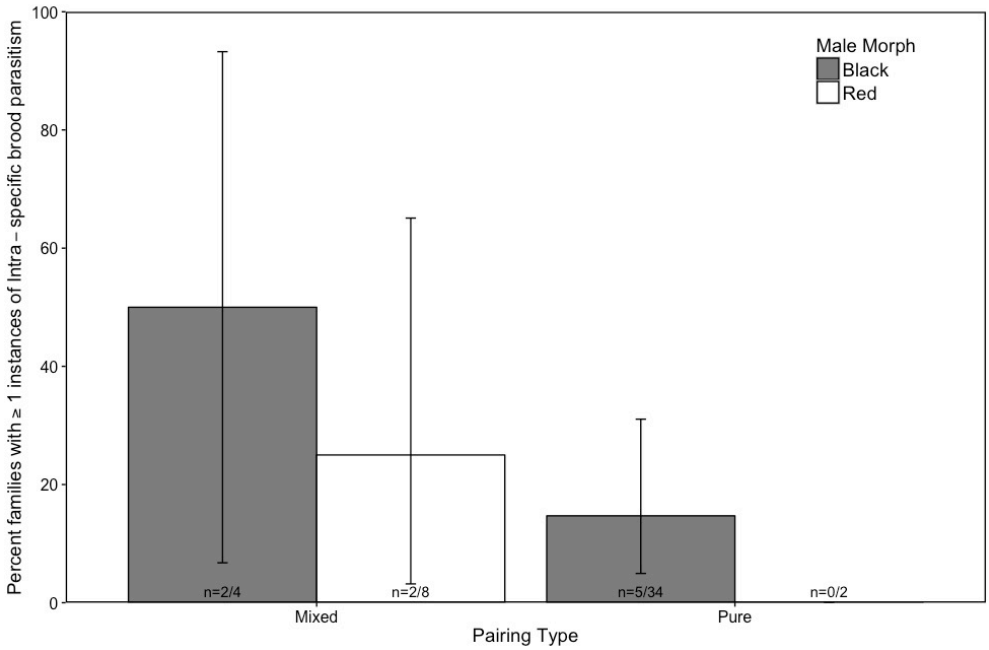

Figure S2: Percent of families with at least one instance of intra-specific brood parasitism according to pairing type, where bar colours represent male morph. Error bars are 95% binomial confidence intervals, and sample sizes are above the x-axis.

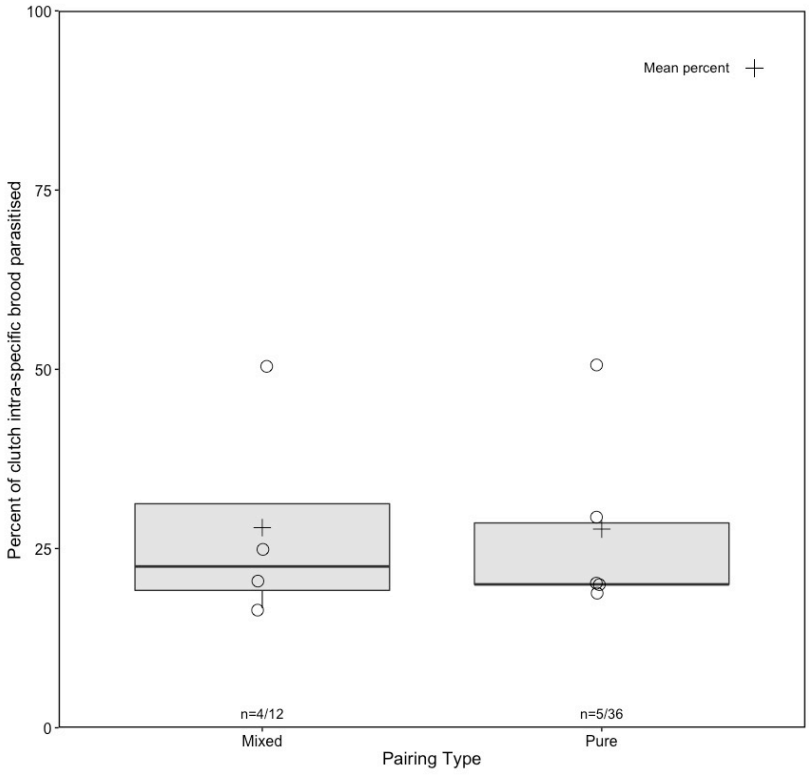

Figure S3: Boxplot showing the median and mean percent of clutch that is the result of intra-specific brood parasitism in mixed and pure pairing types

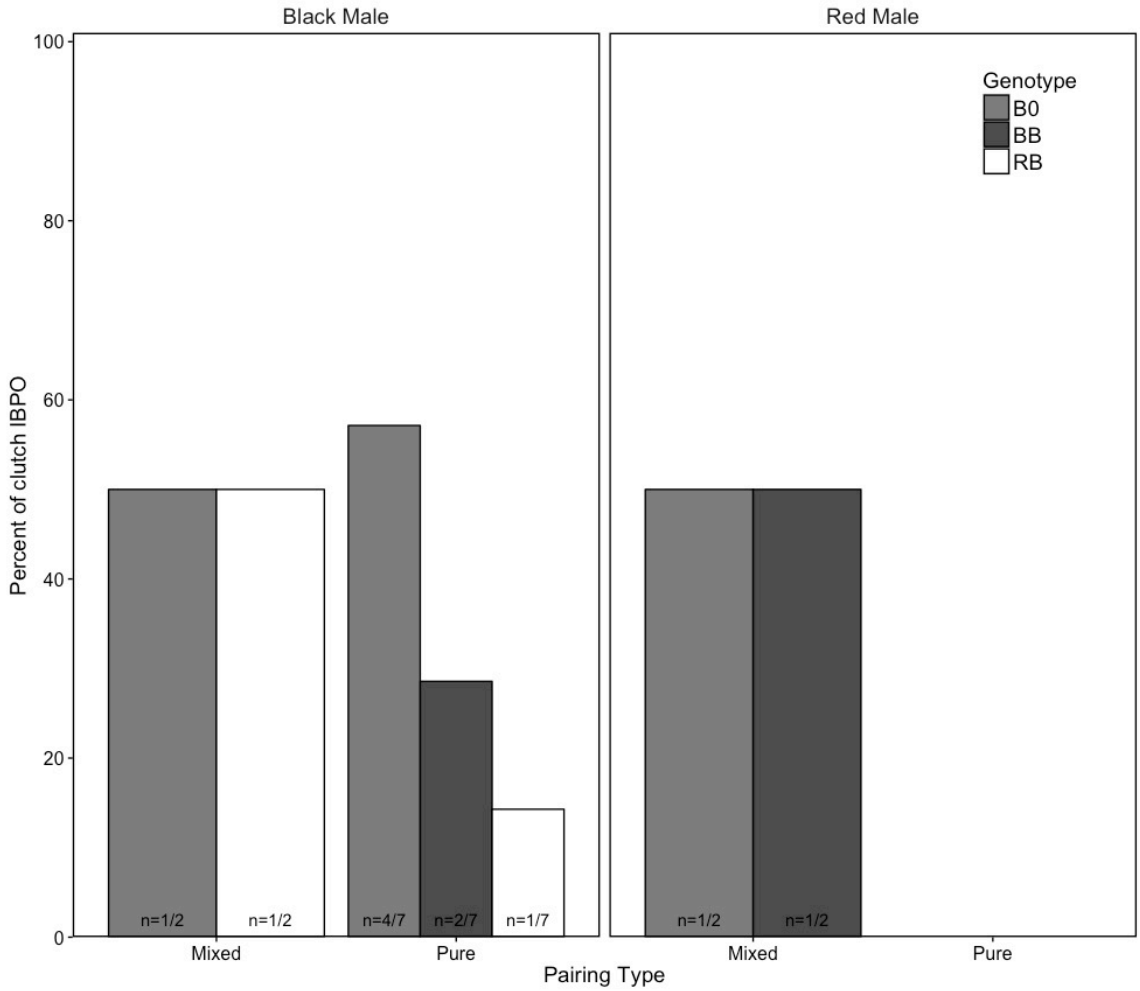

Figure S4: Frequency of genotypes resulting from intra-specific brood parasitism (IBP), where panes represent the morph of the social father of the parasitised nest. Bar colours represent offspring genotypes.

Appendix S4: Offspring morph

All offspring in these data were genotyped for head-colour morph using the Ego 172 PCR assay. If incompatibility is occurring in the wild, fathers of extra-pair young should match the morph of the mother. Separated into to maternal morph categories, we calculated the probability of observing a given observed offspring genotype. We tested against two scenarios: a) that paternity of extra-pair offspring is random (because there is no female preference or sperm competition mechanism), b) that paternity is preferenced toward males of the same head colour as the mother. Due to the small sample size we focused on testing these against the frequency of heterozygote red males (RB) from red mothers, and the frequency of

black females from black morph mothers. An example of calculations for each of the scenarios for heterozygote offspring from red mothers are as follows:

Random mating:

$$\Pr(O = RB) = \Pr(RB) * \Pr(O = RB|M = RB) + \Pr(RR) * \Pr(O = RB|M = RR) + \Pr(BB) * \Pr(O = RB|M = BB)$$

Red male preference:

$$\Pr(O = RB) = \frac{\Pr(RB)}{R} * \Pr(O = RB|\, M = RB) + \frac{\Pr(RB)}{R} * \Pr(O = RB|M = RR)$$

Where O=offspring, R=Red Phenotype, RB=heterozygote red male, RR=homozygote red male.

There was no ability to discriminate between the two scenarios in the case of red mothers, but the proportion of heterozygote offspring was larger than that expected if eggs were only fertilized by red males (Red male preference: binomial exact test obs=0.6, exp=0.25, p=0.096, w=0.74, power=0.38), where the observed patterns are much closer to the expectations under random mating (Random mating:, obs= 0.6, exp=0.44, p=0.66, w=0.33, power=0.11). The expected genotype frequency for black females was not very different between the two scenarios, and both scenarios were unable to be rejected (random mating: obs=0.4, exp=0.44, p=1, w=0.073, power=0.059; black male preference: exp=0.5, p=0.61, w=0.2, power=0.12). This is because the black allele is so common it is difficult to distinguish these scenarios, particularly when the sample size is low.

Appendix S5: Supplementary results for Population Genetics and Incompatibility.

The clustering analysis in STRUCTURE comparing wild and domesticated birds had an LnP(D) plot with the highest likelihood at K=4, and the Evanno method (Evanno *et al.*, 2005) detected a secondary peak at K=4 (Figure 4b, main text). Given this we also plotted the Q-plot for K=4 in Figure S5. Figure S5 shows that the additional clusters are all within the domesticated birds

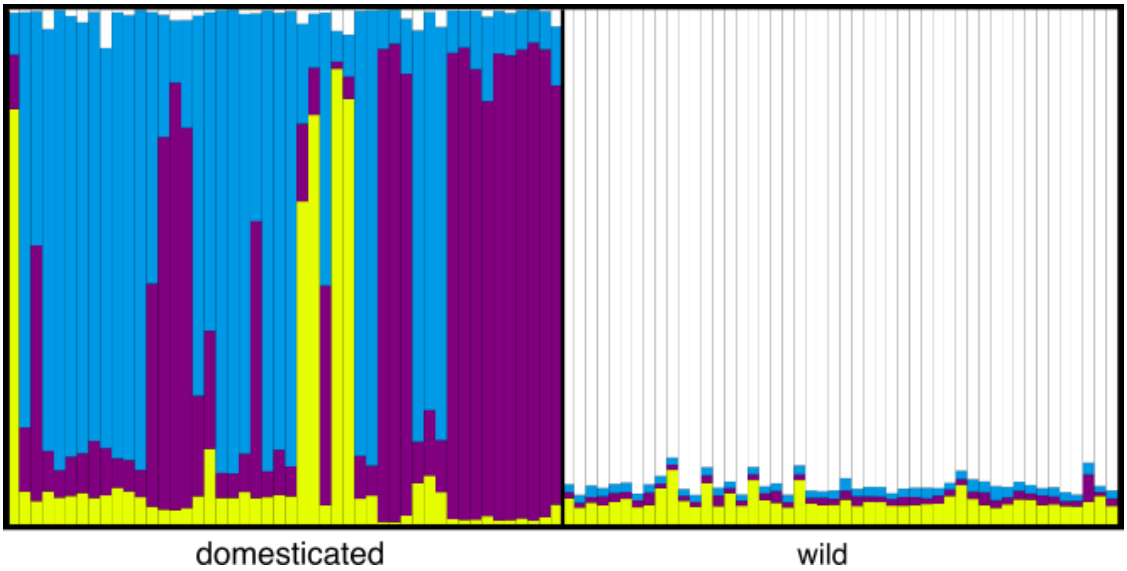

Figure S5: Q-Plot from STRUCTURE analysis for domesticated and wild comparison at K=4.

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Chapter Four

Morph over matter?

Examining body size and age-specific selection on colour morph genotypes in the wild Gouldian finch

Photo: Sarah Pryke

Chapter Four Vignette

Previously, James Brazill-Boast found no discernable effect of incompatibility on fledging success in the wild (Brazill-Boast *et al.* 2013), and the previous chapter found no evidence for strategies to ameliorate incompatibilities. However, the existing datasets had not yet been fully explored for avenues through which incompatibility could manifest. Therefore, using these existing datasets I explored whether mortality due to incompatibility was deferred later in life, through reduction in the frequency of genotypes affected by incompatibility; and whether there were sub-lethal effects of incompatibility via changes in body condition. Body condition can influence risk of mortality, and paired with physiological measures can be used to associate vulnerability with certain processes or seasons (e.g. habitat clearing or moulting) or more generally (Stevenson & Woods 2006; Maute *et al.* 2015).This chapter is the last attempt to use the existing data in the wild to correlate genotypes with predicted effects of incompatibility.

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Abstract

Species with colour polymorphisms often exhibit suites of traits that are correlated with each colour, and are maintained by selection on colour directly or on properties of the correlated traits. Previous work on the domesticated colour polymorphic Gouldian finch revealed a genetic incompatibility between morphs, where the offspring of mixed-morph parents had reduced survival. The interplay of the incompatibility between morphs could be a threatening process to this endangered bird. However, previous work in the wild exploring fledging success, patterns of polyandry and offspring sex ratio was unable to detect any signal of incompatibility with the same strength observed in the domesticated birds. We utilise an allele-specific test for head-colour genotype in the Gouldian finch to explore whether mortality effects of incompatibility are delayed to later in life, as there was no incompatibility effect on fledging success in the wild. Additionally, body condition indices were associated with morph genotypes to explore whether incompatibility has sub-lethal effects. There was no change across age classes in the male genotype frequencies affected by incompatibility. As predicted by the incompatibility experiments in the domesticated birds, there was a male-biased sex ratio in adult birds, but it remains unclear whether this is the result of a genetic incompatibility. Similarly, there was no detectable difference in body condition in either hatchling or adult birds according to their head-colour genotype. Our results suggest that in the wild, there are no delayed mortality and sub-lethal effects of incompatibility between Gouldian finch morphs in the wild.

Keywords

Quality; assortative mating; age-specific selection; body size; genotype frequency

Introduction

Species with sympatric colour morphs often exhibit suites of traits correlated with colour, and are often determined by few genes of large effect (Ford 1945; Huxley 1955; McKinnon and Pierotti 2010; Wellenreuther *et al.* 2014). In a wide array of vertebrates, colour has been associated with alternate life history strategies such as K- and r-selected female side-blotched lizards (*Uta stansburiana*) (Sinervo *et al.* 2000), sexual strategies such as lekking and sneaker males in the Ruff (*Philomachus pugnax*) (Lank *et al.* 1995). Colour morph has also been associated with differences in body size (Almasi and Roulin 2015), stress response (Galeotti *et al.* 2010), and immunocompetence (Pryke *et al.* 2007). The polymorphism may be maintained in the population through ecological drivers such as apostatic selection on colour itself (Bond 2007), selection on the correlated sexual, social or life-history traits (McKinnon and Pierotti 2010), or some combination of the two (e.g. the black sparrowhawk *Accipiter melanoleucus* (Sumasgutner *et al.* 2016)). The interaction of morphs and nature of the correlated traits may decrease population fitness in some polymorphic species (Bolton *et al.* 2015; Bolton, Rollins, *et al.* 2016; Forsman 2016).

Selection against certain colour genotypes has been demonstrated in a number of colour polymorphic species. For example, homozygotes of the inversion polymorphism determining Ruff phenotypes is lethal (Küpper *et al.* 2015), or there may be conflict between the sexes for the optimal colour genotype (Ducret *et al.* 2016). Moreover, assortative colour pairs in the black sparrowhawk have lower offspring survival than disassortative pairs (Sumasgutner *et al.* 2016; Tate *et al.* 2016), reduced survival of inversion homozygotes may explain nearly perfect disassortative mating in the white-throated sparrow morphs (Houtman and Falls 1994; Horton *et al.* 2013). On their own, these effects are no different from the effects of selection on population dynamics (Saccheri and Hanski 2006; Kinnison and Hairston 2007), but in combination with environmental mismatch and behavioural interactions between morphs colour polymorphic species may fall into an ecological or evolutionary trap (Schlaepfer *et al.* 2002; Bolton, Rollins, *et al.* 2016). Indeed, theoretical modelling that includes habitat requirements and behavioural interactions between Gouldian

finch colour morphs revealed parameter space where birds exist in low numbers or drive themselves to extinction (Kokko *et al.* 2014).

The Gouldian finch (*Erythrura gouldiae*) has a sex-linked Mendelian polymorphism for red or black head-colour (Southern 1945), which co-occur at stable frequencies across its range in the monsoon tropics of Australia (Gilby *et al.* 2009). A series of experiments on domesticated Gouldian finches revealed that head-colour was associated with a number of behavioural and physiological traits (Pryke and Griffith 2006; Pryke *et al.* 2007; Pryke and Griffith 2009c). Experiments also showed captive birds mate assortatively with respect to head colour (Pryke and Griffith 2007; Pryke 2010). Compared with assortative parings (same morph), disassortative pairs (mixed morph) have higher offspring mortality, and is strongest in females, in line with Haldane's rule (Pryke and Griffith 2009b). Theoretical modelling suggests the genetic incompatibility and behavioural differences between morphs in domesticated Gouldian finches could be a threatening process in the wild (Kokko *et al.* 2014), which already has a history of population declines (Franklin 1999; Franklin *et al.* 1999). For that reason, it is important to fully evaluate the extent to which such effects may occur in wild Gouldian finches. In the wild, Brazill-Boast *et al.* (2013) found no effect of mixed morph families on offspring fledging rate. Nor was there any evidence for morph assortative mating, the selection of compatible partners through polyandry, biased offspring sex-ratios, or a reduction in heterozygote offspring in a wild population (Bolton *et al.* 2017). Genetic techniques have allowed selection on the underlying colour genotypes to be measured in real time (e.g. Gratten *et al.* 2008; Des Roches *et al.* 2017). Here, an allele specific test for head-colour is applied to adults and offspring in wild Gouldian finches (Kim 2011), to explore whether there is evidence for local selection on head-colour across the range, and whether incompatibility defers incompatibility selection to later in life, or it manifests as sub-lethal effects.

Previous work has shown morph frequencies to be equivalent across the range (Franklin and Dostine 2000; Gilby *et al.* 2009), but does not account for any local selection on

genotype frequencies in male Gouldian finches, which may occur due to difference intensities of incompatibility for example. For the first time, head-colour genotypes are directly quantified across the range. In the laboratory, mixed morph pairs, as well as red females paired with heterozygote red males exhibited reduced offspring survival (Pryke and Griffith 2009b), but incompatible pairs in the wild showed no evidence of reduced fledging success (Brazill-Boast, Griffith, *et al.* 2013). Heterozygote male offspring can only be derived from "incompatible" pairings, and may suffer increased mortality later in life if the effects seen in the laboratory are present in the wild (Pryke and Griffith 2009b). Shifts in the age of viability selection due to incompatibility selection is tested by: (i) testing for a reduction in the genotype derived from mixed morph pairings (heterozygote males) and (ii) a reduction in the incompatibility effected sex (females) across hatchling, immature fledged and adult age classes.

Body condition reflects an individual's energy reserves and potentially its overall quality, and has been associated with fitness consequences in some species (Cotton *et al.* 2006; Roulin 2016). Therefore, any sub-lethal effects of incompatibility in the Gouldian finch may manifest through differences in overall body condition. Indeed, experiments on domesticated Gouldian finches showed that mixed morph parents invested less in provisioning their offspring (Pryke and Griffith 2009a; Pryke and Griffith 2010). Differences in body condition owing to incompatibility is explored by testing: (i) whether body condition is reduced in heterozygote red males in adult and offspring Gouldian finches, (ii) whether the offspring of disassortative pairs have lower body condition. Constraints on mate availability may force individuals in poor condition may to make sub-optimal decisions with respect choosing a mate with an assortative head-colour, or conversely, incompatibility between morphs may directly influence stress levels and hence body condition (Griffith *et al.* 2011). Therefore, we tested whether there was evidence of condition assortative mating, and whether incompatible pairing types consistently had lower body condition than compatible pairs.

Methods

Sample collection

Between 2004 and 2013 Gouldian finches were mist-netted banded and bled at waterholes in five localities in Western Australia and Northern Territory (Figure S1; Table 1). Adult birds from Australian Wildlife Conservancy's Mornington Sanctuary and all birds from the Northern Territory formed part of a larger study on the stress physiology of wild Estrildid finches and details on sampling can be found elsewhere (Maute *et al.* 2013; Legge *et al.* 2015).

Individual and family data was collected from Gouldian finches breeding in 2008 and 2009 at Wyndham in the eastern Kimberley, Western Australia (S15°340', 128°090'). This is a natural breeding site, which was supplemented with special cavity shaped nest-boxes that allow access to the nest chamber (Brazill-Boast *et al.* 2010; Brazill-Boast, Pryke, *et al.* 2013). The details of the monitoring protocol are described elsewhere (Brazill-Boast *et al.* 2010; Brazill-Boast *et al.* 2011; Brazill-Boast, Pryke, *et al.* 2013), but important details are reiterated here. Hatchlings in nestboxes were monitored every 2-4 days and were banded and bled between 14 days and fledging (after day 18). Maternity and paternity of hatchlings were assessed by adult presence at nest and later verified using a molecular parentage analysis (Bolton *et al.* 2017). Birds were also mist-netted and bled at nearby waterholes every 1-2 weeks to capture other adults and fledged juveniles in the population, with additional adult birds caught at waterholes in Wyndham between 2010 and 2013.

All individuals were given unique band combinations supplied by the Australian Bird and Bat Banding Scheme. Adults were assigned sex and head-colour morph according to their plumage, and this can be done equivocally. Blood samples were drawn from all individuals by puncture of the brachial vein with a 26-gauge needle. All individuals were given unique band

Table 1: Number and percent of Gouldian finch head-colour genotypes in each of the five sampling localities. The range of years sampled are provided next to each sampling locality.

	Black-head female Z^rW	Red-head female Z^RW	Black-head male Z^rZ^r	Red-head male Z^RZ^r	Red-head male Z^RZ^R
Mornington (2005-2009)	122 (41.64%)	13 (4.44%)	114 (38.91%)	36 (12.29%)	8 (2.73%)
Wyndham (2008-2013)	126 (36.84%)	19 (5.55%)	151 (44.15%)	45 (13.16%)	1 (0.29%)
Bradshaw (2007-2009)	20 (33.90%)	2 (3.39%)	25 (42.37%)	10 (16.95%)	2 (3.39%)
Delamere (2007-2009)	25 (39.68%)	3 (4.76%)	23 (36.51%)	12 (19.05%)	0
Yinberrie Hills (2007-2009)	22 (33.33%)	5 (7.58%)	25 (37.88%)	12 (18.18%)	2 (3.03%)
Overall (2005-2013)	315 (38.27%)	42 (5.10%)	338 (41.10%)	115 (13.97%)	13 (1.58%)

combinations supplied by the Australian Bird and Bat Banding Scheme. Adults were assigned sex and head-colour morph according to their plumage, and this can be done equivocally. Bloods were drawn from all individuals by puncture of the brachial vein with a 26-gauge needle. A total volume of less than 60uL was taken and stored in either 95% ethanol or Queens lysis buffer. The mass (0.1g) and tarsus length (0.01mm) was measured for all 14 day old offspring and adults at Wyndham in 2008 and 2009, and for all adults at all sites in the Northern Territory.

The protocols used to collect blood samples and monitor nests were approved by the animal ethics committee at Macquarie University (AEC2007/037, AEC 2007/038, AEC2010/053), Australian Wildlife Conservancy (CAEC/6/2005,AEC 2007/43, AEC 2010/35), and the University of Wollongong (AE06/25).

Genotyping

DNA was extracted from blood samples using a Qiagen PureGene Kit and subsequently used in microsatellite analyses described elsewhere (Bolton, West, *et al.* 2016; Bolton *et al.* 2017). Included in one of the microsatellite multiplexes was an allele-specific test for two consecutive SNPs that segregate nearly perfectly with red and black head-colour (Kim 2011). This marker allows the underlying genotype of an individual to be determined, as there are no colouration differences in heterozygote or homozygote red males. The allele conferring red (R) colouration is dominant to black (r) and is carried on the Z-chromosome (Southern 1945), where a heterozygote red-head male has the notation $Z^{R}Z^{r}$ and a black-head female has $Z^{r}W$.

Primers 2550F and 2718R were used to genotype the CHD locus to determine the sex of hatchlings and fledged juveniles (Fridolfsson and Ellegren 1999).

Frequency

Head-colour in the Gouldian finch is a sex-linked trait (Southern 1945), therefore allele and genotype frequencies in each sex needs to be considered separately (Falconer and Mackay 1996). Whether male, female and combined head-colour allele frequencies differed among locations was tested using pairwise fisher's exact tests with Bonferroni correction. Finally, because previous work found that these five localities were a single genetic population (Bolton, West, *et al.* 2016), whether allele frequencies differed in each sex pooled across sampling localities was tested with a Fisher's exact test. Head-colour genotype frequencies for adult males across the range were tested against Hardy-Weinberg expectations calculated from male allele frequencies using a chi-squared test. Under a scenario of morph incompatibility, there would be a depletion of heterozygote red males across the range.

If there is selection against offspring from incompatible pairings later in life, then the frequency of heterozygote males may decline with age. Moreover, given that female offspring were more affected by incompatibility in the domesticated experiments, the fraction of females in the population should decline with age if the incompatibility occurs in the wild. This was tested by comparing the frequency of head colour genotypes and sex-ratio in hatchling (chick), juvenile and adult age classes for individuals caught at Wyndham in 2008 and 2009. Sample sizes for each genotype and age class are presented in Table S2. The age class approach was applied because mark-recapture methods are unable to reliably distinguish survival and dispersal because banding effort is so low in the region, and recapture rates are very low between years at a single site in this apparently very mobile species (Bolton, West, *et al.* 2016).

The hatchling dataset contained family structure from sibling and half-sibling nest-mates, which could potentially over-estimate genotype frequencies as certain families were sampled more often. Therefore, an *ad hoc* resampling method was used to remove family non-independence by resampling one individual (from a pool of siblings and half-siblings) from each family (unique parent pair). All 51 families were resampled 1000 times, and all tests were conducted on each resample of chicks against the juvenile and adult datasets, and

the frequency of significant results amongst the 1000 resamples was used to assess whether there was a difference in frequency between hatchlings and other age classes. If more than 5% of the resampled tests were significant at $p<0.05$ then the null hypothesis for no change in frequency was rejected. Fisher's exact tests were used to compare the frequency of genotypes between age classes, and deviation from sex ratio parity was assessed with a binomial exact test.

Body condition

If there are sub-lethal effects of incompatibility, or head-colour genotype specific fitness then some individuals in the population may have lower body condition. The residual from an ordinary least squares regression (OLS) between body mass and tarsus length (body size) was used as a crude measure of body condition (*mass ~ tarsus length*). Residuals were calculated based on separate regressions on adults caught at Wyndham and in the Northern Territory because they were measured by different observers, and another separate regression was used for the nestlings measured at day 14. An individual with positive integer for condition indicates a larger mass than predicted from its tarsus length. This method for measuring body condition has been criticized for not being explicit in which physiological processes it measures and are rarely validated, and suggest that the use of raw mass measurements are more informative (Schamber *et al.* 2009; Labocha and Hayes 2012). However, the birds used here from the Northern Territory were part of a larger study on body condition and seasonal stress physiology in Gouldian and other sympatric Estrildid finches, which found body condition indices correlated well with changes in specific measures of fat and muscle content (Maute *et al.* 2013). Further, residual condition index and body mass were regressed in these data to assess the relationship between them and the model had an adjusted R^2 of 0.979, therefore there was very little difference between the two measures. The body condition measures were used to answer a number of research questions and the hypotheses and analyses used are presented in dedicated paragraphs.

Heterozygote male Gouldian finches come from either mixed morph matings, or red females paired with heterozygote red males; both of these mating types had evidence for genetic incompatibility in the experiments on domesticated birds (Pryke and Griffith 2009b). Therefore, if there are sub-lethal effects of incompatibility in the wild, then heterozygote red males may show lower body condition than other adults. This was tested on adult birds caught during the breeding season at Wyndham and in the Northern Territory (Maute *et al.* 2013), and the total sample size of adult birds with body condition measures are presented in Table S3. The sample sizes are smaller than the frequency analysis because not all adults and chicks were measured. The data for body condition according to head-colour genotype was heteroscedastic (non-constant variance) (Levene's test: Df=4, F=2.714, p=0.030), therefore a one-way Welch's ANOVA was used to assess differences in quality between genotypes.

The difference in body condition according to head-colour genotype was also explored in the day 14 nestlings from Wyndham. The chick dataset was limited to only include sibling and half-siblings as determined by previous paternity testing, as the parentage of intra-specific brood parasite chicks was not resolved (Bolton *et al.* 2017). This yielded a final dataset of 170 chicks across 41 families (Table S3).

Here, a linear mixed effects model (LMM) was applied in R package '*nlme'* (Pinheiro *et al.* 2016). *Family ID* included as a random intercept to account for non-independence in families (*chick condition ~ chick genotype* + (1|*Family ID*)), and models were checked for homoscedasticity and normality of residuals by visual inspection of graphs on fitted values vs. residuals and Q-Q Plots. After the model was verified, an ANOVA was run on the final model to assess the final effect of head-colour genotype on body condition. Marginal R^2 for fixed and random effects were calculated according to Nakagawa and Schielzeth (2013).

Previous work found that there was no evidence of positive assortative mating according to head-colour in the Gouldian finch at Wyndham (Bolton *et al.* 2017). However, high quality individuals may be able to choose mates with the same head-colour, while low-quality individuals are forced to pair sub-optimally with respect to head-colour (Griffith *et al.*

2011), so sub-optimal pairing types (mixed morph pairs) would be expected to have average body condition. First, the tendency for individuals to form pairs with others of similar body condition was tested on pairs of birds where body condition was measured in both birds (31 pairs). This was initially tested using an OLS regression (*male condition ~ season + female condition*). *Season* was included as a cofactor in the model to account for a lower mean condition of birds measured in 2009. The model was checked for the assumptions of homoscedasticity normality of residuals by visual inspection of residuals and Q-Q plots. A number of different variance structures were explored with respect to *female condition* and the *season* variable, and tested against the null using Log-likelihood tests. The final model was weighted to allow for exponential variance across *female condition* (function: '*varExp'*), and was significantly improved from the null model without accounting for variance structure (Likelihood ratio=4.082, p=0.043). Finally, to test whether there was an influence of head-colour pairing on the condition of parents, a Welch's ANOVA was used. Two pairing categories were removed from this comparison due to low sample size ($Z^RW + Z^rZ^r$ n=2, $Z^RW + Z^RZ^r$ n=1).

In the domesticated population, mixed morph pairs invested less in their offspring compared to pure pairs (Pryke and Griffith 2009a). Therefore, in addition to any sub-lethal effects of the morph incompatibility itself (Pryke and Griffith 2009b), strategic differential investment behaviour might be predicted to lower the body condition in the offspring from mixed morph pairs (Pryke and Griffith 2009a). Additionally, the effect of mean parental condition was explored with respect to offspring condition in a LMM, which accounted for the mean difference in condition according to *season,* and used *family ID* as a random intercept in the model. Three models were used to explore the effects of parental condition and parent pairing type on offspring condition (Table 2, models 1-3), accounting for the effect of *season* on parental condition. Models 4-5 explore the effect of parental *pairing type* on offspring condition, these models utilised a larger dataset because it did not require condition measurements for both parents. Sample sizes and model forms are provided in Table 2. The

Table 2: Results from linear mixed effects models exploring the effects of parental condition and parental genotypes on offspring condition at Wyndham. Models 1-3 include testing the effects of parental condition (dependent on *season*) on offspring body condition, and is a smaller dataset because not all nests had measurements for both parents (column 2), but models 4-5 explore the effects of parent pairing type on offspring condition. For each model, an ANOVA was used to assess the significance of the fixed effect variables (cols 3-5), and the marginal R^2 as a measure of response variable explained by both the fixed and random effects.

Model Form	Number chicks/ Number families	Variable	F	p	Fixed effects Marginal R^2	Random effects Marginal R^2
(1) Chick condition ~ 1 + (1|Family ID)	129/31		-	-	-	0.756
(2) Chick condition ~ Season + mean parental condition + (1|Family ID)		Season	15.219	0.0006	0.330	0.428
		Parent condition	6.345	0.018		
		Parent pairing	2.562	0.078		
(3) Chick condition ~ Pairing type + Season + mean parental condition + (1|Family ID)		Season	15.544	0.0006	0.396	0.367
		Parent condition	7.711	0.010		
(4) Chick condition ~ 1 + (1|Family ID)	170/41		-	-	-	0.708
(5) Chick condition ~ Pairing type + (1|Family ID)		Pairing type	2.658	0.063	0.123	0.599

proportion of variance in the response explained by the fixed and random effects in the models were calculated as the marginal R^2 (Nakagawa and Schielzeth 2013). The effect of *nest initiation day* on chick quality was explored, but was never significant in explaining variation in chick quality when accounting for the random effect of *Family ID*. This is because *Family ID* and *Initiation day* are highly correlated, because there were so few repeated clutches.

Results

Frequency

There was no difference in allele or genotype frequencies across the five sampling localities as measured by pairwise Fisher's exact tests (Table S1). When pooling the samples across the sampling localities, there was no evidence that male and female allele frequencies differed (black males 791/932 vs black females 315/357, Fisher's exact test p=0.13). Similarly, male genotype frequencies did not differ from Hardy-Weinberg expectations (χ^2= 0.47,p=0.49, F_{IS}=0.03). The genotype frequency at each sampling locality is provided in Table 1.

Changes in genotype frequencies amongst the age classes was tested by resampling one chick from each of 51 families 1000 times and conducting a Fisher's exact test, with total sample sizes for each genotype and age class in Table S2. There was no evidence for a reduction in heterozygote frequency across all three age classes (proportion of significant Fisher's exact tests=0.001). The power to assess differences in heterozygote frequency in chicks and adults/juvenile age classes was high (>0.8) in most of the tests across resamples (Figure S3). Similarly, there was no evidence for a change in allele frequency between juvenile and adult age classes (Fisher's Exact test p=1, power=0.99, h=0.95). There was no evidence for a change in the frequency of red or black females though age classes (proportion of significant Fisher's exact tests=0). However, tests across chick resamples comparing head-colour frequency in female chicks and adults/juveniles had much lower power, owing to much

smaller effect sizes (Figure S3). The change in genotype frequencies in each sex across age classes are summarised in Figure 1.

From 1000 samples of one chick per family (51 families), binomial exact tests found no evidence that the sex ratio differed from parity (proportion of significant binomial exact tests=0.058), but the effect size, and consequently, power was generally low (Figure S4). There was also no evidence of sex ratio deviation in the juvenile age class, with similarly low effect sizes (binomial exact test, 30/62, p=0.45, h=0.03, power=0.08). However, there was a departure from parity in the adult age class, where 56% of the population was male (binomial exact test: 142/251 p=0.04, h=0.13, power=0.55). These results are summarised in Figure S5.

Condition

A Welch's ANOVA was run to account for heteroscedasticity to explore whether there was any relationship between adult body condition and head-colour genotype. There was no effect of head-colour genotype on adult body condition (Df=(4, 25.507), F=0.84, p=0.51; Figure 2). There was much variation in body condition according to head-colour genotype in the chick dataset (Figure 3a), but the linear mixed effect model found no effect of genotype on body condition (df=(4,125), F=1.45, p=0.22; Figure 3b), where the random effect for *Family ID* explained 69.1% of the variation, whilst the genotype of the chick explained only 1.7% of the total variance.

After accounting for variation in body condition between 2008 and 2009, there was still a significant relationship between *female condition* and the body condition of her partner (ANOVA: *season*: df=, F=8.40, p=0.007; *female condition*: df=, F=7.90, p=0.009; Figure 4a). The coefficient estimates from this GLS model are presented in Table S4. However, Welch's ANOVA found no evidence that mixed-morph pairs ($Z^RW+Z^rZ^r$) had a lower average body condition than the same-morph pairs ($Z^rW+ Z^rZ^r$) (Df=(1, 7.72), F=0.005, p=0.94), but was unable to be tested for the remaining two pairs ($Z^rW+Z^RZ^r$ and $Z^RW+Z^RZ^r$) because of low sample size (Figure 4b).

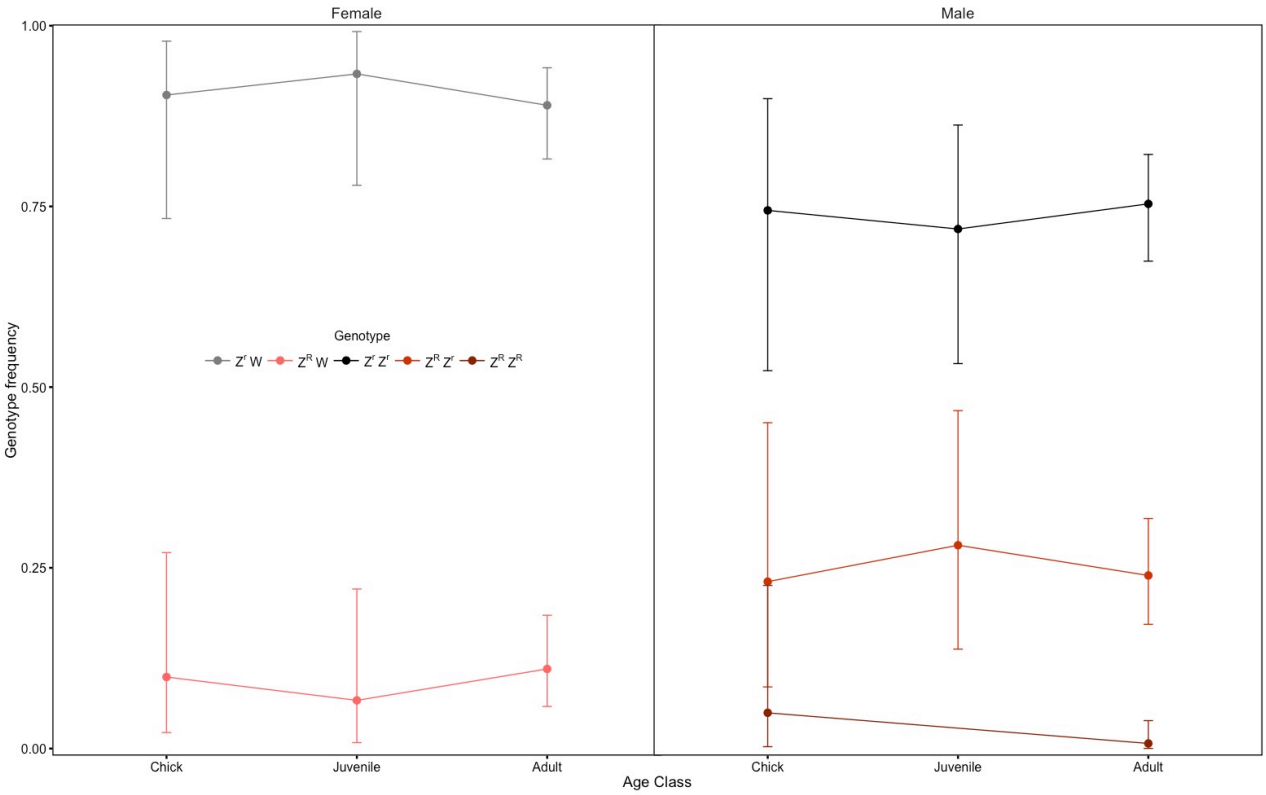

Figure 1: Genotype frequencies with 95% binomial confidence intervals for male and female Gouldian finches at Wyndham in each age class. The values for chicks represent the mean of the frequency and its upper and lower binomial confidence intervals across 1000 resamples of 51 families.

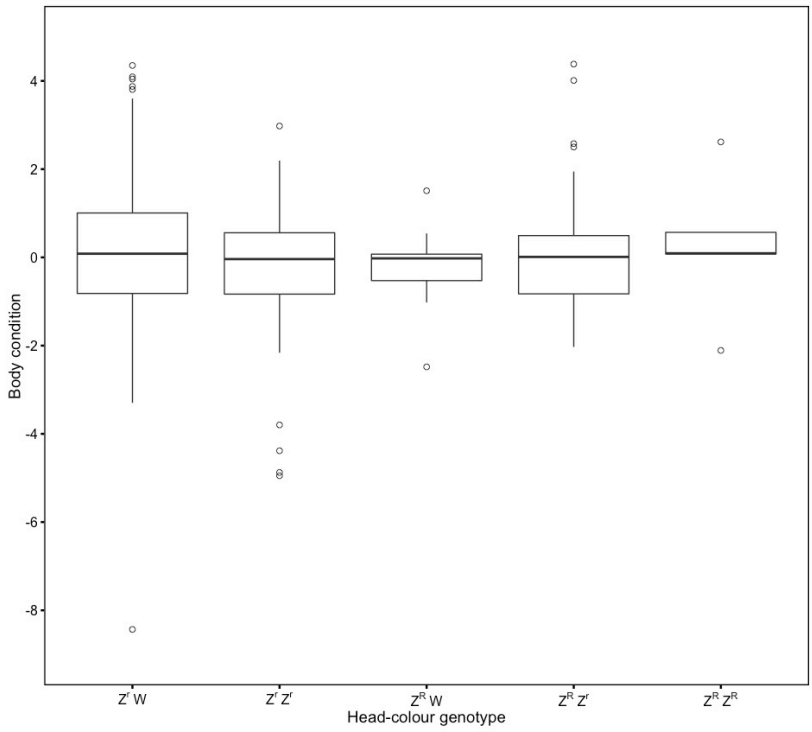

Figure 2: Boxplot of body condition according to head-colour genotype in adult Gouldian finches in Wyndham and the Northern Territory.

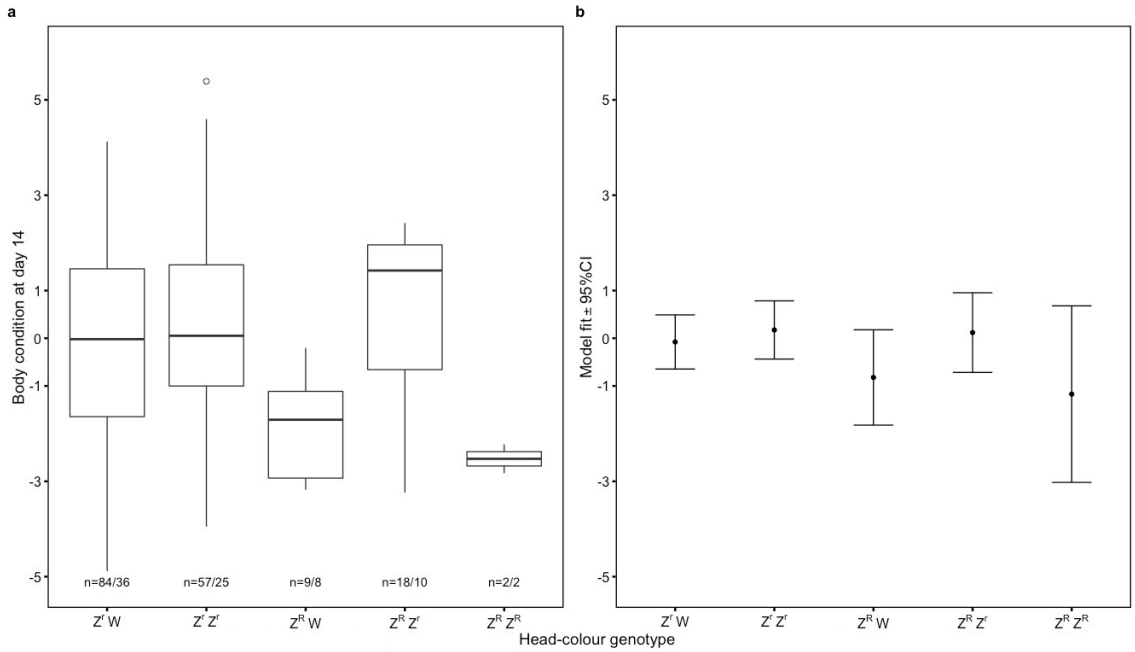

Figure 3: Panel a) shows raw of data body condition of day 14 chicks according to their head-colour genotype, above the x-axis is the sample sizes of the number of chicks/number of families from which those chicks were derived, there were a total of 41 unique families. Panel b) shows the fitted values of body condition according to head-colour genotype in the linear mixed model accounting for family structure.

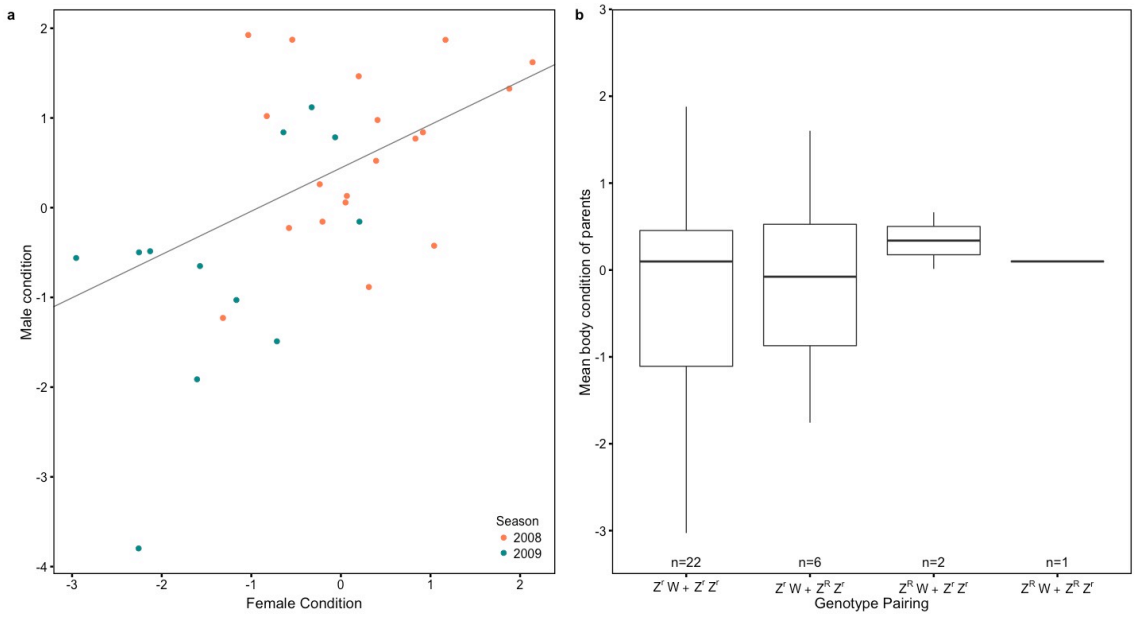

Figure 4: Panel a) shows the body condition of each individual in a mated pair at Wyndham. The regression line is the result of a GLS regression accounting for mean condition according to season and non-constant variance (*male condition ~ Season + female condition*). Panel b) shows the distribution of mean body condition of parents according to head-colour pairing.

The relationship between body condition of offspring and their parents was explored using a linear mixed effects model (LMM), account for differences in quality between 2008 and 2009 and using *family ID* as a random intercept. There was a significant effect of mean parental condition, even after controlling for differences according to *season* (Table 2, model 2; Figure 5a). When comparing with the null model (1), the addition of *season* and *mean parental condition* did not explain additional variance in chick condition (marginal R^2), but partitioned the variance explained by *family ID*. The addition of *pairing type* into these models with parental condition (3) explained little extra variance (0.5%). Corroborating this, ANOVA of fixed effects variables on models 3 and 5, found no significant effect of parental *pairing type* on offspring condition (Table 1; Figure 5b-c). Variable coefficients for model 3 are presented in Table S5.

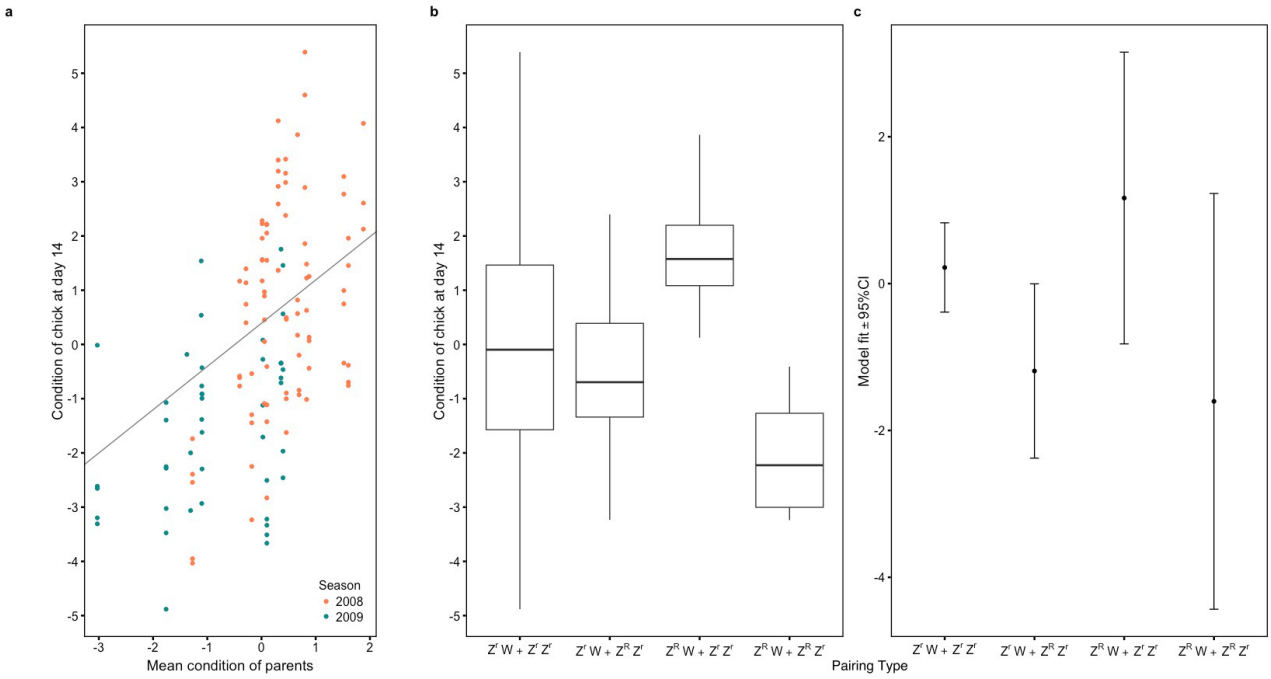

Figure 5: Panel a) shows the relationship between the mean body condition of both parents on offspring body condition, where the regression line is based on the linear mixed model accounting for condition differences between 2008 and 2009 using *Family ID* as a random intercept (Table 1, model 3). Panel b) shows the distribution of chick body condition according to the pairing type of their parents, and panel c) shows the fitted values and 95% confidence intervals for chick body condition for each pairing type according to linear mixed effect model 5, accounting for *Family ID* as a random effect

Discussion

Gouldian finch head-colour frequencies were assessed across five geographically disparate sampling localities using an allele-specific test (Kim 2011), in order to measure incompatibility selection. The frequency and body condition of heterozygote red males was used as a proxy for mortality owing to incompatibility. Across all five sampling localities there was no evidence for a reduction in heterozygote males with respect to expected Hardy-Weinberg proportions, nor a difference in their frequency across age-classes at the Wyndham locality. Similarly, heterozygous adult males and nestling males were of equivalent body condition to the other head-colour genotypes, and when accounting for random variation according to individual families, there was no detectable influence of parental head-colour on offspring condition. This suggests that if there are any sub-lethal effects of incompatibility, they are not manifested through differences in body condition. Indeed, when accounting for random variation according to individual families, there was no detectable influence of parental pairing type on offspring condition. Moreover, the previously found differences in life-history between morphs, differing ability to acquire and defend high quality nest-sites (Brazill-Boast, Griffith, *et al.* 2013), in the wild do not have a detectable effect on morph specific survival or the body condition indices used here.

In the experiments on domesticated birds, female offspring of incompatible pairs experienced higher mortality rates than their brothers. In support of a deferred mortality effect, we found that there was a significantly male biased sex ratio, but only in the adult sample (1.3 male:1 female). This may be a mechanism that explains significantly male biased adult sex-ratios that have been reported at some locations (Tidemann *et al.* 1992; Woinarski and Tidemann 1992), but male bias is not significant across all locations (Franklin and Dostine 2000). Although this result with adult sex ratio lends some credence to the hypothesis of deferred effects of incompatibility in the female offspring, it must be considered with caution. Many other bird species have male biased adult sex ratios caused by female-biased mortality and dispersal (Clarke *et al.* 1997; Donald 2007), and therefore the male-biased sex-ratio in the Gouldian finch may be due to completely unrelated processes.

Although our results do not provide any support for the effect of morph genotype or incompatibility on age-dependent mortality or body condition, they do not completely exclude such effects. The effect size of differential morph survival, or condition, may be very small. Modelling work has shown that even with strong selection against heterozygotes (s=0.1), achieving sufficient power to detect shifts in genotype frequency using Hardy-Weinberg tests requires thousands of individuals (Lachance 2009). Indeed, the effect sizes of assortative mating and sex-specific genotype deficits in barn owls (*Tyto alba*) at the MC1R melanin-colouration locus were quite small (<15%), and were only detectable due to the large multi-year dataset used (Ducret *et al.* 2016). One reason incompatibility was detected in the domesticated birds is because they used balanced experimental designs (Pryke and Griffith 2009b). In the wild, it is almost impossible to get sufficient power to test these questions because head-colour alleles are sex-linked and occur at unequal frequency in the wild, which means that some genotypes and mated pairs will be poorly represented even under random mating (Southern 1945; Franklin and Dostine 2000; Bolton *et al.* 2017).

When individuals vary in quality, individuals in socially monogamous species may have limited options in mating with their first mate preference, or the mate preferences of an individual may be informed by their own condition (Härdling and Kokko 2005; Cotton *et al.* 2006; Griffith *et al.* 2011). Here we explored how two modalities of quality, overall body condition and head-colour morph, correlated with mating outcomes in the Gouldian finch. In line with theoretical predictions (Härdling and Kokko 2005), and empirical findings (Holveck and Riebel 2010; Montiglio *et al.* 2015), there is a pattern of condition assortative mating in the wild Gouldian finch, and that parental condition reflects the quality of the offspring. On the other hand, the covariation of condition in adult partners and their offspring could readily be explained by a shared environment and may not reflect mating preferences given that condition was not measured at the time of mate-choice. Assortative mating patterns do not reflect back on pairing frequency with respect to genotype (Bolton *et al.* 2017), nor do we find evidence here that mixed morph parents, or their offspring, are consistently lower

condition than the parents and offspring from same morph pairs. This is in contrast with previous work on the domesticated Gouldian finch, which found higher baseline levels of stress hormone corticosterone (CORT) in females in mixed morph pairs (Griffith *et al.* 2011). However, this study considered only baseline CORT, which does not have a consistent relationship with fitness (Bonier *et al.* 2009); only stress-induced CORT levels were correlated with condition indices in the closely related Zebra finch (*Taeniopygia guttata*), where a small change in condition was associated with large changes in peak CORT (Crino *et al.* 2016). Therefore, measuring the physiological stress response may yield larger effect sizes for any mal-adaptive pairings in the wild. Furthermore, domesticated Gouldian finch morphs showed differing physiological responses to social environment and diet (Pryke *et al.* 2007; Pryke *et al.* 2012), which is worth investigating in the wild birds given their alternate strategies with regard to reproductive resources (Brazill-Boast, Griffith, *et al.* 2013).

An examination existing data in wild Gouldian finches shows no evidence of body condition differences or age specific mortality according to genotype or incompatibility selection. The most powerful way to approach the question of genotype specific mortality would be to track cohorts of individuals from hatching through adulthood. In the present analyses, any signal of age specific genotype selection may have been lost through the inclusion of multiple cohorts of individuals, particularly in the adult age class. Sadly, tracking the survival of individual Gouldian finches beyond fledging is highly impractical. After the breeding season local populations either perish, or more likely move out of range of radio-tracking or recapture efforts, with few (if any) individuals being recaptured or observed in subsequent years (Woinarski and Tidemann 1992; Legge *et al.* 2015; Bolton, West, *et al.* 2016). Given the scale, and remoteness of the environment over which Gouldian finches are likely to range, it will require an intensive and regionally coordinated effort to achieve enough mark-recapture data to use in models that account for missing data (e.g. Des Roches *et al.* 2017). In the meantime, directly studying the physiological responses (such as CORT levels) of parents and offspring in same and mixed morph pairs may prove more insightful, but will

again be limited by the low natural availability of informative crosses ($Z^rW+Z^RZ^r$ and $Z^RW+Z^RZ^r$).

Based on the current collection of work on the Gouldian finch (Pryke and Griffith 2009b; Brazill-Boast, Griffith, *et al.* 2013; Bolton *et al.* 2017), the most conservative conclusion is that the red and black morphs are only incompatible in the domesticated population. The domestication process itself may be responsible for genetic incompatibility between morphs, through genetic bottlenecks, selective breeding and breeding populations isolated by morph. Previous work found that there is genome-wide admixture between morphs in the wild (Kim 2011), but there was evidence of reduced gene-flow between domesticated morphs (Bolton *et al.* 2017). Therefore, future work would be more fruitful to investigate the origin of incompatibility in the domesticated population, and explore other mechanisms maintaining Gouldian finch morphs in the wild.

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Appendix

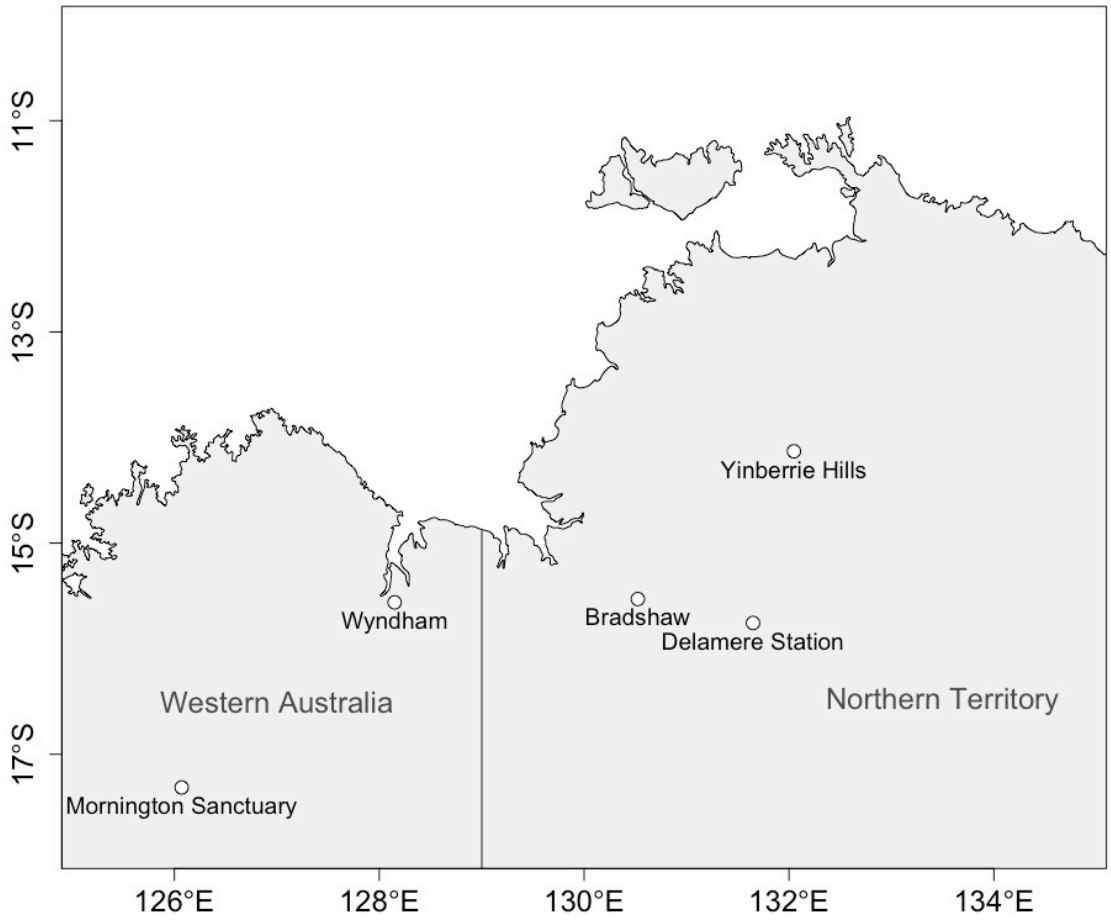

Figure S1: Map of northern Australia, showing the locations at which Gouldian finches were mist-netted. Individuals in Wyndham were collected by JBB, all sites in the Northern Territory were collected by KM, and Australian Wildlife Conservancy's Mornington Sanctuary were collected by SL and KM**.**

Table S1: Bonferroni corrected p-values for pairwise fisher tests in comparison of morph frequencies across the five sampling localities. Where test (1) is the p-value for differences in female allele/genotype frequency, (2) male allele frequency, (3) combined male and female allele frequency, and (4) changes in the frequency Z^rZ^r genotype.

	Mornington				Wyndham				Bradshaw				Delamere			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Wyndham	1.00	1.00	1.00	1.00												
Bradshaw	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
Delamere	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
Yinberrie Hills	1.00	1.00	1.00	1.00	1.00	0.47	0.42	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table S2: Summary of individuals in each age class by genotype at Wyndham in 2008 and 2009. The chick dataset includes only chicks that are siblings and half-siblings and the sample is across 51 families, from each one individual was resampled randomly to remove effects of family structure.

Phenotype	Genotype	Chick	Juvenile	Adult
Female: black-head	Z^rW	119	28	97
Male: black-head	Z^rZ^r	82	23	107
Female: red-head	Z^RW	12	2	12
Male: red-head	Z^RZ^r	25	9	34
Male: red-head	Z^RZ^R	2	0	1

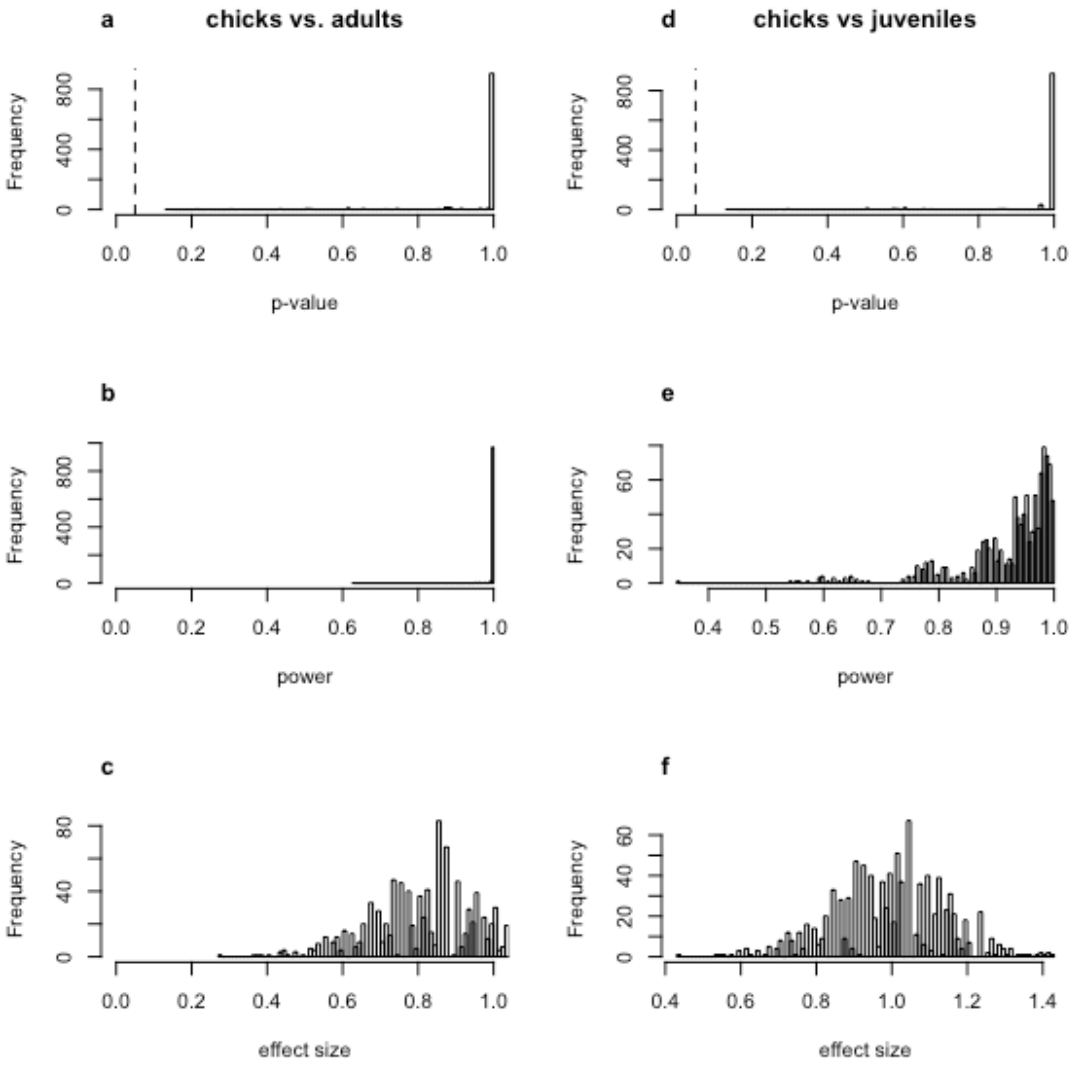

Figure S2: The left hand side corresponds to Fisher's exact tests on changes in heterozygote (Z^RZ^r) genotype frequency between chicks and adults where, panel a) shows the distribution of p-values, where the proportion of significant results=0. Panel b) shows the statistical power for each test, and c) is the effect size of each comparison. The right hand side corresponds to Fisher's exact tests on changes in heterozygote (Z^RZ^r) genotype frequency between chicks and juveniles where panel d is the distribution of p-values, and the proportion of significant results=0. Panel e) is the statistical power for each test, and panel f) is the effect size of each comparison.

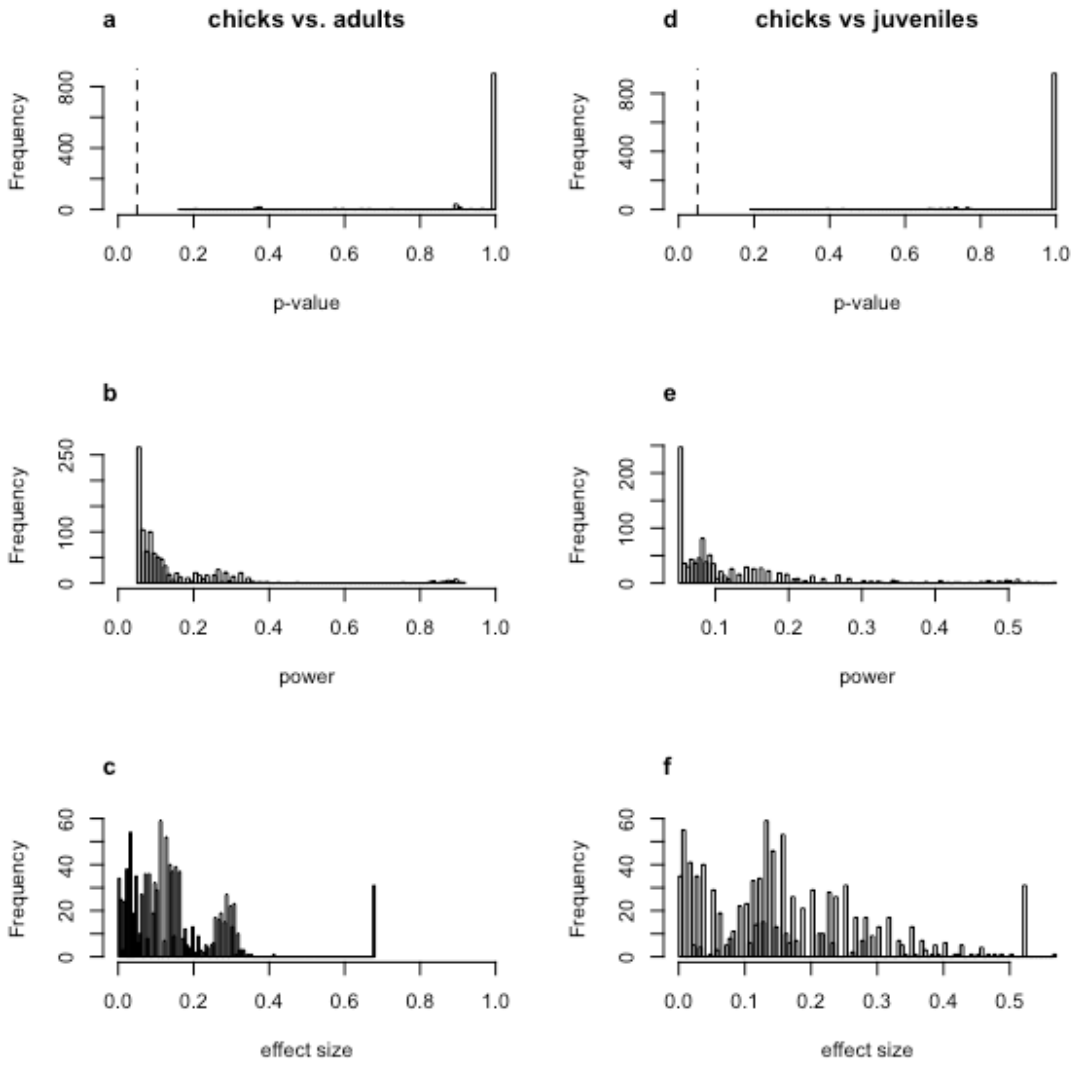

Figure S3: The left hand side corresponds to Fisher's exact tests on changes in head colour allele frequency in females between chicks and adults where, panel a) shows the distribution of p-values, where the proportion of significant results=0. Panel b) shows the statistical power for each test, and c) is the effect size of each comparison. The right hand side corresponds to Fisher's exact tests on changes in head-colour frequency between chicks and juveniles, where panel d) is the distribution of p-values, and the proportion of significant results=0. Panel e) is the statistical power for each test, and panel f) is the effect size of each comparison.

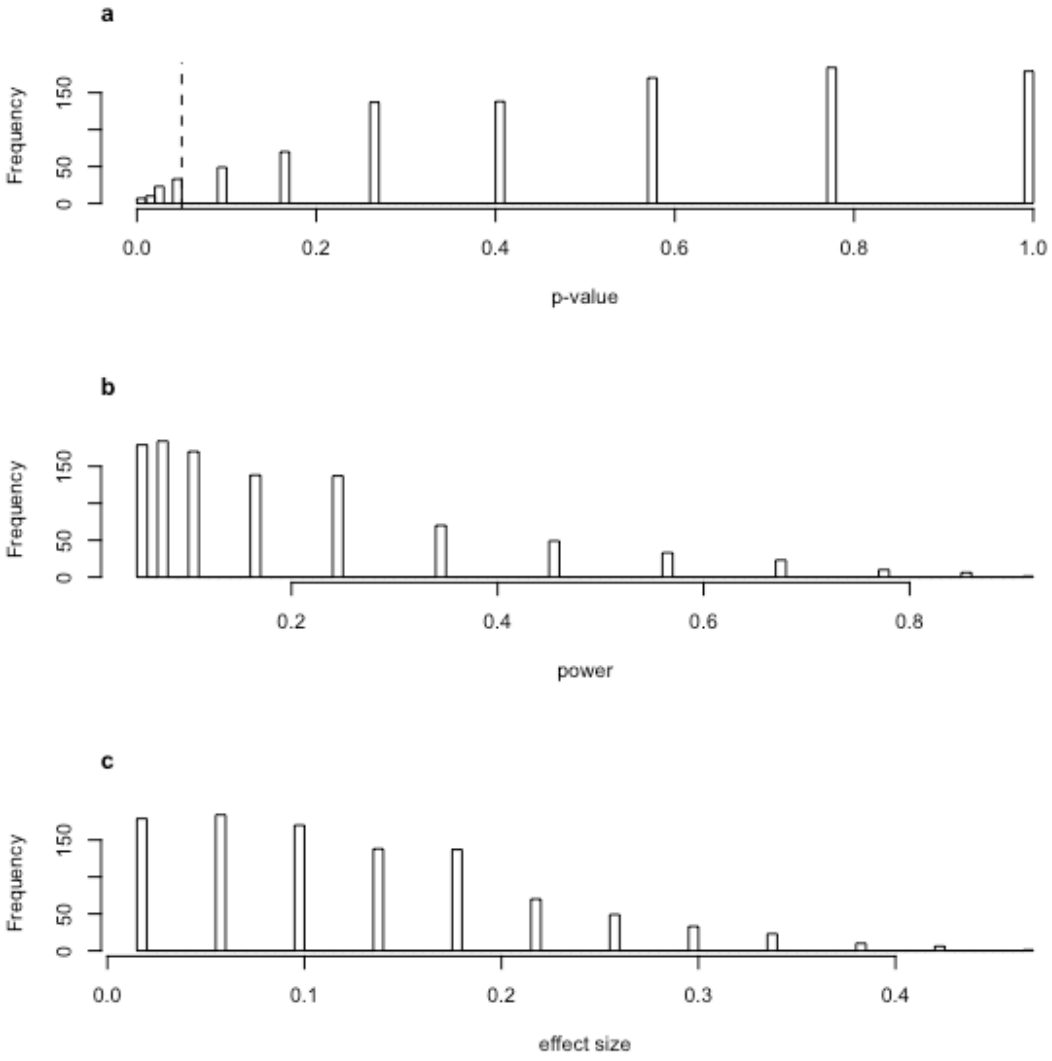

Figure S4: Panel a) distribution of p-values for binomial exact test for deviation of sex ratio from parity in the chick dataset=0.073. Panel b) shows the power of each test, and c) shows the effect size.

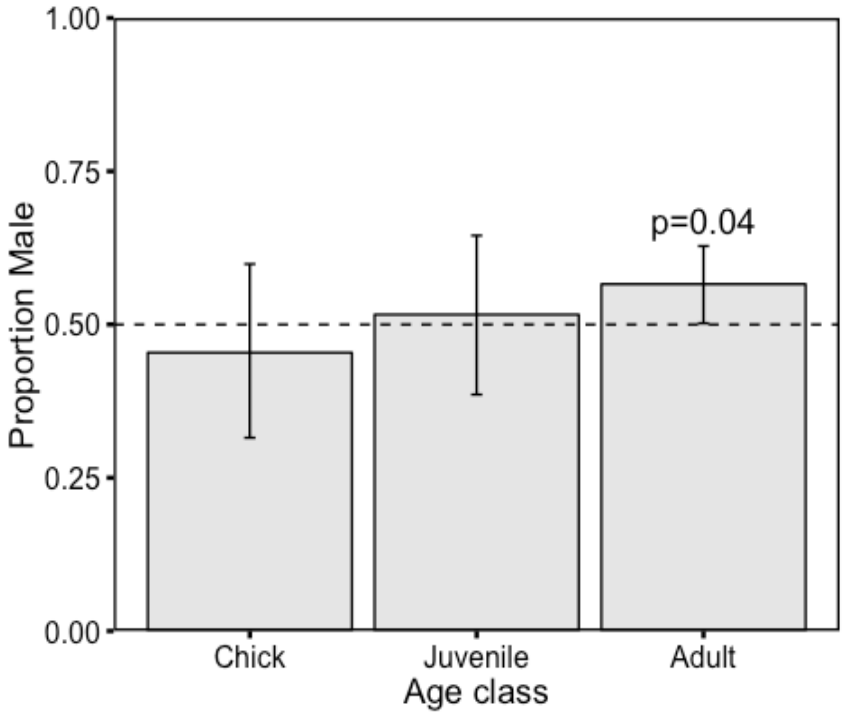

Figure S5: Proportion male offspring with 95% binomial confidence intervals in each age class. The frequency of males represents the mean proportion of males and its 95% binomial confidence intervals across 1000 resamples.

Table S3: Sample sizes of Gouldian finches with body condition measurements, per head-colour genotype as determined by molecular marker for head-colour genotype. Also includes sample sizes for each genotype in the chick dataset, where body size data was taken on day 14 chicks from 41 of the original 51 families

Genotype	Wyndham	Northern Territory	Wyndham chicks
Z^rW	74	53	84
Z^rZ^r	78	62	57
Z^RW	8	9	9
Z^RZ^r	25	28	18
Z^RZ^R	1	4	2

Table S4: Variable coefficients from Generalised Least Squares (GLS) regression exploring whether there is assortative mating with respect to body condition in pairs at Wyndham (*male condition ~ season + female condition*)

	Estimate	Standard error	t-value	p-value
Intercept	0.443	0.215	2.059	0.049
Season=2009	-0.360	0.462	-0.778	0.443
Female condition	0.483	0.172	2.807	0.009

Table S5: Output from linear mixed model 3 exploring the effect of mean parental condition and pairing type on offspring condition (*chick condition ~ season + mean parental condition + pairing type* + (1|*Family ID*))

	Estimate	Standard error	t-value	p-value
(Intercept)	0.620	0.390	1.589	0.115
Season=2009	-0.970	0.687	-1.411	0.171
Mean parent condition	0.835	0.301	2.777	0.010
Pairing: $Z^rW+Z^RZ^r$	-1.310	0.663	-1.974	0.059
Pairing: $Z^RW+ Z^rZ^r$	0.684	1.045	0.654	0.519
Pairing: $Z^RW+Z^RZ^r$	-2.146	1.450	-1.480	0.151

Chapter Five

Three molecular markers show no evidence of population genetic structure in the Gouldian finch (*Erythrura gouldiae*)

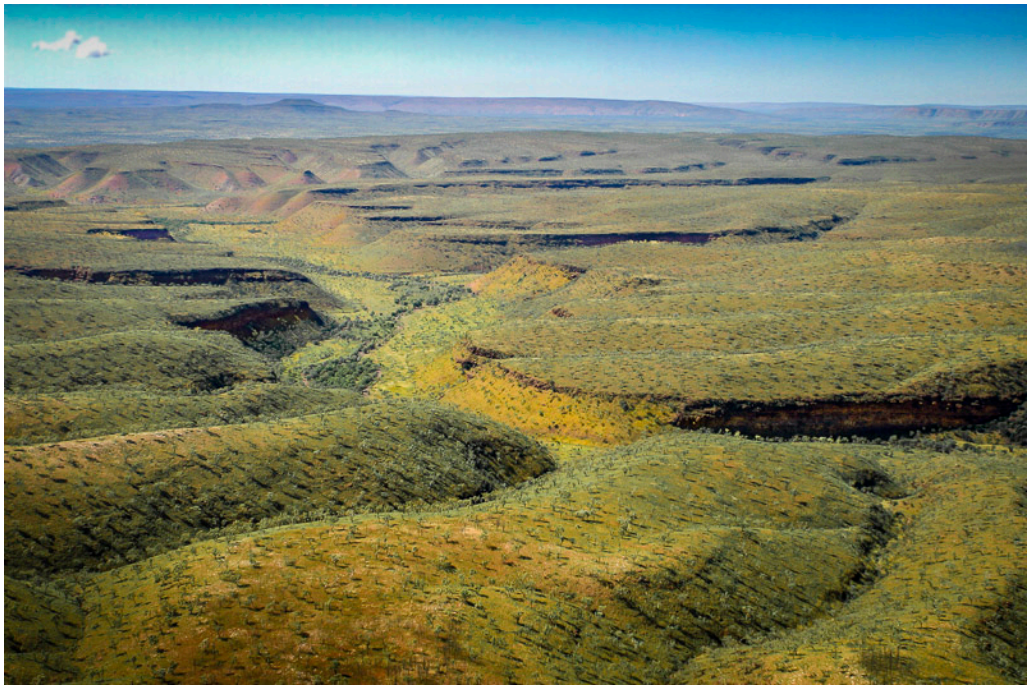

Photo: Mike Fidler

Chapter Five Vignette

The assessment of genetic diversity and gene-flow are a core part of species conservation management. Characterising the amount of genetic diversity, and its geographic patterns are essential in predicting risk factors of drift and inbreeding. It gives an indication of how far individuals are capable of moving across the landscape, and therefore how susceptible a particular region is to loss of further genetic diversity. In this chapter I use the most extensive sampling and powerful genetic datasets to date to explore genetic diversity and patterns of geographic structure in the contemporary populations of the Gouldian finch. The key finding is there is evidence for unfettered gene-flow across the western part of the range, spanning more than 700km, which is suggestive that individuals may be capable of moving long distances.

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PLOS | ONE

RESEARCH ARTICLE

Three Molecular Markers Show No Evidence of Population Genetic Structure in the Gouldian Finch (*Erythrura gouldiae*)

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Abstract

Assessment of genetic diversity and connectivity between regions can inform conservation managers about risk of inbreeding, potential for adaptation and where population boundaries lie. The Gouldian finch (*Erythrura gouldiae*) is a threatened species in northern Australia, occupying the savannah woodlands of the biogeographically complex monsoon tropics. We present the most comprehensive population genetic analysis of diversity and structure the Gouldian finch using 16 microsatellite markers, mitochondrial control region and 3,389 SNPs from genotyping-by-sequencing. Mitochondrial diversity is compared across three related, co-distributed finches with different conservation threat-statuses. There was no evidence of genetic differentiation across the western part of the range in any of the molecular markers, and haplotype diversity but not richness was lower than a common co-distributed species. Individuals within the panmictic population in the west may be highly dispersive within this wide area, and we urge caution when interpreting anecdotal observations of changes to the distribution and/or flock sizes of Gouldian finch populations as evidence of overall changes to the population size of this species.

Introduction

Robust estimates of population parameters, such as size and connectivity, are of vital importance to effective conservation and wildlife management. Connectivity describes the movement of individuals, genes and behaviour between regions or groups of individuals, and the degree of connectivity can be used to define populations in a genetic and demographic sense [1]. There is a long history of directly assessing total population size, population growth rates and regional connectivity using methods such as visual observation, mark-recapture and radio

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Competing Interests: The authors have declared that no competing interests exist.

tracking [2,3]. These methods are not always logistically feasible or reliable in species that have cryptic behaviour, inhabit remote areas, have large geographic distributions, or persist at low densities [3]. Population size and connectivity estimates derived from these methods may be additionally confounded when individuals or groups are highly mobile (e.g. nomadic or migratory), or numbers fluctuate seasonally [4]. In these situations, population genetics may fill knowledge gaps or improve estimates of population size and connectivity.

The Gouldian finch (*Erythrura gouldiae*) is one such species that could benefit from population genetic investigation, because the absence of robust population estimates has hindered conservation decision making. In the 1970s, Gouldian finch populations experienced strong declines (up to 87%), and became restricted to a few small areas in Western Australia and Northern Territory, and were virtually extirpated from Queensland [5,6]. First identified as Threatened by IUCN Red List in 1988, the Gouldian finch was recently down-listed to Near Threatened by the IUCN, based on population data compiled from largely *ad hoc* observations [7]. Many of these observations come from resident or bird watcher observations that report large flocks (400 and >1000) in geographically distant locations [7]. These observations are unreliable for estimation of total population size because observations are not carried out systematically throughout the range, and occur mostly in the accessible dry season when birds aggregate around the diminishing watering holes. Lack of systematic surveys means that the same individuals or entire flocks may be counted more than once on different days and in different locations, and depending on vagility even geographically distant observations may be pseudoreplicated. Furthermore, these observations of large flocks are largely juveniles, who either die before breeding or never return to their natal area, and may not be representative of the number of breeding pairs in the local area [8].

The Gouldian finch is distributed across the savannah woodlands of the wet-dry tropical regions of Australia, and across a number of major biogeographic boundaries [9,10] including the Ord Arid Intrusion, which has been previously identified as important in maintaining subspecific variation in the related long-tailed finch [11]. Therefore, we might expect there to be population genetic structure corresponding to these barriers, depending on the species' ability to disperse. There are conflicting reports about the movement capacity of the Gouldian finch, which may vary according to the season. Early reports suggest migrations within North Queensland in and out of breeding grounds [12,13], More recently, there have been anecdotal reports of birds travelling long distances between localities and outside the breeding range [7]. In a banded population of Gouldian finches studied at Australian Wildlife Conservancy's Mornington Sanctuary, in the Kimberley, Western Australia, the maximum distance between re-sightings and recaptures was 20 km [14], and radio-tracking suggests birds can move within a 3 km radius within a day [8]. Australian Bird and Bat Banding Scheme records show the average recovery distance for banded birds within and between years is 1 km. Although the typical banding activity in remote areas in Australia tends to be highly focused on a particular area, and most birds are re-trapped in that area, and very rarely re-trapped at other remote sites due to no, or very low banding effort at other sites. At locations where Gouldian finches are regularly banded, the return rates between years are low compared to co-distributed Estrildid finches (1%-17% in the Gouldian finch, 15–60% in long-tailed finches), and show much variation in the total number of individuals in any given year ([8,14,15], S1 Appendix). It is unknown whether low recapture rates represent high mortality, long distance movement patterns or some combination of these.

Previous population genetics analyses on the Gouldian finch has suggested a lack of any genetic structure and high gene-flow, but relatively limited sampling and the genetic markers used have not allowed for robust conclusions [16,17]. The first population study on this species found no significant differences in allele frequency in the Myoglobin intron from samples taken across the geographic range (three sites in the west and a sample of seven birds from

Georgetown in Queensland) [16]. However, a single coding locus may have reduced diversity due to selection and does not provide adequate statistical power to draw conclusions about population connectivity. More recently, data from six microsatellite loci and mitochondrial control region sequences from samples collected at two geographically distant localities (one in Western Australia and one in Northern Territory) indicated no evidence of structure [17]. It is possible that structure may not have been detected in this study due to poor geographic coverage of the species' distribution [18], and the relatively small number, and characteristics of the loci employed may have lacked sufficient power to detect weak differentiation [19]. Additionally, while this study explored historical gene-flow, it is of greater use to management to compare these estimates to those from methods capable of assessing current gene-flow [17]. In this paper, we perform a thorough investigation of Gouldian finch population genetics, to determine whether population structure exists, particularly across the major biogeographic boundary of the Ord Arid Intrusion and across the Kimberley Plateau. We used mitochondrial control region sequences, sixteen microsatellite markers, and 3,839 SNP loci to infer levels of genetic diversity and differentiation across the Gouldian finch range. From these data, we ask whether locations that are reliable for catching and sighting Gouldian finches should be considered separate management units. Finally, we explore these results in the context of diversity in a co-distributedAustralian finch, and the consequences of our results for conservation of the Gouldian finch.

Methods

Sample collection

Samples were collected from six locations across the range of the Gouldian finch in Australia between 2004 and 2013 (Fig 1). The sampling localities focus on areas of historical and contemporary high abundance in the Gouldian finch, reflected in the heat-map based on occurrence density in Fig 1 from Atlas of Living Australia data [20]. These data are from 1987

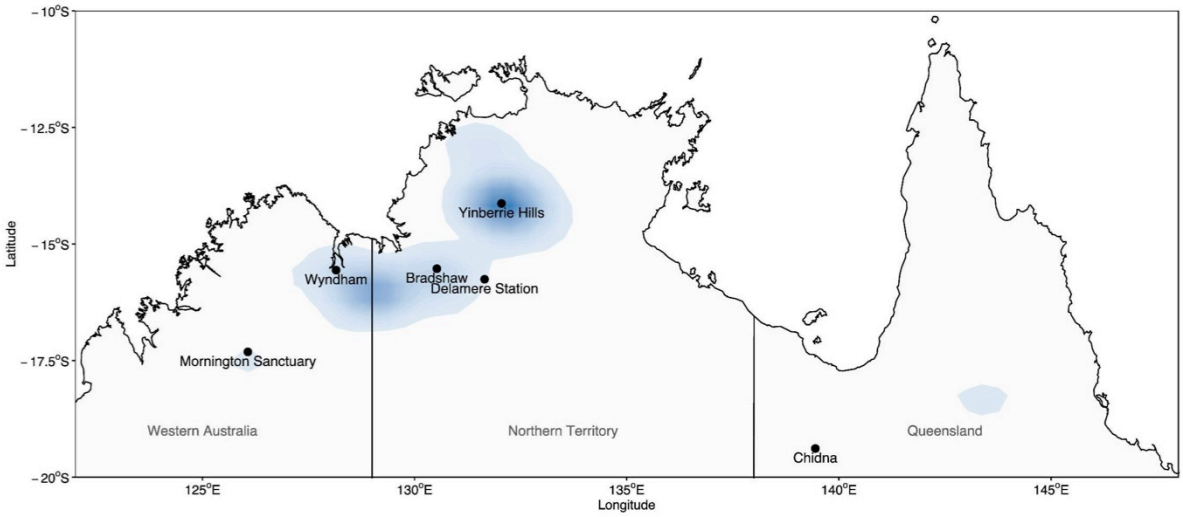

Fig 1. Map of the north of Australia, showing the locations blood samples were collected between 2004 and 2013. Heat map indicates the density of Gouldian finch presence data from Atlas of Living Australia [20] since trapping became illegal in 1987, where darker blue indicates high occurrence density. Background map reprinted from [22] under a CC BY 4.0 license, with permission from the Australian Bureau of Statistics, Original Copyright 2011.

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onwards, when Gouldian finch trapping was banned. We refer to these five sites (Mornington through Yinberrie Hills) in this area as "the western range". Considerably fewer individuals were caught at Chidna because Gouldian finches appear to be less abundant in the east [7]. The majority of birds were caught in the breeding season (late wet season; January-August). Birds at all sites except Wyndham were banded and bled next to waterholes. Birds were caught using a mist-net over a few hours after dawn. Under Australian federal law, all birds must be fit with a unique metal band supplied by the Australian Bird and Bat Banding Scheme (ABBBS), and the handler must be certified by the ABBBS as a competent handler and bander. Blood was drawn by puncturing the brachial vein with a 26-gauge needle and collecting a sample volume of <60μL with a capillary tube. Samples taken for the purposes of genetics and were bled immediately after capture, those taken for hormone analysis were taken between 5 and 60 minutes after capture [21].The birds were restrained using ringers grip, no anaesthetics were administered as the bleeding procedure takes less than two minutes per bird. Blood samples were stored in 95% ethanol or Queen's Lysis Buffer.

As part of a larger study to compare breeding ecology of two sympatric hollow-nesting finches, the site at Wyndham was supplemented with nest-boxes and the breeding of Gouldian and long-tailed finches was monitored [15,23,24]. Birds were caught at nests with hand nets when nestlings were more than 14 days old to avoid nest desertion. Birds were also caught at nearby waterholes with mist-nets and provided with unique bands and bled as described above. Under both scenarios birds were confined for less than ten minutes. For comparison with long-tail finches at the same site [15], we provide recapture rates for different life-history stages of the Gouldian finch across six years (S1 Appendix).

For different analyses and molecular techniques we used different subsets of samples, based on quality and quantity of DNA extract. For each analysis, we aimed for similar sample sizes across all five extensively sampled populations, because sample size differences can bias some estimates of genetic differentiation [25].

Molecular methods and analysis of genetic data

Gouldian finch DNA was extracted from blood samples using a Qiagen PureGene Kit, and subsequently used in microsatellite, mtDNA and genotyping-by-sequencing analyses.

Microsatellites. Because we had access to a large number of samples over a 10 year period, microsatellite analyses were conducted with two different subsets of individuals to answer different questions about genetic structure. We used 93 breeding individuals from the Wyndham population in 2008 and 2009 [23,24,26] to examine fine-scale genetic structure. All other analyses are based on a random subset of individuals matched to the sample size of the smallest locality (49 individuals), plus an additional six individuals from Chidna in Western Queensland. Twenty-two microsatellite loci were amplified across five multiplexes, according to protocols listed in S2 Appendix. These 22 loci were checked for amplification consistency, null alleles, and conformity to Hardy-Weinberg Equilibrium (S2 Appendix) using ARLEQUIN v.3.5.2.2 [27]. Linkage disequilibrium between loci was tested in GENEPOP v4.2 [28]. Hardy-Weinberg and linkage disequilibrium were corrected for multiple testing using Bonferroni correction. Loci that were inconsistent or violated assumptions were removed from downstream analyses.

We then used ARLEQUIN to tabulate the overall allelic richness (N_A), observed and expected heterozygosities (H_O, H_E), and GenAlex v6.502 to calculate private alleles [29]. Differences in allelic richness and heterozygosity in each of the five major sampling localities was conducted by pairwise Wilcoxon sign-rank tests, and Bonferroni corrected for multiple testing. Measures of allelic richness are sensitive to differences in sample size, so allelic richness

per locus at the five major localities was rarefied to match the sample size at Chidna using ADZE 1.0 [30]. The rarefied richness values were then compared using pairwise Wilcoxon sign-rank tests with Bonferroni correction.

To obtain measures of global differentiation and inbreeding, we conducted an Analysis of Molecular Variance (AMOVA) in ARLEQUIN [27] with 10,000 permutations. We calculated the number of private alleles per population using GenAlex [29,31]. We used pairwise F_{ST} and AMOVA (global F_{ST}) to estimate genetic structure between sampling localities in ARLEQUIN [27], with 10000 permutations to identify statistical significance. Given that F_{ST} can under-estimate differentiation at highly polymorphic loci [32–34], for all pairwise comparisons we also calculated Jost's D [35] using R package '*DEMEtics*' with p-values calculated by 1000 bootstrap resamples [33]. Continuous populations may not exhibit evidence of pairwise or global differentiation, but may show a pattern of increased differentiation with distance—isolation by distance (IBD). We measured IBD in ARLEQUIN using a Mantel test against a matrix of pairwise geographic distances between sampling localities, with 10000 permutations [27]. We also conducted individual-based genetic clustering analyses because sampling localities may not reflect actual populations [36]. These techniques are useful because they find genetic clusters without *a priori* population definitions. We used STRUCTURE v 2.3.4 [37] to estimate the number of genetic groups in our dataset. We compared the effect of the location as a prior (LOCPRIOR: [38]) against the standard model with no location prior on the resulting genetic clusters. The location prior does not define populations strictly *a priori*, but considers individuals that are sampled together to be more likely from a genetic cluster [38]. This method is sensitive to subtle population structure, but will not falsely detect structure [38]. We used admixture models with correlated allele frequencies. The length of the burn-in was 100,000, followed by 1,000,000 MCMC, with K (number of clusters) set between 1–10 and 10 iterations per value of K. K was determined by comparison of plots of Ln P(D) and ΔK [39] using STRUCTURE HARVESTER v0.6.94 [40].

As an independent assessment of number of genetic clusters, we also ran a population genetic model-free ordination clustering method in R-package '*adegenet*' [41]. These ordination techniques have the advantage that it does not rely on any particular population genetic model (such as minimising deviation from Hardy-Weinberg equilibrium as in STRUCTURE) to discern the number of clusters [41,42]. We used the *find.clusters* function to select clusters, which reduces the genetic data into Principal Components and runs a k-means clustering analysis (where k is the number of clusters) and weights results according to the Bayesian Information Criterion (BIC), and we retained all principal components for this analysis. Subsequently, we performed a Discriminant Analysis of Principal Components (*dapc*) using R-package '*adegenet*' [41,42], which takes *a priori* clusters and maximises the distances between them. We also ran *dapc* using the collection localities as prior groupings in an effort to explore geographic structuring by maximising the multivariate distances between sampling localities. Using the selected clusters, we ran model validation on ability of the model to correctly assign individuals to their clusters using *optim.a.score* and *xvalDapc* functions. For more information please see S4 Appendix.

Some movement information we have on Gouldian finches, based on band recoveries and radio-tracking, suggests that many individuals may be restricted to a 5km area, at least over short periods. Low recovery rates in the banding data suggest very limited natal philopatry in these birds, but we used genetic data as an independent test of this. If movement is limited, and natal philopatry is high, we might expect to see some evidence of spatial structure on the scale of a few kilometres. Spatial autocorrelation takes pairwise genetic distances and pairwise geographic distances between individuals and provides a measure of autocorrelation (r)–and by proxy genetic similarity—between them [43]. Under restricted dispersal, geographically

proximate individuals should have shorter genetic distances between them, and will show a signature of positive autocorrelation at this spatial scale. We used this spatial autocorrelation approach to investigate restricted movement at the local scale. Spatial autocorrelation was conducted on individuals nesting in 2008 and 2009 over ~12km in our Wyndham study site [24,26]. Significant autocorrelation (either positive or negative) was determined by 1,000 bootstrap resamples against 1,000 permutations of a null hypothesis constituting no spatial structure [43]. All analyses were conducted in conducted in GenAlex v6.502 [29,31], and were partitioned according to sex to investigate whether there was sex-biased dispersal [44].

mtDNA. For a subset of individuals (sample sizes in Table 1), we sequenced mitochondrial control region domain 1. We amplified a 330bp segment using primers and protocols developed in the closely related long-tailed finch [11]. Final sequences were checked using python programme SEQTRACE v 0.9.0 [45].

We examined the mitochondrial genetic diversity from a subset of individuals across the five western populations, plus we explored the five individuals from Chidna in the east. Haplotype richness (H), haplotype diversity (*h*) and nucleotide diversity (π) was calculated using

Table 1. Summary of various measures of genetic diversity with (±) sampling standard deviation for microsatellite, mitochondrial and SNP datasets.

Parameter	Mornington	Wyndham	Bradshaw	Delamere	Yinberrie Hills	Chidna	Overall
Microsatellites							
N	49	49	49	49	49	6	251
N_A	13.88	14.13	14.31	14.31	14.63	5.69	21.81
N_{PA}	1.06	0.88	1.31	1.06	1.12	0.18	NA
H_O	0.77 (±0.12)	0.77 (±0.13)	0.76 (±0.15)	0.79 (±0.14)	0.77(±0.14)	0.80 (±0.15)	0.77 (±0.12)
H_E	0.81 (±0.12)	0.81 (±0.11)	0.81 (±0.12)	0.81 (±0.11)	0.81 (±0.11)	0.80 (±0.14)	0.81 (±0.11)
F_{IS}	0.04*	0.04**	0.06***	0.02	0.05**	0.04*	0.04**
Mitochondrial control region							
N	32	35	25	32	23	5	152
S	6	9	10	5	6	4	14
H	8	10	12	5	8	3	20
H_R	6.8 (± 0.96)	7.8 (± 1.11)	11.4 (± 0.66)	4.7 (± 0.46)	NA	NA	NA
H_P	1	2	6	0	2	0	NA
h	0.68 (± 0.07)	0.80 (± 0.04)	0.83 (± 0.07)	0.71 (± 0.06)	0.71 (± 0.09)	0.70 (± 0.22)	0.76 (± 0.07)
$\pi x10^2$	0.37 (± 0.07)	0.50 (± 0.07)	0.59 (± 0.10)	0.39 (± 0.07)	0.51 (± 0.08)	0.61 (± 0.21)	0.47 (± 0.03)
Genotyping-By-Sequencing SNPs							
N	52	47	48	53	48	3	251
N_S	3817.7 (±18.9)	3816.0 (±20.9)	3826.9 (±10.2)	3818.4 (±20.5)	3823.3 (±17.9)	3827.7 (±4.0)	3820.3 (±18.3)
S	3838	3836	3837	3835	3837	2497	3839
H_O	0.30 (±0.17)	0.30 (± 0.17)	0.30 (±0.17)	0.30 (± 0.17)	0.30 (±0.17)	0.48 (± 0.26)	0.30 (± 0.16)
H_E	0.30 (± 0.15)	0.30 (±0.15)	0.30 (± 0.14)	0.30 (± (0.15)	0.30 (± 0.14)	0.47 (± 0.11)	0.30 (± 0.14)

The table describes each population, the number of individuals used in the analysis (N), and the observed heterozygosity (H_O), expected heterozygosity (H_E), number polymorphic sites (S). Diversity measures specific to the microsatellites are: the average no alleles per locus (richness) (N_A), number of private alleles per locus (N_{PA}), and Inbreeding Coefficient (F_{IS}), with degree of significance indicated by number of asterisk. Diversity measures specific to the mitochondrial data are: raw number of haplotypes (H), rarefied number of haplotypes to n = 23 (H_R), private haplotypes (H_P), haplotype diversity (*h*), and nucleotide diversity (π). Measures specific to SNPs are the average number of sites across individuals in a population (N_S). NA indicates the parameter was not calculated for that population, either due to sample size constraints, or it was not a relevant parameter.

* is p<0.05

** is p<0.005

*** is p<0.0005

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DnaSP v5.10.1 [46]. We calculated the rarefied haplotype richness (H_R) based on the smallest sensible sample size (at Yinberrie Hills, n = 23) was calculated in Analytic Rarefaction 2.0 [47].

We conducted an AMOVA and population pairwise genetic differentiation measures in ARLEQUIN. We calculated differentiation between sampling localities using haplotype frequencies (F_{ST}) and nucleotide diversity ϕ_{ST} calculated with the Kimura 2-parameter model. All significance tests were run with 10,000 permutations. In addition, we examined the evolutionary relationships between haplotypes using a median-joining network ($\varepsilon = 0$, [48]) using the programme PopART (http://popart.otago.ac.nz).

Genotyping-by-sequencing. We also used a reduced-representation next generation sequencing approach to obtain SNPs from across the genome. We sequenced 285 individuals that included a subset of individuals from our six populations (Mornington = 56, Wyndham = 57, Bradshaw = 57, Delamere = 56, Yinberrie Hills = 55, Chidna = 4). Populations were randomised across three plates, and 12 individuals were duplicated across all three plates, to ensure there were no lane or library effects, which can cause artificial substructure in the data.

We sent DNA extracts to Cornell Institute of Genomic Diversity for library preparation and sequencing according to their in-house Genotyping-by-Sequencing (GBS) methodology [49]. This is a reduced representation approach, similar to RADseq [50], that sequences short sections of the genome downstream from restriction enzyme cut-sites. We used restriction enzyme *Pst1*; *in silico* digests using '*SimRAD*' suggested this enzyme yields 952,644 cut-sites across the genome of the related Zebra finch *Taeniopygia guttata* [51,52]. Each plate was multiplexed into three lanes on an Illumina HiSeq 2500 (100bp single-end reads).

Raw reads were processed into SNP genotypes using the reference-free bioinformatics pipeline designed for this particular methodology: Universal Network Enabled Analysis Kit (UNEAK) [53] in TASSEL 3.0 [54]. Reads must have been recorded at least five times to be included as a tag for further analysis, and an error tolerance rate of 0.03 was used to identify reciprocal tag pairs for SNP calling [53]. SNPs with a minimum minor allele frequency (mnMAF) of 0.00, 0.01 and 0.05 were called across the entire dataset using TASSEL. We compared the results of the three mnMAFs, and they did not affect the interpretation so we chose the most conservative dataset (mnMAF = 0.05). All resulting SNPs were subsequently filtered using VCFtools [55] according to the following quality criteria: a) minimum read depth of five reads per genotype; b) sites with an outlying number of reads, as these can represent gene duplications or repetitive regions. An arbitrary threshold of >28X was chosen based on the frequency histogram of the number of reads; c) removing individuals with outlying high and low average sequencing depths, and overall heterozygosity by removing the bottom and top ten percent of individuals in these categories. Then the data were filtered for missingness according to site and individual criteria: a) sites not represented in >80% of individuals [56]; b) individuals with >50% or >30% missing genotypes. Filtering individuals by >30% or >50% missing genotypes did not affect the results, so we present the data based on those with more individuals (>50%).

We measured heterozygosity, number of polymorphic sites per population using ARLEQUIN v.3.5.2.2 [27]. We conducted an AMOVA and pairwise F_{ST} between sampling localities using pairwise distance for the underlying distance matrix in ARLEQUIN [27], and 10,000 permutations.

Genetic clustering analysis was conducted in a Bayesian clustering programme FASTSTRUCTURE v1.0 designed to process large SNP datasets quickly [57]. This programme is based on a variational Bayesian inference framework, which does not necessitate user set sampling of parameter space (e.g. MCMC reps). As a preliminary step to detect strong genetic structure in the SNP data, we ran models with 1 to 10 genetic clusters (K), using a 'simple' or flat prior of population specific allele frequencies. This method reports two new measures of K

that explain the data in different ways, and can provide a range of K values to run further tests with more sensitive priors [57]. As in the microsatellite dataset, we also ran a discriminant analysis on this data to infer genetic clusters.

Mitochondrial richness in co-distributed Australian finches

Genetic diversity is reduced in threatened taxa, relative to non-threatened taxa reflecting population bottlenecks and small population sizes [58–61]. We used data from the same section in the mitochondrial control region from a related and co-distributed finches to provide context for the measures of genetic diversity. We compared genetic diversity in the co-distributed, but 'Least Concern' status long-tailed finch (*Poephila acuticauda*) using previous data from the mitochondrial control region [11]. Measures of allelic richness are more sensitive to population size changes than diversity or heterozygosity [62], but must be corrected for sample size [63]. We rarefied haplotype richness of the long-tailed finch (n = 274) to match the smaller sample of the Gouldian finch (n = 152) in the program Analytic Rarefaction 2.0 [47]. Point estimates of rarefaction cannot be directly compared statistically, but we used the rarefaction sampling variance and 95% confidence intervals as a guide for whether mitochondrial control region haplotype richness was different between the different species. Estimates of diversity are much less sensitive to sample size, and therefore we directly compared uncorrected haplotype and nucleotide diversity estimates. Providing a sufficiently large sample size, central limit theorem predicts that the nucleotide diversity and haplotype diversity sampling variance derived from theory will approximate a normal distribution [64], which allowed us to use a t-test to compare diversity indices.

Results

After quality filtering of the microsatellite dataset (S2 Appendix), we retained 16 of 22 loci that were in Hardy-Weinberg Equilibrium (after Bonferroni correction $p<0.000625$) (Table B in S2 Appendix). No pairs of loci were in linkage disequilibrium after Bonferroni correction (Table C in S2 Appendix).

Summary diversity statistics for the microsatellite data are presented in Table 1. Within collection localities, the microsatellite allelic richness was between 13.8 and 14.6 in the five major populations, and pairwise Wilcoxon sign-rank tests found no significant differences in richness at our five major sampling localities (Bonferroni corrected p-values = 0.36–1). Pairwise tests on rarefied richness to include the smallest sample at Chidna also found no significant difference (all Bonferroni corrected pairwise p-values = 1). Similarly, there was no significant difference in observed or expected heterozygosity between any of the localities (all Bonferroni corrected pairwise p-values = 1). For the uncorrected pairwise p-values please see Tables A and B in S3 Appendix.

The sample sizes for populations using mtDNA were smaller than those employed in the microsatellite analysis. Of the 330bp fragment amplified, 14bp were polymorphic and we identified 20 haplotypes (KX858950-KX585969). There was considerable variation in levels of mitochondrial richness between the collection localities (Table 1). Bradshaw locality had the highest private haplotype count and contained 60% of the total observed haplotypes, and had higher haplotype richness than the site with next highest richness (Wyndham rarefied to n = 25, H_R = 8.2, 6.14–10.0 95% CI, Bradshaw raw H = 12). The haplotype diversity was not significantly higher ($t_{1.93}$, df = 35.15,p = 0.062), but nucleotide diversity was significantly higher in Bradshaw than Wyndham ($t_{3.87}$, df = 40.26, p = 0.00039).

Genotyping-by-sequencing (GBS) yielded 735,164,326 raw reads across three Illumina lanes containing 96 samples each, with an average of 2,552,653.91 reads per individual or 1.6X reads per predicted site. After stringent filtering, we retained 3839 SNPs with a minimum site

depth of 5X and average 13.5X across 251 individuals. Like the microsatellites, measures of genetic diversity were consistent across the five main sampling localities (Table 1). No sampling localities had private alleles, but this is expected due to the high minimum minor allele frequency filtering.

Population structure

Analysis of molecular variance (AMOVA) on the microsatellite data showed that most genetic variation was contained within individuals, with less than 1% of variation attributed to between population differences, and 4.2% among individuals within populations. Inbreeding coefficients (F_{IS} and F_{IT}) were low but statistically significant (F_{IS} = 0.042, p = 0.00; F_{IT} = 0.043, p = 0.00) based on permutation tests, and individual population inbreeding coefficients (F_{IS}) are not consistent across populations (Table 1). SNP results showed similarly low variation between populations, but there was no indication of inbreeding (F_{IS}) using these markers (F_{ST} = -0.0001,p = 1; F_{IT} = -0.093, p = 1; F_{IS} = -0.093, p = 1). AMOVA based on mtDNA nucleotide diversity and haplotype frequency yielded slightly higher between population differentiation, but still with only 1.9% of genetic variation explained among sampling localities.

All measures of pairwise genetic differentiation between the collection localities was negligible and statistically non-significant after Bonferroni correction (Tables C-E in S3 Appendix). Haplotype frequencies between Chidna and all other populations were moderately differentiated (average Chidna Pairwise F_{ST} = 0.20, standard deviation = 0.046), and nucleotide diversity was significantly differentiated (average ϕ_{ST} = 0.25, standard deviation = 0.085) except with closest neighbour Yinberrie Hills, but none of these comparisons were significant after Bonferroni correction (Table D in S3 Appendix). The lack of population structure at mtDNA can be visualised in the median-joining network (Fig 2), which shows no pattern of unique haplotypes shared between different regions.

Mantel tests showed evidence of Isolation-by-Distance (IBD) in the mitochondrial dataset (ϕ_{ST}, β = 0.00034, p = 0.026; F_{ST} β = 0.00027, p = 0.039), and a significant effect in the microsatellite dataset (β = $9x10^{-6}$, p = 0.0064) and SNP datasets (β = $1x10^{-5}$, p = 0.04), but the regression coefficients in both analyses was small. When the poorly sampled and distant Chidna locality was removed from the analysis there was no significant effect in the microsatellite dataset (β = 0, p = 0.53), SNP dataset (β = 0, p = 0.43), or mitochondrial dataset (ϕ_{ST}, β = 0.000021, p = 0.27; F_{ST} β = -0.000004, p = 0.43).

There was no evidence of spatial genetic clustering from the STRUCTURE analysis. The results did not differ meaningfully with or without the use of the LOCPRIOR model [38], so we only present the latter results here. In the LOCPRIOR model, if the parameter *r* is less than one, it suggests that the location information is informative to the ancestry of the location [38]. Across all our repetitions, the mean r inferred was well above 1 (mean r = 11.94, standard deviation r = 1.26). The log probability of the data (genotypes given K clusters, LnP(D)) indicates the best model fit is for a single cluster (Fig 3C). If the rate of change in LnP(D) is used to infer number of clusters [39], then we find that the optimal number of clusters (ΔK) is two (Fig 3B), but this method is only able to make inferences about clusters greater than or equal to two. Indeed, the LnP(D) plot only shows a strong drop off in model fit after K = 2. Therefore, we plotted the ancestry proportions for each individual given two clusters. All individuals are equally admixed (Fig 3A) across the range, supporting a single genetic cluster. Furthermore, the fastSTRUCTURE method on the SNP dataset found the optimal number of clusters was one at both measures of K (Fig 3D).

In the microsatellite and SNP dataset, the k-means clustering method *find.clusters* in 'adegenet'[41] found that the lowest BIC was for one cluster (K = 1)(Figs A and D in S4 Appendix).

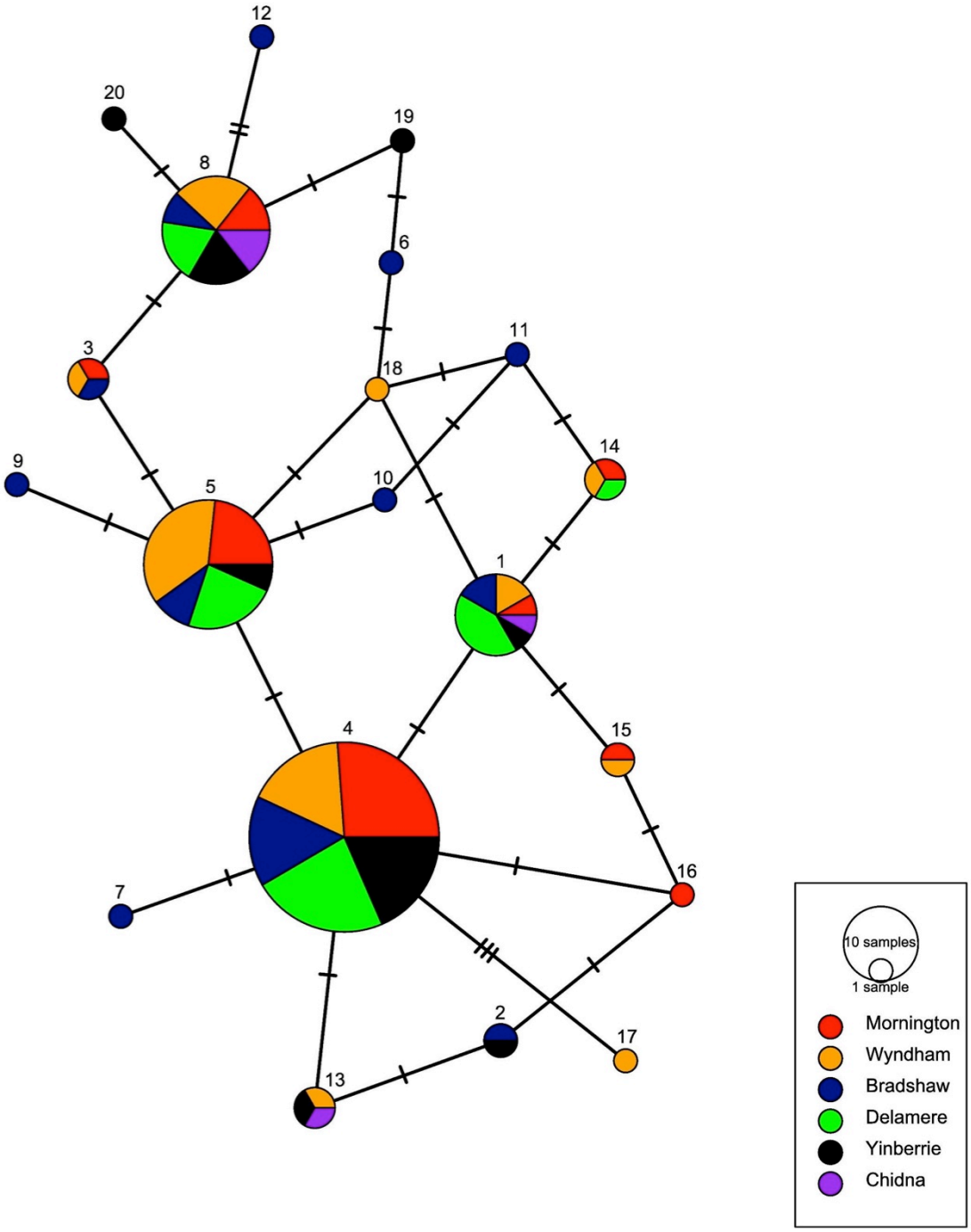
12
20
19
8
6
3
11
18
14
9
5
10
1
4
15
16
7
2
17
13
10 samples
1 sample
Mornington
Wyndham
Bradshaw
Delamere
Yinberrie
Chidna

Fig 2. Median-joining network for mitochondrial control region haplotypes in the Gouldian finch. Colours represent sampling localities, and node circle size represents the number of individuals with that haplotype. Number of strokes joining nodes indicates then number of mutations between two haplotypes.

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When running Discriminant Analysis of Principal Components (DAPC) using collection locality as the grouping factor, we found no evidence of separation between all six localities in the microsatellite dataset. Yet, in the SNP data DAPC was able to distinguish the three samples from Chidna (Fig 4). For more details on the procedures and model validation see the S4 Appendix.

The spatial autocorrelation also revealed no pattern of fine scale genetic structure (Fig A in S3 Appendix). When the sexes were considered separately in the spatial autocorrelation there was also no evidence of sex-bias in spatial genetic structure patterns (results not shown).

Mitochondrial richness in co-distributed Australian finches

The haplotype richness observed in the Gouldian finch fell within the confidence intervals of the rarefied long-tailed finch estimate (n = 152, H_R = 21.2, 17.19–25.23% CI). Conversely, haplotype diversity and nucleotide diversity were significantly higher in the long-tailed finch (*h*, $t_{-9.04}$, df = 321.68, $p<2.2x10^{-16}$; π, $t_{-24.01}$, df = 212.64, $p<2.2x10^{-16}$).

Discussion

Genetic diversity is an important indicator of future adaptive potential and risk of inbreeding [65]. In the Gouldian finch, there was no evidence in SNP and microsatellite markers for differences in genetic diversity between populations, but the population at Bradshaw had higher mitochondrial richness and diversity than the other populations. This is not the result of a confluence between haplotypes that are abundant or private to localities to the east and west, as most haplotypes are found throughout the range, and appears to be driven mostly by a higher number of private haplotypes. This pattern in allelic richness may represent a gradient of genetic diversity between range core (Bradshaw) and toward the range edge populations (See Fig 1 observations from Atlas of Living Australia) [20,66]. Across the sampled range, mitochondrial haplotype richness does not appear to be different between the Gouldian finch and related and 'Least Concern' long-tailed finch (*Poephila acuticauda*), but mitochondrial diversity estimates are significantly lower in the Gouldian finch than the long-tailed finch (*P. acuticauda*). Indeed, the widespread zebra-finch (*Taeniopygia guttata*) has similarly high estimates of diversity at mtDNA locus ND2 as those observed the long-tailed finch [11,67]. Broadly, the lower haplotype diversity in Gouldian finches supports a "Threatened" conservation status. However, measures of nucleotide diversity do not correlate well with population size or bottleneck intensity [68–70], therefore lineage specific processes (such as mutation rate or life-history) might be more important in determining mitochondrial diversity between these two species. Therefore, we caution against conclusions about the population status of the Gouldian finch until formal analyses of effective population size have been conducted.

We found evidence of heterozygote reduction (F_{IS} and F_{IT} = 0.04) in the microsatellite dataset, but not the SNP dataset. This discrepancy is potentially because of large sampling variances associated with these highly polymorphic markers, in conjunction with our modest number of markers and population sampled, and therefore expected heterozygosities from SNP data may be more accurate [71,72]. Further, small microsatellite marker sets are not good predictors of pedigree inbreeding except in highly structured populations with high levels of consanguineous matings, and therefore may not correlate well with inbreeding depression ([72,73] but see [74]). That we find no evidence of increased F_{IS} & F_{IT} inbreeding coefficients in the more

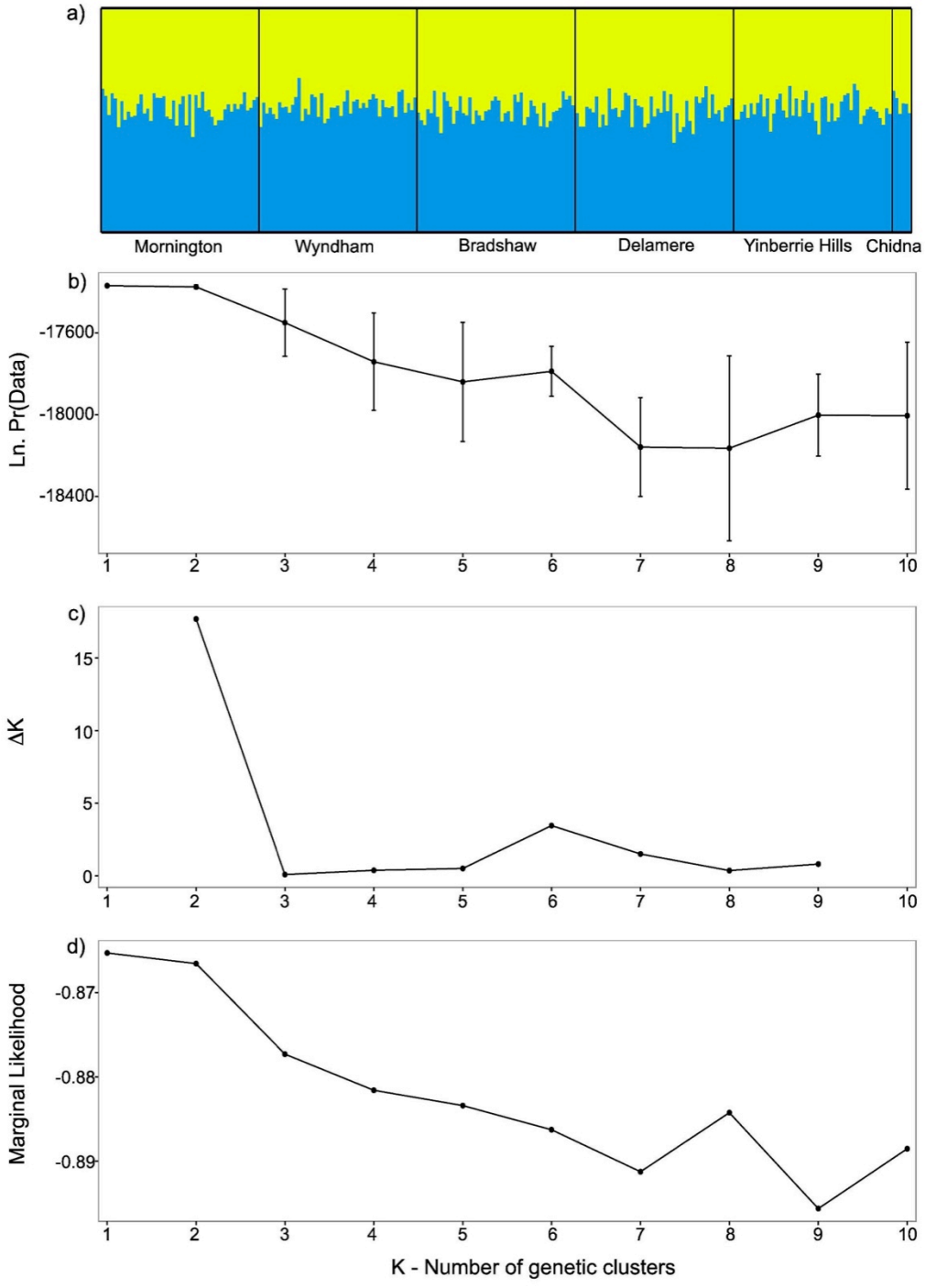
a)
Mornington
Wyndham
Bradshaw
Delamere
Yinberrie Hills
Chidna
b)
Ln. Pr(Data)
-17600
-18000
-18400
1
2
3
4
5
6
7
8
9
10
c)
ΔK
15
10
5
0
d)
Marginal Likelihood
-0.87
-0.88
-0.89
K - Number of genetic clusters

Fig 3. Results from Bayesian clustering analysis using STRUCTURE (a-c) [37] and d) fastSTRUCTURE [57]. Part a) shows equal membership probability plot for each individual plotted for two clusters; b) log probability of data (LnP(D)) showing K = 1; c) the optimal number of genetic clusters according to the Evanno et al method; d) output of marginal likelihoods from fastSTRUCTURE showing optimal K = 1.

doi:10.1371/journal.pone.0167723.g003

representative and statistically powerful SNP dataset suggests that inbreeding may not occur at significant levels in the wild Gouldian finch. Indeed, field observations suggest consanguineous matings may be rare, due to very low numbers of recaptured individuals between years, suggesting high levels of dispersal ([8,14], S1 Appendix). Further, although banding data indicate that individual movements occur over small spatial scales, our spatial autocorrelation data indicate that relatedness is not spatially structured within the Wyndham sampling site. Together, the genetics and banding data suggest that birds become more mobile after the breeding season, or that adult mortality is high and juveniles are highly dispersive.

We found no evidence of genetic differentiation in the western range of the Gouldian finch from Mornington to Yinberrie Hills, a distance of 730 km, despite the bio-geographic complexity and vast distances involved in this part of Australia [10]. Indeed, we find no fine-scale genetic structure at the landscape scale in Wyndham, nor evidence of Isolation-by-Distance across the five major sampling localities (spanning 730km) across our three marker types. Notably, our sampling spans a number of biogeographic breaks including the Ord Arid Intrusion (Wyndham area) and the Victoria Gap, which have been associated with genetic discontinuities within a number of species with different dispersal capacities [10,75,76]. However, we found no evidence of a genetic discontinuity in the Gouldian finch across these barriers. The Ord Arid Intrusion is associated with separate mtDNA lineages roughly in line with the sub-

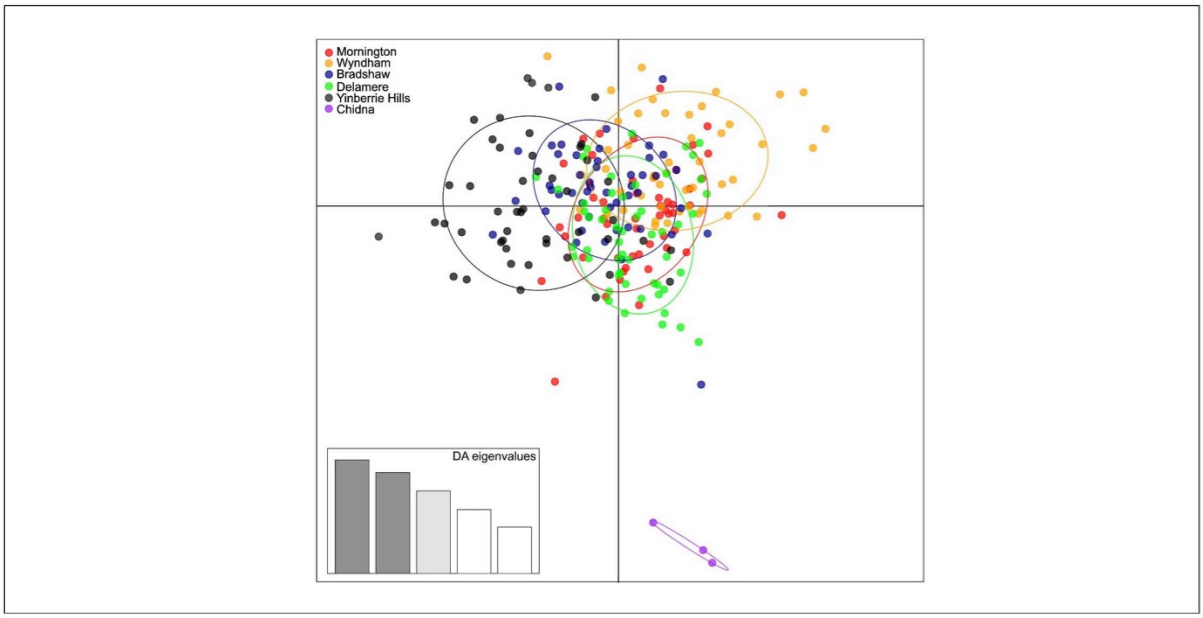

Fig 4. Scatterplot from discriminant analysis of principal components on the Genotyping-by-Sequencing SNP dataset. Points represent individual genotypes, and colours are the sampling localities surrounded by a 95% confidence ellipse. DA eigenvalues represent the amount of genetic variation captured by the discriminant factors plotted as the x- and y- axis.

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species of the long-tailed finch (*P. a. acuticauda* and *hecki*) [11]. This is congruent with our understanding of movement based on mark recapture studies of both species, where long-tailed finches show more site-fidelity than the Gouldian finch ([8,15], S1 Appendix). Further, no genetic structure has been previously detected in the nomadic continental zebra finch, and therefore it is plausible that Gouldian finches are similarly dispersive [77], but equally plausible is an expansion from a single ice-age refugium as seen in many Palaearctic and Nearctic faunas [78].

Genetic differentiation between the western range and our sampling locality in Queensland, Chidna, remains unclear. The Chidna locality was responsible for significant results in Mantel tests for IBD, despite Chidna showing no evidence of significant pairwise differentiation after Bonferroni correction. Discriminant Analysis of Principal Components on the SNP dataset identified Chidna as a distinct cluster, but our validations suggest model instability. However, clustering analyses found no evidence that there was more than one genetic cluster, suggesting Chidna is part of the same population. Because of the small sample size at Chidna it is unlikely that we have captured the true allele frequencies at the location, and any signal of differentiation (or lack-thereof) may be spurious [25,71]. However, anecdotal reports suggest Gouldian finch densities are lower in Queensland [7], which differs in land-management and fire-regimes which are determinants of Gouldian finch abundance [5,14,79,80]. These tracts of unsuitable or poor quality habitat may restrict movement between the western range and Queensland, and within Queensland, which may be severe enough to restrict gene-flow. Therefore, we urge that results of high genetic connectivity in the western range are not extrapolated into Queensland.

The putative decline and fragmentation of the Gouldian finch (the 1960s-1970s) is relatively recent (30–50 years and Gouldian finch generations), and there may not have been sufficient time to detect a reduction in gene-flow [78,81]. The time it takes for a reduction in gene-flow to affect allele frequencies will depend on the migration rate, effective population size of the subpopulations, generation time and overlap and population growth [82,83]. However, modelling has shown a complete cessation of gene-flow can be statistically detectable within two generations using equilibrium estimators (e.g. F_{ST}) for microsatellite sampling schemes equivalent to ours and census population sizes of less than 500 individuals [19]. To our knowledge, there is no equivalent modelling done on high-throughput technology SNP data, but studies suggest that the number of SNPs employed here ought to be sufficient to distinguish very low levels of differentiation (e.g $F_{ST} < 0.05$) [19,84–86]. Compared with previous attempts to measure population structure in the Gouldian finch [16,17], we are confident of our finding of the absence of genetic structure across the western range of the Gouldian finch because we included more sampling localities, analyses with different underlying assumptions, and more powerful genetic markers (SNPs) for detecting subtle differentiation [1,19,84,86].

Populations across the western range of the Gouldian finch are genetically interconnected, and exchange more than sufficient effective migrants to maintain the genetic diversity in each region (irrespective of stringency of $N_e m$) [1,87,88]. But this does not usefully inform the demographic connectivity between regions, as this depends on the subpopulation size and the migration rate between them ($m<0.1$) [1,89,90]. Unless there is detectable differentiation between subpopulations, assignment-test methods such as BayesAss will not be able to measure migration rate between populations [91]. Therefore, ecological data is still useful to infer management units and demographic connectivity between populations [89]. Banding data from Mornington, Wyndham, Newry and Yinberrie Hills indicate very low return rates between years at collection localities ([8,14], S1 Appendix), which suggests that local recruitment (on the scale of the sampling localities) may not be important for maintaining populations. Limited spatial and temporal banding data do not allow inference about Gouldian finch

populations beyond what is possible with the genetic data. Until there is an extremely substantial banding effort (both spatially and temporally), or satellite telemetry is used to monitor Gouldian populations across the range (for which tags are currently not available due to the size of the bird), it will remain uncertain whether Gouldian finches are regularly dispersing long distances, or the lack of population genetic structure comes from a high volume of local migrant exchange [3].

All three molecular markers employed in this study provided congruent evidence about moderately high genetic diversity across the western range of the Gouldian finch, with no evidence of genetic differentiation despite biogeographic barriers. Although these data make it impossible to infer demographic connectivity (migration rate, *m*) between populations, we urge caution in the interpretation of spatially and temporally unsystematic estimates of population from anecdotal reports by bird-watchers. Our findings do not exclude the possibility that individual Gouldian finches may be capable of moving quite long distances. The genetic connectivity between the west and the populations in Queensland remain unresolved, but due to differences in land management practices and Gouldian finch density, movement patterns may be drastically different from what we have observed in the western range. Establishing patterns of genetic connectivity in Queensland remains a priority for adequately assessing the population status of the Gouldian finch in Queensland.

Supporting Information

S1 Appendix. Gouldian finches at Wyndham. Detailed description of methods and recapture rates of Gouldian finches at Wyndham between 2008 and 2013.
(DOCX)

S2 Appendix. Microsatellite methods. Detailed methods for microsatellite amplification and quality checks.
(DOCX)

S3 Appendix. Pairwise diversity, differentiation and spatial autocorrelation results. Figures and tables include uncorrected p-values for differences between each population comparison, pairwise estimates of genetic differentiation and spatial autocorrelation.
(DOCX)

S4 Appendix. K-means clustering and discriminant analysis of principal components. Text and figures describe the detailed methods and model checks associated with the analysis of the microsatellite and SNP data in adegenet.
(DOCX)

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S1 Appendix: Gouldian finches at Wyndham

Previous banding efforts at Yinberrie Hills and Newry showed that there was much variation in the number of banded Gouldian individuals in a given year, and generally low (but variable) recapture rates (Woinarski and Tidemann 1992). Indeed, in the sample of banded birds from Mornington Sanctuary recovery rates were very low, between 5 and 19% (Legge *et al.* 2015). In all banded birds in our sample from Bradshaw, Delamere and Yinberrie Hills, in the Northern Territory, there was only a single recapture at Bradshaw. Below we present a summary of the banded birds and their recapture rates at the Wyndham site in the Eastern Kimberley (Table A). During these years birds were mist-netted at waterholes and nests were monitored at natural cavities and artificial nest-boxes (Brazill-Boast *et al.* 2010; Brazill-Boast *et al.* 2013). The longest duration between recaptures was three years, where band number 87233 was banded in 2010, and recovered again in 2013. One individual, 87237 was captured in 2011, 2012 and 2013. The bird recaptured in 2012 was banded in the nest in 2010.

Table A: Summary of birds banded at Wyndham, Western Australia. Banded breeding pairs include only nests where both parents were uniquely identified by band number. Nestlings are chicks that were banded at 14 days in the nest, and fledglings were independent young banded with mist nets at water holes.

Year	Total Adults	Banded breeding pairs	Pairs from previous year	Adults from previous year	Nestlings banded	Nestlings from previous year	Fledglings banded	Fledglings from previous year
2008	200	34	-	-	130	-	55	-
2009	64	23	0	4 (2.0%)	125	4 (3%)	11	0
2010	20	9	0	2 (3.1%)	48	0	0	0
2011	22	4	0	1 (5.0%)	20	0	47	0
2012	35	6	0	3 (13.6%) + 1§	19	1§ (5%)	71	0
2013	26	10	1	3 (8.6%) + 1§	49	0	2	1(1.4%)

§ Indicates an individual that was re-caught later than one year after its capture, but had no

S2 Appendix: Microsatellite methods

We amplified 22 microsatellite loci across five different multiplexes (Table S2.1), including the first use of primers developed in the Gouldian finch (Kim *et al.* 2015). Multiplexes were amplified using a Qiagen multiplex mix in 5uL reactions.
The step-down thermal cycler protocol for multiplex 1,3,4 and 5 was as follows: 95°C for 15 min, 94°C for 45s denaturation, 70°C, 66°C, 62°C, 62°C, 58°C, 54°C annealing temperatures for 1 minute, 72°C for 1 min extension. These cycles were repeated 8 times per annealing temperature. Final extension was 72°C for 10 min.

The step-down thermal cycler protocol for multiplex 2 was as follows: 95°C for 15 min, 94°C for 30s denaturation, 64°C, 60°C, 56°C, 53°C, 50°C annealing temperatures for 90s, 72°C for 90s. These cycles were repeated 10 times per annealing temperature. Final extension was 72°C for 10 min.

Samples were run at Macrogen Inc. on an ABI 3730 machine using a GS-500 LIZ size standard. Genotypes were scored using GeneMapper 3.7 (Applied Biosystems, Foster City, CA, U.S.A.).

Loci Tgu7 and Tgu3 were excluded because they did not amplify consistently within individuals and between populations. Loci were checked for null alleles using CERVUS 3.0.6 (Kalinowski *et al.* 2007), and deviation from Hardy-Weinberg equilibrium using ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010). We did not include samples from Chidna in these analyses because of the small sample size (N=6). Any locus that had high rates of null-alleles (above 10%) and/or deviated from Hardy-Weinberg in more than two populations were excluded from the further analyses (those marked with an asterisk in Table A). Significant deviation from Hardy-Weinberg was assessed after Bonferroni correction for multiple comparisons at an alpha-level of 0.05 (results not shown).

For the final dataset of 16 loci we present the summary diversity statistics and null alleles as calculated in ARLEQUIN for 49 individuals from each locality (and six from

Chidna) in Table B. No locus combination was significantly in linkage disequilibrium after Bonferroni correction (Table C).

Table A: Microsatellite primers used for the different multiplexes employed in this paper.

Marker	Dye	Multiplex	uM	Reference
Pco2	6FAM	1	0.142	(Saito *et al.* 2001)
Pca7	VIC	1	0.532	(Dawson *et al.* 2000)
Cuu4	NED	1	0.212	(Gibbs *et al.* 1998)
Tgu7*	NED	1	0.638	(Forstmeier *et al.* 2007)
Ind41	6FAM	1	0.638	(Sefc *et al.* 2001)
Titgata2	PET	2	0.334	(Wang *et al.* 2005)
Ind28	6FAM	2	0.234	(Sefc *et al.* 2001)
Tgu11	VIC	3	0.1	(Forstmeier *et al.* 2007)
Ind37	VIC	3	0.8	(Sefc *et al.* 2001)
BF18	6FAM	3	0.2	(Yodogawa *et al.* 2003)
Ase24	6FAM	3	0.334	(Richardson *et al.* 2000)
Tgu3*	NED	3	0.4	(Forstmeier *et al.* 2007)
Ego26	6FAM	4	0.2	(Kim *et al.* 2015)
Ego27*	VIC	4	0.2	(Kim *et al.* 2015)
Ego15	NED	4	0.4	(Kim *et al.* 2015)
Ego29*	PET	4	0.2	(Kim *et al.* 2015)
Ego49	6FAM	5	0.2	(Kim *et al.* 2015)
Ego31*	VIC	5	0.4	(Kim *et al.* 2015)
Ego34	NED	5	0.2	(Kim *et al.* 2015)
Ego52	PET	5	0.2	(Kim *et al.* 2015)
Ego45*	PET	5	0.2	(Kim *et al.* 2015)

Table B: Summary statistics for each microsatellite locus for each population, describing the number of alleles (N_A), Observed Heterozygosity (H_O), Expected Heterozygosity (H_E), Deviation from Hardy-Weinberg by exact test (P) where bolded values are significant at $p<0.05$, but none are significant after Bonferroni correction ($p<0.000625$).

Population	Locus	N_A	H_O	H_E	P
Mornington	Ego15	22	0.896	0.938	0.5814
	Ego26	11	0.796	0.858	0.0541
	Ego31	13	0.776	0.817	0.1501
	Ego34	12	0.771	0.801	0.3241
	Ego49	35	0.918	0.953	0.7182
	Ego52	9	0.837	0.786	0.9749
	Ase24	6	0.551	0.653	0.2309
	BF18	12	0.673	0.813	0.2066
	Ind37	16	0.830	0.874	0.3751
	Tgu11	6	0.653	0.645	0.0046
	Cuu4	14	0.787	0.869	0.1993
	Ind28	7	0.667	0.761	0.0523
	Ind41	26	0.915	0.945	0.3365
	Pca7	18	0.918	0.927	0.2336
	Pco2	3	0.571	0.535	0.0273
	Titgata02	12	0.750	0.824	0.0956
Wyndham	Ego15	19	0.659	0.850	**0.0105**
	Ego26	14	0.898	0.888	0.4876
	Ego31	13	0.776	0.833	0.2006
	Ego34	13	0.681	0.828	**0.0050**
	Ego49	35	0.918	0.950	0.0606
	Ego52	8	0.776	0.769	0.6567
	Ase24	7	0.653	0.691	0.4118
	BF18	11	0.673	0.755	**0.0226**
	Ind37	16	0.896	0.913	0.5997
	Tgu11	5	0.633	0.602	0.4427
	Cuu4	12	0.771	0.867	0.1219
	Ind28	8	0.688	0.736	0.3697
	Ind41	26	0.878	0.946	0.1844
	Pca7	21	0.959	0.926	0.5387
	Pco2	4	0.510	0.592	0.2551
	Titgata02	14	0.918	0.856	0.3161
Bradshaw	Ego15	19	0.878	0.911	0.2241
	Ego26	12	0.939	0.880	0.8809
	Ego31	13	0.837	0.861	0.1645
	Ego34	17	0.755	0.831	0.7037
	Ego49	37	0.959	0.953	0.0938

Population	Locus	N_A	H_O	H_E	P
	Ego52	10	0.755	0.800	0.1454
	Ase24	6	0.469	0.610	0.1165
	BF18	11	0.592	0.761	0.0281
	Ind37	16	0.857	0.914	0.6709
	Tgu11	7	0.551	0.610	0.2427
	Cuu4	12	0.673	0.835	0.0737
	Ind28	8	0.714	0.758	0.8325
	Ind41	27	0.878	0.941	0.6016
	Pca7	18	0.878	0.908	0.1263
	Pco2	3	0.592	0.577	1.0000
	Titgata02	13	0.898	0.858	0.9113
	Ego15	19	0.913	0.901	0.7962
	Ego26	14	0.918	0.861	0.8284
	Ego31	10	0.708	0.771	0.0963
	Ego34	14	0.898	0.840	0.4814
	Ego49	37	0.918	0.954	0.1532
	Ego52	9	0.633	0.750	**0.0268**
	Ase24	6	0.551	0.632	0.3308
Delamere	BF18	12	0.694	0.822	**0.0040**
	Ind37	16	0.878	0.882	0.5994
	Tgu11	7	0.653	0.622	0.8376
	Cuu4	11	0.898	0.856	0.9064
	Ind28	8	0.714	0.757	0.0572
	Ind41	25	0.837	0.942	0.4988
	Pca7	24	0.959	0.944	0.4063
	Pco2	4	0.551	0.611	0.6738
	Titgata02	13	0.918	0.868	0.5757
	Ego15	22	0.837	0.878	0.1233
	Ego26	12	0.939	0.877	0.1203
	Ego31	14	0.776	0.848	**0.0238**
	Ego34	14	0.735	0.792	0.1145
	Ego49	39	0.959	0.941	0.9934
	Ego52	8	0.673	0.783	0.0117
	Ase24	7	0.571	0.644	0.3590
Yinberrie	BF18	12	0.612	0.752	0.1000
Hills	Ind37	16	0.837	0.904	0.2669
	Tgu11	8	0.653	0.643	0.5655
	Cuu4	14	0.776	0.876	0.0590
	Ind28	6	0.714	0.737	0.4206
	Ind41	26	0.959	0.953	0.5406
	Pca7	20	0.918	0.925	0.1121
	Pco2	4	0.551	0.597	0.1525
	Titgata02	12	0.837	0.856	0.4371
	Ego15	7	1.000	0.933	1.0000
Chidna	Ego26	5	0.833	0.833	0.9448
	Ego31	3	0.667	0.621	1.0000

Population	Locus	N_A	H_O	H_E	P
	Ego34	3	0.667	0.712	1.0000
	Ego49	9	1.000	0.955	1.0000
	Ego52	4	0.833	0.682	0.7919
	Ase24	4	0.800	0.733	1.0000
	BF18	5	0.667	0.803	0.3035
	Ind37	6	0.800	0.889	0.6172
	Tgu11	4	0.500	0.455	1.0000
	Cuu4	7	1.000	0.911	1.0000
	Ind28	5	0.667	0.742	0.5176
	Ind41	8	0.667	0.939	0.0817
	Pca7	8	0.833	0.924	0.4955
	Pco2	4	0.833	0.712	0.7921
	Titgata02	9	1.000	0.955	1.0000

Table C: Results from linkage disequilibrium analysis conducted in GenePop, bolded p-values are those significant at $p<0.05$, but no comparisons were significant after Bonferroni correction for multiple testing ($p<0.0004132$).

Locus1	Locus2	P-Value
Ego15	Ego26	0.845067
Ego15	Ego31	0.668469
Ego26	Ego31	0.106509
Ego15	Ego34	0.694017
Ego26	Ego34	**0.02957**
Ego31	Ego34	0.9421
Ego15	Ego49	0.192741
Ego26	Ego49	0.22047
Ego31	Ego49	0.073453
Ego34	Ego49	0.686806
Ego15	Ego52	0.48442
Ego26	Ego52	0.696252
Ego31	Ego52	0.627829
Ego34	Ego52	0.240781
Ego49	Ego52	0.334443
Ego15	Ase24	0.899588
Ego26	Ase24	0.85959
Ego31	Ase24	**0.002716**
Ego34	Ase24	0.156194
Ego49	Ase24	0.552941
Ego52	Ase24	0.831776
Ego15	BF18	0.360148
Ego26	BF18	0.181914

Locus1	Locus2	P-Value
Ego31	BF18	0.260903
Ego34	BF18	0.1785
Ego49	BF18	0.110341
Ego52	BF18	0.373995
Ase24	BF18	0.06989
Ego15	Ind37	0.13613
Ego15	Ind37	0.13613
Ego26	Ind37	0.305382
Ego31	Ind37	0.744387
Ego34	Ind37	0.716983
Ego49	Ind37	0.585006
Ego52	Ind37	0.700925
Ase24	Ind37	0.955974
BF18	Ind37	0.430907
Ego15	Tgu11	0.489194
Ego26	Tgu11	**0.006512**
Ego31	Tgu11	0.835162
Ego34	Tgu11	0.065263
Ego49	Tgu11	0.276819
Ego52	Tgu11	0.325607
Ase24	Tgu11	0.204872
BF18	Tgu11	0.426137
Ind37	Tgu11	0.340764
Ego15	Cuu4	0.108394
Ego26	Cuu4	0.175472
Ego31	Cuu4	0.349392
Ego34	Cuu4	**0.041076**
Ego49	Cuu4	0.730567
Ego52	Cuu4	**0.035861**
Ase24	Cuu4	0.559942
BF18	Cuu4	0.423064
Ind37	Cuu4	0.519046
Ego15	Cuu4	0.516717
Ego15	Ind28	0.620335
Ego26	Ind28	0.932433
Ego31	Ind28	0.727195
Ego34	Ind28	0.896817
Ego49	Ind28	0.083154
Ego52	Ind28	0.357335
Ase24	Ind28	0.064868
BF18	Ind28	0.120058
Ind37	Ind28	0.548723
Ego15	Ind28	0.669828
Ego15	Ind28	0.430285
Ego15	Ind41	0.536852
Ego26	Ind41	**0.000541**

Locus1	Locus2	P-Value
Ego31	Ind41	0.61758
Ego34	Ind41	0.261592
Ego49	Ind41	0.796281
Ego52	Ind41	0.236599
Ase24	Ind41	0.617847
BF18	Ind41	0.674755
Ind37	Ind41	0.095534
Ego15	Ind41	0.769595
Ego15	Ind41	0.901013
Ego15	Ind41	0.330448
Ego15	Pca7	0.948381
Ego26	Pca7	0.519102
Ego31	Pca7	0.215805
Ego34	Pca7	0.465952
Ego49	Pca7	0.374468
Ego52	Pca7	0.098997
Ase24	Pca7	0.553526
BF18	Pca7	0.434907
Ind37	Pca7	**0.029283**
Tgu11	Pca7	0.330017
Cuu4	Pca7	**0.041938**
Ind28	Pca7	0.746738
Ind41	Pca7	0.361844
Ego15	Pco2	0.2242
Ego26	Pco2	0.662943
Ego31	Pco2	0.516495
Ego34	Pco2	0.524357
Ego49	Pco2	0.095903
Ego52	Pco2	0.258396
Ase24	Pco2	0.41678
BF18	Pco2	0.972699
Ind37	Pco2	0.678384
Tgu11	Pco2	0.490333
Cuu4	Pco2	**0.023427**
Ind28	Pco2	0.668428
Ind41	Pco2	**0.047054**
Pca7	Pco2	0.130533
Ego15	Titgata02	0.583406
Ego26	Titgata02	0.74044
Ego31	Titgata02	**0.008375**
Ego34	Titgata02	0.764913
Ego49	Titgata02	0.154271
Ego52	Titgata02	0.265703
Ase24	Titgata02	0.520606
BF18	Titgata02	0.280592
Ind37	Titgata02	0.678128

Locus1	Locus2	P-Value
Tgu11	Titgata02	0.945373
Cuu4	Titgata02	0.458788
Ind28	Titgata02	0.29573
Ind41	Titgata02	0.104625
Pca7	Titgata02	0.300377
Pco2	Titgata02	0.096827

Table A: P-values for pairwise Wilcox sign-rank tests on microsatellite allelic richness between each of the sampling localities. Below the diagonal reflects the difference in richness on the data rarefied to match the sample size of Chidna. Above the diagonal, is the pairwise p-values between the five major sampling localities of the same sample size. Bolded P-values are significant before Bonferroni correction, but none were significant after Bonferroni correction.

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**
Mornington		0.574	0.360	0.578	**0.036**
Wyndham	0.53		0.680	0.583	0.345
Bradshaw	0.63	1		0.765	0.502
Delamere	0.71	0.82	0.86		0.579
Yinberrie Hills	0.32	0.63	0.82	0.82	
Chidna	0.32	0.53	0.53	0.40	0.40

Table B: P-values for pairwise Wilcox sign-rank tests on microsatellite heterozygosities between each of the sampling localities. Below the diagonal is the difference in observed heterozygosity. Above the diagonal is for expected heterozygosity. All P-values presented are the raw (not Bonferroni corrected) values, but none were significant before or after correction.

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**	**Chidna**
Mornington		0.90	1.00	0.94	0.94	0.46
Wyndham	0.83		0.78	0.86	0.80	0.78
Bradshaw	0.74	0.84		0.63	0.94	0.82
Delamere	0.59	0.53	0.38		0.98	0.60
Yinberrie Hills	0.86	0.93	0.75	0.31		0.94
Chidna	0.59	0.78	0.60	0.86	0.52	

Table C: Estimates of differentiation (F_{ST} and Jost's D) from sixteen microsatellite markers from the six sampling localities. Below the diagonal is pairwise F_{ST}, where p-values are bolded at $p<0.05$, but no comparison was significant after Bonferroni correction ($p<0.0051$). Above the diagonal are Jost's D, emboldened are p-values calculated by 1000 bootstrap resamples in DEMEtics, but none were significant after Bonferroni correction. Negative F_{ST} and D estimates are corrected to zero

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**	**Chidna**
Mornington		0.008	**0.029**	0.016	0.022	0.027
Wyndham	0.001		**0.020**	0.011	0.010	0.039
Bradshaw	**0.004**	0.002		**0.029**	0.000	0.000
Delamere	0.002	0.001	0.003		0.000	0.000
Yinberrie Hills	0.002	0.000	0.000	0.000		0.025
Chidna	0.014	0.012	0.004	0.001	0.007	

Table D: Pairwise F-ST based on mitochondrial control region haplotype frequencies (below diagonal); Pairwise Φst based on nucleotide diversity in each population using the Kimura 2 Parameter model above the diagonal. Values in bold are significant at p=0.05, but no comparison was significant after Bonferroni correction (p<0.0033). All negative F_{ST} results were corrected zero.

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**	**Chidna**
Mornington		0.00	0.00	0.00	0.01	**0.19**
Wyndham	0.01		0.00	0.00	0.02	**0.33**
Bradshaw	0.01	0.00		0.00	0.00	**0.15**
Delamere	0.00	0.00	0.00		0.02	**0.30**
Yinberrie Hills	0.03	0.00	0.00	0.00		0.10
Chidna	**0.15**	**0.27**	**0.16**	**0.21**	**0.19**	

Table E: Below diagonal: Pairwise F_{ST} from Arlequin resulting from 3839 SNP dataset, negative values are corrected to zero. Significant differences (p<0.05) are emboldened, but after Bonferroni correction (p<0.0033), no result was statistically significant against 10100 permutations.

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**
Mornington					
Wyndham	0.000				
Bradshaw	0.000	0.000			
Delamere	0.000	0.000	0.000		
Yinberrie Hills	0.000	0.000	0.000	0.000	
Chidna	0.007	**0.011**	0.005	0.009	0.005

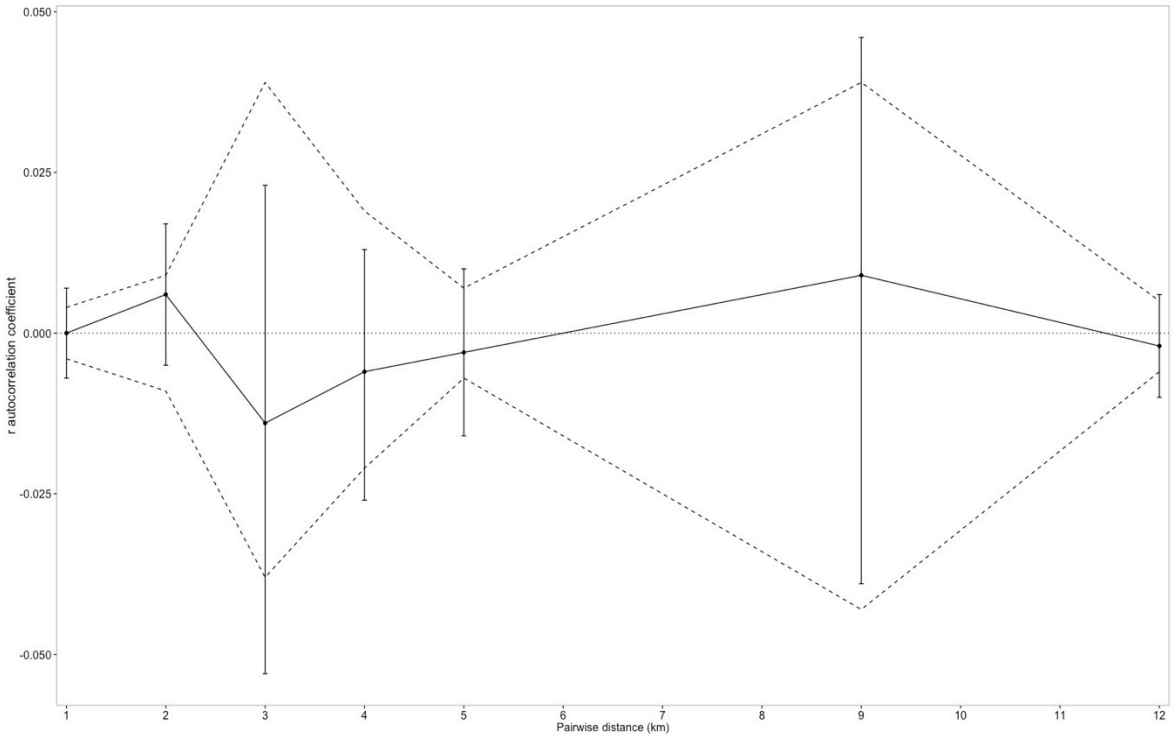


Figure A: Correlogram from spatial autocorrelation analysis, showing no spatial structure at the local scale within the Wyndham population. As there were no sex differences only the pooled results are presented. The unbroken line (r) represents the autocorrelation coefficient from the comparison between genetic and geographic distance matrices, and error bars are the bootstrap 95% confidence intervals about the *r* estimate for that distance class. The dashed lines represent the upper and lower 95% confidence intervals for the null hypothesis of no spatial structure (around r=0, dotted line) generated by permutation of samples across the distance classes

S4 Appendix: K-means clustering and Discriminant Analysis of Principal Components in '*adegenet*'

We followed the recommendations of the developers using the dapc tutorial online (Jombart and Collins 2015)

Discriminant Analysis on microsatellite dataset

In order to infer the number of populations without *a priori* sampling information, we ran the function *find.clusters* in R-package adegenet (Jombart 2008) on the dataset with 49 randomly subsampled individuals per population plus six from Chidna. This function turns the original genotypic data into uncorrelated principal components, then scores the clustering solutions for different numbers of clusters using the Bayesian Information Criterion. Because we found the optimum number of clusters to be one (K=1) (Figure A), we only proceeded with Discriminant Analysis of Principal Components (DAPC, function *dapc*) using the sampling locality as a prior (Jombart *et al.* 2010).

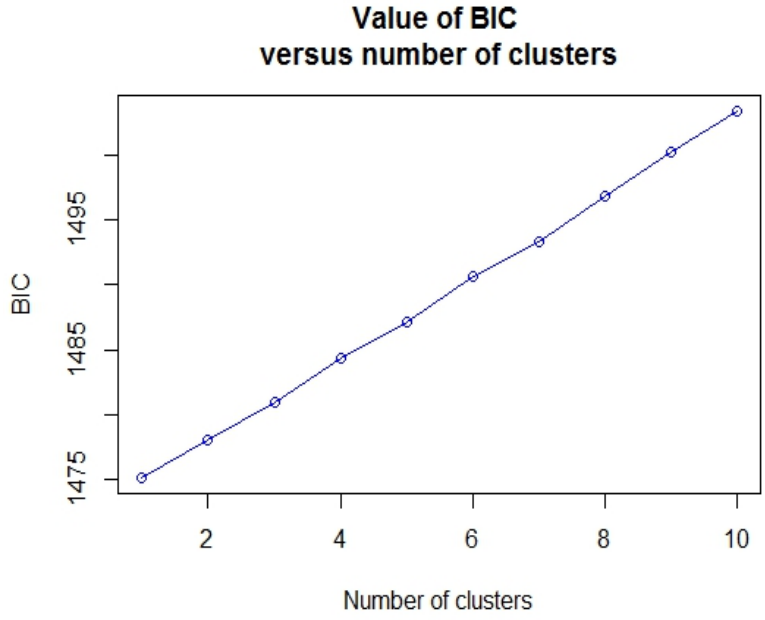


Figure A: Results of K-means clustering method *find.clusters* for microsatellite data, with each clustering solution and its corresponding BIC score

To ensure the discriminant analysis with population priors was not overfit by retaining too many principal components we ran two model checks using a "full" model including all principal components, linear discriminants among the optimal four clusters identified previously (Jombart *et al.* 2010).

On this full model we ran two procedures that maximises the ability to assign individuals to clusters reliably. Function *optim.a.score* is essentially a permutation test, which maximises the "a-score", by comparing the number of assignments to "real" number of clusters to randomised clusters. The a-score is a measure the proportion of assignments back to the prior clusters or to random clusters, and is essentially a measure of 'over-fitting' of the model. We ran 100 simulations per increase of 16 principal components.

Function *xvalDapc* also attempts to optimise genuine assignment power over random assignments, and does so by cross-validating against training and test subsets of data. We ran this using the default settings which uses 90% of the data as a training dataset, and tested on the remaining 10%. The a-score optimisation showed that no number of retained principal components gives a high assignment success, but there is an elevated success rate between when retaining 80 to 160 principal components (maximum=127) (Figure Bii). The cross-validation procedure showed equal assignment success across all principal components. For the final discriminant analysis we selected 80 principal components, because adegenet also recommends retaining principal components that are less than the number of individuals divided by three (Jombart 2008; Jombart and Collins, 2015).

The final model using 80 retained principal components revealed considerable overlap between individuals from different sampling localities (Figure Ai), and the final model was able to reassign 59% of individuals back to the original sampling population, but the a-score for this configuration was very low (<5%). The poor a-score results and the optimisation procedures carried out suggest it is virtually impossible to find a model that does not over-fit the data. Therefore, sampling locality does not have an important effect on the clustering of this data.

We then proceeded with the *dapc* analysis using sampling locality as a prior, retaining 80 principal components and all linear discriminant functions, as in the microsatellite analysis. These results show that all western populations are clustered together, with some separation evident in the Eastern population at Chidna (Figure S4c).

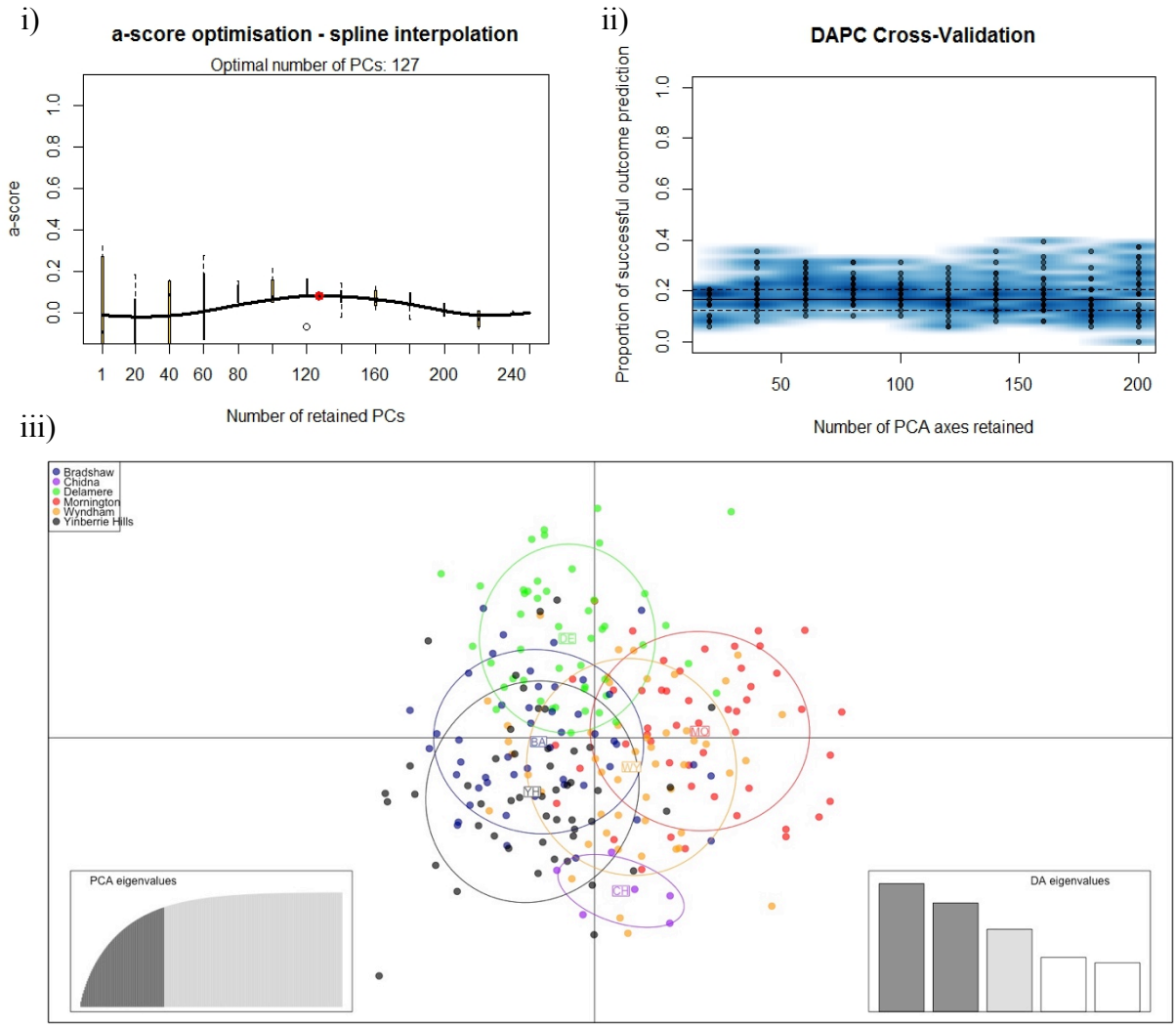


Figure B: Discriminant Analysis of Principal Components (DAPC) model optimisation for the best number of principal components of microsatellite data given the sampling localities. Part i) shows the results from the a-score optimisation procedure and ii) shows the results from the cross-validation analysis. Part iii) is the scatterplot of the final DAPC model with sampling locality as a prior, where points are individual genotypes, colour-coded by their original sampling locality and surrounded by a 95% confidence ellipse. DA and PCA Eigenvalues represent the amount of genetic variation captured by the analysis, and the first two discriminant factors are plotted as the x- and y- axis.

Discriminant analysis on SNP dataset

The *adegenet* package is also able to run the same analyses on SNP datasets, and essentially ran the same procedure as above. Unlike the microsatellite dataset, the contribution to the total variance per additional principal component did not reach an asymptote, and remained roughly linear (Figure C). Further, in finding the best clustering solution, here was a roughly linear increase in BIC with increase in number of clusters (Figure D), with the lowest BIC at a single cluster. Given *find.clusters* suggested that one cluster was the optimal configuration we did not proceed with DAPC from the *find.clusters* result.

Like the microsatellite data, we explored how the genetic variation was partitioned among our sampling localities using it as a grouping prior in dapc. First we validated the best number of principal components to use using *optim.a.score* function, which was unable to identify an optimal number of PCs to retain (Figure Ei) We then ran a cross-validation analysis as in the microsatellite dataset, but was again unable to find a number of principal components that correctly predict the group membership of clusters (Figure Eii). We then proceeded with the *dapc* analysis using sampling locality as a prior, retaining 80 principal components and all linear discriminant functions, as in the microsatellite analysis. These results show that all western populations are clustered together, with some separation evident in the Eastern population at Chidna (Main text Figure 4).

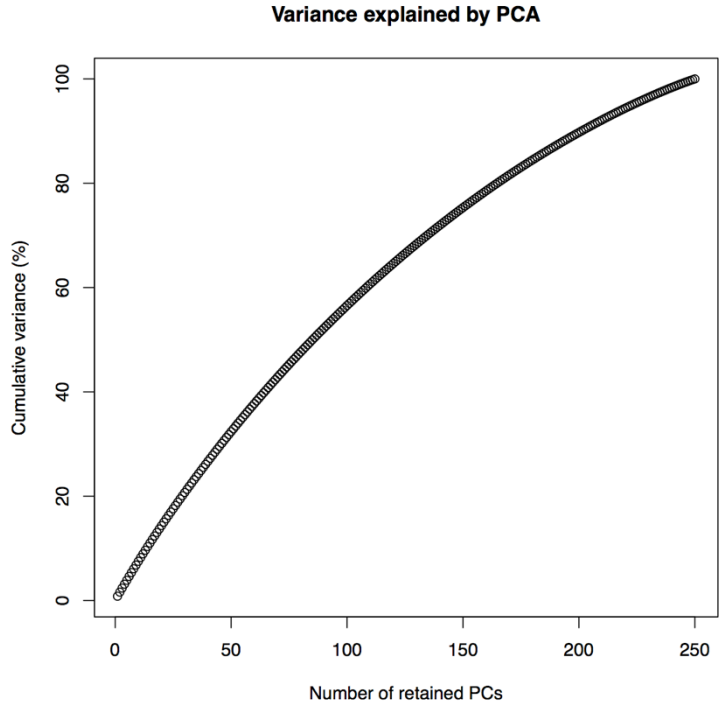


Figure C: How the principal components of the SNP dataset represent the variance within the dataset.

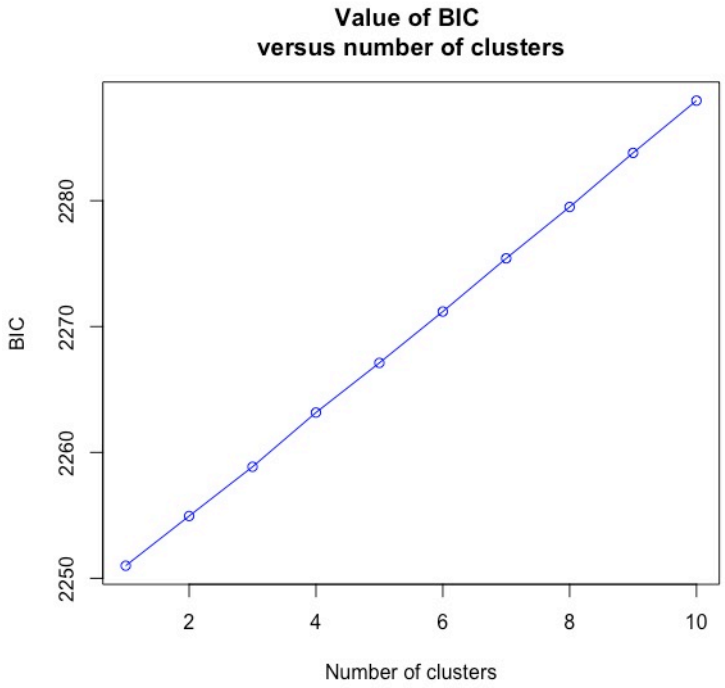
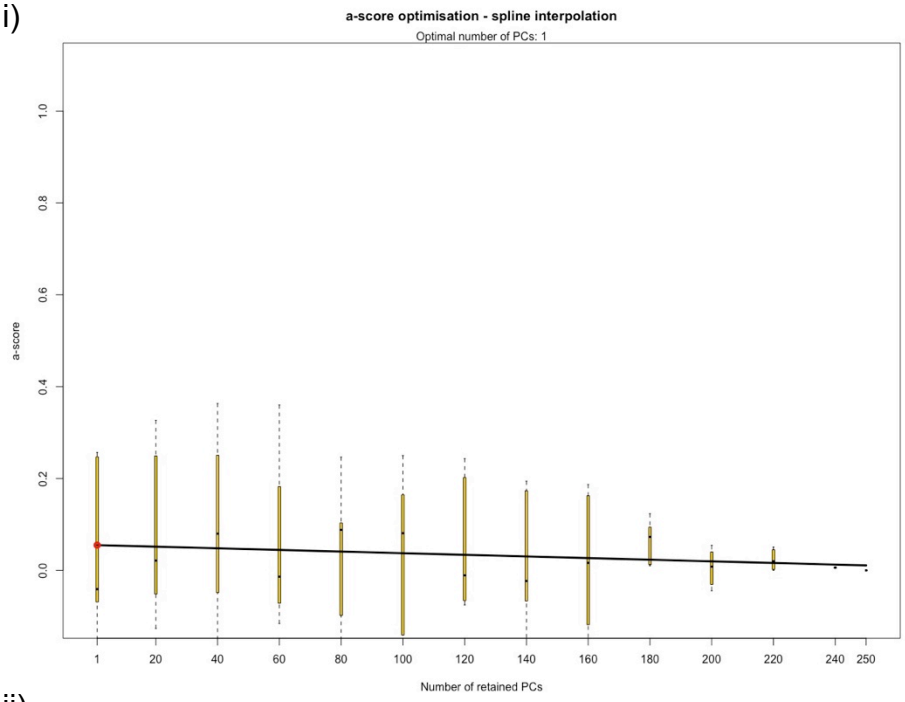


Figure D: results of *find.clusters* analysis on SNP data, with each clustering solution given a BIC score.

i)



ii)

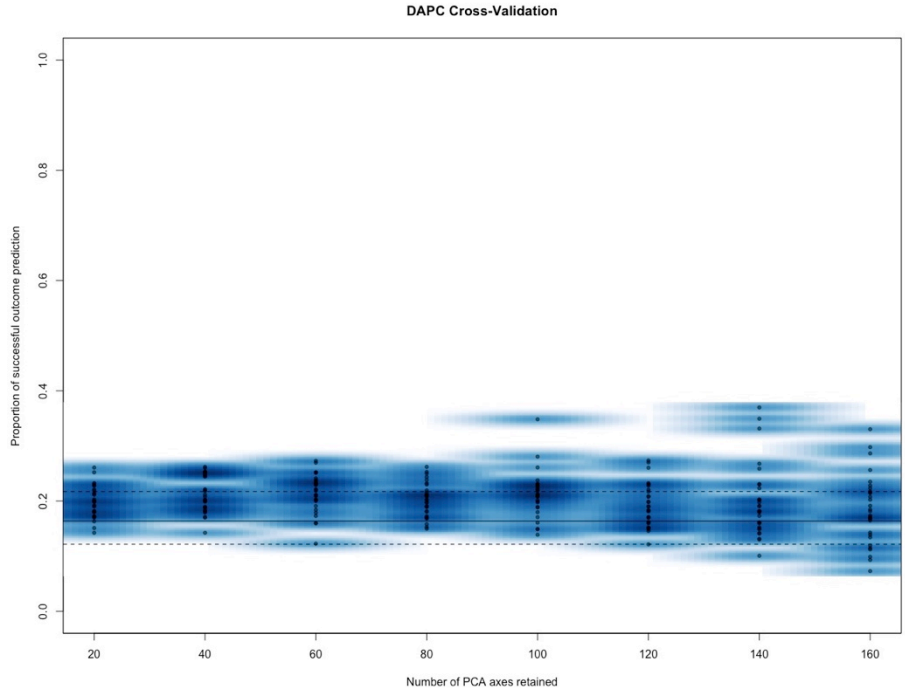


Figure E: Results of i) a-score optimisation on the SNP dataset, and ii) the cross-validation analysis on SNP data

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Chapter Six

Genetic diversity through time and space

Diversity and demographic history from natural history specimens and serially sampled contemporary populations of the threatened Gouldian finch (*Erythrura gouldiae*)

Photo: Mike Fidler

Chapter Six Vignette

The previous chapter provided substantial evidence that the Gouldian finches occupying Western Australia and the Northern Territory are a single genetic population. Some measures of genetic diversity were lower than the common, sympatric long-tailed finch (*Poephila acuticauda*), but were not as severely depleted of genetic diversity as some other endangered species (Frankham *et al.* 2010). Knowledge of the population structure and diversity prior to putative anthropogenic impacts can give conservation managers a target for what is considered normal levels of gene-flow and genetic diversity for a particular species. Furthermore, quantifying the degree of population decline can be useful to understand the demographic consequences of certain anthropogenic impacts.

In this chapter, I use genetic data from contemporary and historical samples to assess loss of genetic diversity over the twentieth century, and changes in gene-flow patterns. I explicitly assess the degree of population decline, and quantify the susceptibility of the population to inbreeding and genetic drift by calculating the effective population size in the contemporary population using multiple methods.

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Abstract

Declines in population size can compromise the viability of populations through a reduction in the effective population size (N_e). N_e describes an ideal population's propensity for inbreeding and loss of alleles due to genetic drift. Temporal population genetic data can be a powerful tool for testing the hypothesis of severe population declines leading to a reduced N_e. The Gouldian finch (*Erythrura gouldiae*) is a flagship for the conservation of species from the Australian monsoonal savannas. The Gouldian finch underwent severe population declines in the twentieth Century due to land use changes associated with European colonization. Genetic data from Gouldian finch samples from natural history collections dating prior to land use changes were compared with contemporary populations to estimate the severity of decline and any associated changes in gene flow. These data show that Gouldian finch decline was not as severe as some sources suggest, and that population genetic connectivity has not changed as a result of any landscape changes in the twentieth Century. Multiple estimators of current N_e using genetic data from consecutive years suggest the Gouldian finch N_e is likely between a few hundred and a few thousand individuals, within the range considered of conservation concern. This work has identified the need to genetically characterize populations in Queensland, and to understand critical demographic parameters (e.g. lifespan) in the Gouldian finch. Understanding these factors is vital to further improve genetic estimates of population size, in order to guide appropriate conservation management in this species.

Keywords

Museum skins; historical biodiversity; Estrildidae; biogeography; bottleneck;

Introduction

The decline and fragmentation of wildlife populations can measurably impact genetic diversity and pose a direct threat to population viability through inbreeding, genetic drift and reduced evolutionary potential (Frankham, 2005; Frankham et al., 2014). The extent to which a population is vulnerable to these factors will depend on its effective population size (N_e): the size of an idealized population that experiences genetic drift and inbreeding at the same rate as the population observed at size N (Crow and Kimura, 1970). The use of temporally sampled population genetic data can be an invaluable tool for discerning the causes and severity of demographic declines and fragmentation (Habel et al., 2014; Ramakrishnan and Hadly, 2009). Such approaches require population sampling over a number of time periods, or sampling before and after some event of hypothesized significance, such as human disturbance, and use population genetic techniques to measure changes in genetic diversity and gene flow.

Temporal genetic approaches often opportunistically utilise natural history collections and fossils over the scale of hundreds to thousands of years (Holmes et al., 2016; Navascués et al., 2010; Wandeler et al., 2007), and can disentangle the effects of recent human interference versus ancient demographic events. Disentangling these effects can have direct impacts for conservation management. For example, the Iberian brown bear (*Ursus arctos*) was managed as a distinct evolutionary unit on the basis of modern genetic data delimiting isolated, and putatively ancient populations (Valdiosera et al., 2008). However, comparisons of ancient and modern Iberian brown bear DNA revealed that population isolation was relatively recent and a composite of bottlenecks and cessations of gene-flow due to human activities in the past 500 years (Valdiosera et al., 2008). Over shorter time-scales the use of natural history collections has documented significant reductions in genetic diversity owing to anthropogenic over-exploitation or disturbance in the nineteenth and twentieth Centuries (Larsson et al., 2008; Mondol et al., 2013; Nyström et al., 2006; Weber et al., 2000). These studies can inform conservation management by determining 'normal' (i.e. historical) levels of genetic variation (Pacioni et al., 2015), and how that variation was distributed

geographically (Hoeck et al., 2009; Paplinska et al., 2011), and can be used to track the effectiveness of management actions such as reintroductions (Bristol et al., 2013). Such approaches are so powerful for detecting demographic and connectivity changes that they have been advocated for long-term genetic monitoring of conservation relevant organisms (Antao et al., 2011; Schwartz et al., 2007).

Australia provides a useful test case for these types of questions, because European arrival dramatically changed the landscape and is associated with a suite of extinctions (Woinarski et al., 2014). The predilection of the colonizing British for museum collecting has enabled contemporary scientists to test the effects of European colonization on a variety of species. These studies have revealed extinctions of local populations owing to habitat degradation (Austin et al., 2013), and loss of genetic diversity since the introduction of novel predators in pre-historic and colonial times (Pacioni et al., 2015). Conversely, the Tasmanian devil (*Sarcophilus harrisii*) and koala (*Phascolarctos cinereus*) have experienced dramatic population declines due to persecution and disease, but also have shown evidence of low genetic diversity prior to European colonization (Bruniche-Olsen et al., 2014; Morris et al., 2013; Tsangaras et al., 2012). No work has explicitly used population genetics on museum specimens to address recent (within 200 years) demographic histories in Australian tropical savanna species (Catullo et al., 2014; Joseph et al., 2011). This is despite evidence suggesting that the highly diverse fauna of the Australian tropical savannas may be very sensitive to over-grazing, the introduction of non-native grasses and predators, and fire-regime changes that have occurred over the past 100 years (Franklin, 1999; Franklin et al., 2005; Woinarski et al., 2011, 2001; Woinarski and Ash, 2002), where granivorous birds in particular have experienced severe population declines (Franklin, 1999).

The Gouldian finch (*Erythrura gouldiae*) is a granivorous bird once distributed across the majority of Australia's tropical savanna that showed large population declines in the twentieth Century. Anecdotal reports from the early to mid-twentieth Century suggest the Gouldian finch was abundant, found in similar numbers to currently abundant long-tailed and

masked finches (*Poephila acuticauda* and *P. personata*) (Evans and Fidler, 2005; Heuman, 1926; Smedley, 1904). Analysis of historical records shows that Gouldian finches have declined in abundance (up to 40-50% decline in reporting rate), and spatial extent (42% decline in grid-cells occupied); the location and timing of decline is associated with intensification of land-use (primarily pastoral) by Europeans (Franklin, 1999; Franklin et al., 2005). The decline has been most severe in Queensland, which has the highest human population density of the tropical states (Evans and Fidler, 2005; Franklin, 1999; Tidemann et al., 1993). Obligate reporting from avicultural finch trappers in Western Australia in the 1970's indicate that the decline may have been more severe, with an 87% decline in Gouldian finches caught until Gouldian finch trapping was banned in 1982 (and subsequently trapping for all finches in 1986) (Franklin et al., 1999; Tidemann, 1996). Despite this evidence of decline, the Gouldian finch recently had its threat-status down-graded from 'Endangered' to 'Near Threatened' by the IUCN, based on estimates of current population size that suggest the Gouldian finch population is stable or even increasing (Garnett et al., 2011). These estimates come from a series of *ad hoc* observations by bird watchers, and relatively few systematic surveys of birds visiting water holes at two locations separated by 730km (Garnett et al., 2011). There is currently no reliable data about size of Gouldian finch populations. Previous examination of contemporary populations of the Gouldian finch indicates that there is no genetic structure across the range (Bolton et al., 2016; Esparza-Salas, 2007; Heslewood et al., 1998). However, these studies provide no indication as to whether the observed connectivity is a recent phenomenon (such as population movement due to displacement or habitat degradation (Caplins et al., 2014; Welch et al., 2012)), and provide information on neither the magnitude of population decline nor current effective population size.

For the first time, museum skins from across the historical range of the Gouldian finch were used to explicitly assess genetic diversity and geographic structure prior to the observed decline in the mid twentieth Century. Population structure in the contemporary population was previously characterised elsewhere (Bolton et al., 2016), but the status of the populations

in Queensland remained unresolved due to the difficulty of sampling contemporary populations in this region. Here the historical genetic structure is investigated in the Gouldian finch to determine whether population changes over the twentieth Century affected population connectivity and to resolve whether Queensland populations were historically genetically unique. Historical samples were directly compared to contemporary samples to characterise changes in genetic diversity, identify the existence and intensity of any genetic bottleneck in the twentieth Century, and determine whether the population has recovered. To this end, effective population size was estimated in the contemporary populations using large, temporally discrete datasets. The results are discussed in the context of other population declines and the conservation consequences for the Gouldian finch.

Methods

Sampling

Historical Gouldian finch samples were obtained from toe-pads of skins accessioned in museums (Museum Victoria, South Australia Museum, and the American Museum of Natural History (see Table A1 for accession details), prior to the reported population decline described above (1890-1920). The Gouldian finch's range covers the monsoon tropics of northern Australia so samples from the states of Western Australia, the Northern Territory and Queensland were used to represent the western, central and eastern extent of this range respectively. Samples with explicit locality information were prioritised, and a total of 36 skins were sourced from Queensland, 34 from the Northern Territory, and 34 from Western Australia. Sampling localities fell broadly into five regions that correspond roughly to biogeographic zones: Western Kimberley, Eastern Kimberley, Top End, Western Queensland, and Cape York (Figure 1). Throughout this text, "sampling region" will refer to these sub-regions within each state, and are analysed in addition to the state level groupings.

Modern samples were caught between 2004 and 2013 from five locations in Western Australia and the Northern Territory, and a small sample from Queensland (Figure 1); detailed sampling protocols are outlined in previous work (Bolton et al., 2016). For all

analyses directly comparing diversity between historical and contemporary datasets, we used subsets of previously published microsatellite (n=251) and mtDNA (n=152) datasets (Bolton et al., 2016). N_e estimates were derived from a larger set of individuals in the contemporary population sampled over multiple sites and years (Table A2).

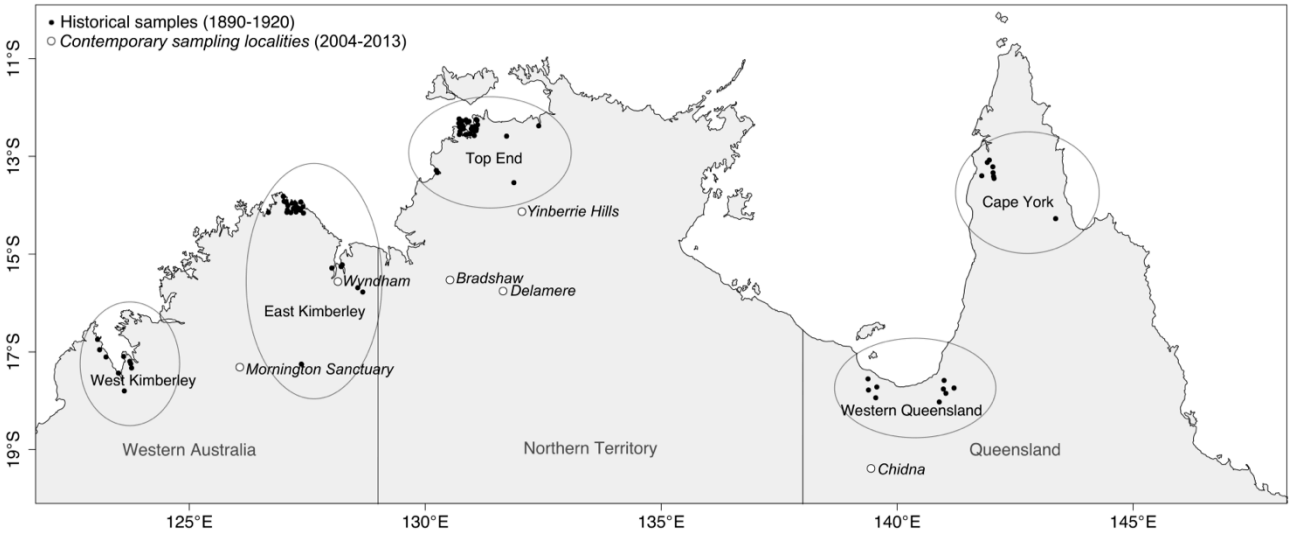

Figure 1: Sampling of Gouldian finches in historical (black dots) and contemporary populations (white dots and italic text). Historical samples are grouped in some analysis by state as an analogue for western, central and eastern extent of the distribution, and where available subdivided further by geographical regions: West Kimberley, East Kimberly, Top End, Western Queensland and Cape York. Plot only shows historical samples for which explicit geographic locality information was included, and specimens with the same co-ordinates have been artificially spaced out to give an indication of sampling density in an area and is not intended as a precise representation of the sampling co-ordinates

Laboratory procedures and genotype scoring

To ensure that historical samples were free from contamination from contemporary DNA, all extractions were conducted in a separate clean-room, at the Australian Centre for Ancient DNA, University of Adelaide; no contemporary bird specimens or DNA were ever used in this room, and workflows included regular decontamination, and disposable PPE. For more information on these standard protocols used please see Joseph et al. (2016). In this

facility, DNA was extracted from toe-pads using the DNeasy Tissue Kit (Qiagen, Valencia, California, USA), with protocol modifications outlined elsewhere (Joseph et al., 2016). Negative controls were included in the extraction process to detect any contamination. All negative extraction controls were included in the downstream PCRs.

Aliquots of the historical DNA samples were amplified in a dedicated space in a laminar flow cabinet at Macquarie University that had never contained or been used for amplification of any avian DNA. To ensure PCRs were free from contamination, the workspace was decontaminated with sodium hypochlorite and UV radiation after each use, and the user wore dedicated PPE that had never been used for handling contemporary bird samples or DNA.

The protocols used to extract and amplify samples of contemporary Gouldian finches are described by (Bolton et al., 2016) with some modifications for the historical samples: microsatellite multiplexes 1-3 were unmodified; multiplex 4 and 5 were amplified in 5ul reactions with final concentrations of Immolase Taq 1U, ImmoBuffer 1X, 1mM dNTPs, 4mM $MgCl_2$, 0.2uM per primer, and 0.8ug/uL of bovine serum albumen. Final PCR reactions used positive controls from museum specimens used to develop the PCR conditions, negative controls from the extraction process and a template free PCR negative control. To check for repeatability at least 20% of samples were sequenced twice, in addition to problematic samples and where novel alleles were identified. In total, we achieved an average of 1.4 successful amplifications per genotype. All multiplexes were run on an ABI3730. Genotypes were scored in GeneMapper (Applied Biosystems, Foster City, CA, U.S.A.). Loci with more than 30% missing data were removed from the dataset, as were individuals with more than 50% missing data. After filtering, our data set contained nine loci across 101 individuals, from the 16 loci used previously on contemporary samples (Bolton et al., 2016). Four of these loci were removed because they were not in Hardy-Weinberg Equilibrium (Table S3), leaving five loci for downstream analysis. The contemporary dataset was reduced to match the five loci successfully typed in the historical samples.

For historical samples, a ~220bp fragment of the mitochondrial control region was amplified using previously developed primers GouldmtF and GouldmtR (Esparza-Salas, 2007). Total volume of PCR reactions was 12uL, with final concentrations of Platinum Taq High Fidelity 1U, 1X Buffer, 0.25mM dNTPs, 2mM $MgSO_4$, 0.4uM of each primer and 1ug/uL of bovine serum albumen. Thermal cycler protocol was 94°C for 2min, then 50 cycles of 94°C for 15s, 52°C 15s, 68°C for 30s, and a final extension of 68°C for 10 min. Amplicons were sequenced on an ABI 3730. Sequences were inspected using SeqTrace (Stucky, 2012), and any polymorphisms that were not found in the sample of contemporary individuals were sequenced twice to verify the authenticity of putatively novel polymorphisms.

Historical diversity and structure

Using data from historical samples, microsatellite heterozygosity (H_O, H_E) and allelic richness (N_A) were calculated in ARLEQUIN v3.5 (Excoffier and Lischer, 2010), and private alleles (N_{PA}) were calculated in GENALEX v6.5 (Peakall and Smouse, 2012). Each diversity estimate was calculated for each state and sampling region (subsection of sampling state, see Figure 1) in the historical dataset. These were compared with the same diversity estimate calculated from the matching subset of five loci in the contemporary population (Bolton et al., 2016). Deviation from Hardy-Weinberg equilibrium in the historical dataset was calculated in Arlequin using an exact test with a Markov Chain length of 1,000,000 and 100,000 dememorization steps. Linkage disequilibrium between loci in the historical dataset was tested in GENEPOP v4.2 (Raymond and Rousset, 1995).

Mitochochondrial DNA (mtDNA) haplotype richness (H), haplotype diversity (*h*) and nucleotide diversity (π) was calculated in DnaSP for the entire historical sample, and each state, region and time bin (Librado and Rozas, 2009). Mitochondrial DNA genetic structure according to sampling state (analogue for east, central and west) was estimated using a median-joining network (ε=0; Bandelt *et al.*, 1999) implemented in the program POPART (http://popart.otago.ac.nz). Differentiation between sampling regions and states was also

explored using AMOVA in Arlequin by comparing haplotype frequencies (F_{ST}) and sequence divergence using pairwise difference (ϕ_{ST}).

Using the microsatellite dataset, geographic structure was explored using an analysis of molecular variance (AMOVA) and the *a priori* geographic groupings of state and region. This was conducted in Arlequin with 10,000 permutations (Excoffier and Lischer, 2010). Pairwise F_{ST} for each population grouping was also calculated. We also explored historical genetic structure in the microsatellite dataset without using *a priori* populations using the Bayesian clustering method implemented in STRUCTURE (Pritchard et al., 2000). We used the admixture model with a burn in of 100,000 over 1,000,000 MCMC iterations with ten replicates per simulated cluster (K) 1-10. Number of K was determined using both Ln P(Data) and ΔK using STRUCTURE HARVESTER v0.6.94, as the ΔK does not allow evaluation of K=1 scenario (Earl and VonHoldt, 2012; Evanno et al., 2005; Pritchard et al., 2000). Q-plots were generated with CLUMPP v1.1.2 and DISTRUCT v1.1 and were used to verify the number of clusters from the two K selection methods (Jakobsson and Rosenberg, 2007; Rosenberg, 2004).

Diversity through time

Evidence for a genetic bottleneck in the Gouldian finch was explored by comparing estimates of genetic diversity between the historical and contemporary samples using microsatellite and mtDNA datasets. Given that there was no evidence of genetic structure in either the contemporary or historical dataset (see results), all samples in the historical dataset were compared with all samples in the contemporary dataset.

Population bottlenecks can reduce microsatellite genetic diversity and allelic richness (number of alleles), but because allelic richness estimates are sensitive to differences sample size (Leberg, 2002), a rarefaction approach was used to calculate the corrected allelic richness for the smaller historical sample in ADZE 1.0 (Szpiech et al., 2008). Differences in rarefied microsatellite allelic richness and heterozygosity were compared between historical and contemporary samples using a Wilcox signed-rank test. Further, if there was a significant

change in allele frequencies between historical and contemporary samples (owing to a bottleneck, for example), then there should be genetic differentiation between our two sampling time periods (Skoglund et al., 2014). This was tested using AMOVA and STRUCTURE analyses as described in the previous section.

To statistically compare whether there was a change in mtDNA haplotype richness (H) between historical and contemporary samples, a custom resampling (without replacement) approach was used to rarefy the contemporary sample to the historical sample size (n=104) and a p-value was obtained by comparing the observed historical richness against the resampled distribution (1000 resamples). This was modified from a custom R-script '*shuffleAndExtract'* function, and haplotype richness was calculated using R-package '*haplotypes*' (Aktas, 2015; Tovar, 2012). Rarefaction curves were also generated using ANALYTIC RAREFACTION 2.0 (Holland, 2012). Estimates of haplotype (*h*) and nucleotide diversity (π) were compared using a t-test, assuming the sampling variance conforms to a normal distribution. Parsimony network displaying the haplotypes lost and shared between the historical and contemporary populations were generated using R script '*TempNet'* (Prost and Anderson, 2011).

Demographic history

We tested for evidence for genetic bottlenecks in the contemporary and historical population using a heterozygosity excess test implemented in the program BOTTLENECK v1.2 (Piry et al., 1999), using the two-phase model (TPM) with proportion of single step mutations at 90% with a variance of 30% among multi-step mutations. Analyses were run with 10,000 iterations and significance of heterozygosity excess was tested using the Wilcoxon sign-ranked test. Genetic bottlenecks can also leave a signature in the ratio of number alleles to the allele size range (the M-ratio), where a bottleneck depletes the number of alleles relative to the total allelic size range of the microsatellite (Garza and Williamson, 2001). We calculated the M-ratio using Arlequin, and considered a depletion in the number of alleles to occur when $M<0.68$ (Garza and Williamson, 2001).

The mtDNA sequence data was tested for deviation from neutrality as evidence of demographic changes. Tajima's D is sensitive to selection and demographic changes and measures the difference between the observed nucleotide diversity (π) and the population mutation rate based on the infinite sites model (θ_S), and if there is no selection or demographic changes then D=0 (Tajima, 1989). This was measured in Arlequin, and significant deviation from zero was assessed using 1000 random samples under the null hypothesis. Fu's F statistic is similar to Tajima's D, but is more sensitive to demographic changes (Fu, 1997; Ramos-Onsins and Rozas, 2006). Fu's F was calculated in Arlequin, and significance was assessed using 1000 random samples generated under the null hypothesis, where $p<0.02$ is considered a significant deviation from neutrality (Fu, 1997). The mismatch distribution is the distribution of the number of pairwise differences between pairs of haplotypes, and when a population has experienced a demographic or spatial expansion the distribution of these differences will have a single mode (rather than multi-modal under the neutral expectation) (Excoffier, 2004; Rogers and Harpending, 1992). Significant deviation from observed and expected distributions was assessed using the appropriate parametric bootstrap approach for the spatial and demographic models using 1,000 replicates.

A more comprehensive approach to understanding the demographic history of the Gouldian finch was taken using Approximate Bayesian Computation (hereafter ABC), where alternate demographic scenarios were modeled using DIYABC 2.1.0 (Cornuet et al., 2014). This allowed us to test explicit scenarios about the timing and intensity of genetic bottlenecks by simulating scenarios within set priors of timing and population size, and comparing summary statistics from the simulated to observed datasets. The historical and contemporary datasets were modeled together (5 microsatellite loci and 221bp mtDNA), to increase sample sizes they were pooled into a single 'historical' and 'contemporary' dataset separated by 100 generations. Here, the generation time of the Gouldian finch is set to one year, because the average age at first reproduction in the sympatric long tailed finch (*Poephila acuticauda*), which has a similar body size and is ecologically similar, was 330 days (van Rooij and

Griffith, 2011). Captive Gouldian finches become sexually mature within 9 months, and usually breed in the first breeding season after they were hatched (Evans and Fidler, 2005). However, the Gouldian finch generation time in the wild is uncertain because recapture rates are so low, so the same analyses were repeated assuming a two year generation time, but it did not affect the results.

Because there was no geographic genetic structure in the contemporary or historical populations, simulations included demographic changes to a single population. These simulated a "control" situation where the population underwent no demographic change (Figure 3, SC1), and a recent bottleneck with and without recovery (Figure 2 SC2 and SC3). Northern Australia was affected by cycles of aridity during glacial maxima of the Pleistocene, which likely caused population retractions as in other Australian flora and fauna (Bowman et al., 2010; Byrne et al., 2008), so scenarios were also compared that included an ancient bottleneck (8,000-500,000 generations ago) with and without a recent bottleneck (SC4 and SC5) (Figure 2). Informed by the observation of population declines in the twentieth Century (Franklin, 1999; Franklin et al., 1999), the prior for the timing of the recent bottleneck (t) was uniformly distributed between 10 and 90 generations/years ago, with a duration (t_{bn}) uniformly distributed between 1 and 50 generations. The bottleneck size prior (N_{bn}) was uniformly distributed between 10 and 1000 individuals to represent a bottleneck more severe than the 87% decline observed by trappers (Tidemann, 1996). The prior for contemporary effective population size ($cN_{\#}$) was uniformly distributed between 200 and 8,000 and was based on estimates of current breeding population (1000-2400 individuals) (Garnett et al., 2011). The lower bound of the prior was set much lower than this because population size fluctuation, variance in reproductive success and sex ratio bias could make the contemporary effective size much lower. The upper bound was set at 8,000 to allow for the possibility that the published estimate of population size was severely underestimated due to low sampling effort in this remote area. Details of the summary statistics used are described in Appendix 4, and all demographic priors and mutational models are in Table S11. Each demographic

scenario was simulated 1 million times (5 million simulations total). Alternative scenarios were compared by calculating the posterior probability of each scenario by comparing the number of simulations closest to the observed dataset using i) the direct approach using the 500 simulated datasets closest to the observed and ii) a weighted polychotomous logistic regression deviations between the observed and closest 1% (10,000) simulated datasets. Parameters for posterior distributions for each parameter in the chosen scenario was estimated using local linear regression on 1% of simulations (10,000) closest in parameter space to the observed dataset, with a logit transformation applied to parameter values. For more information on priors, Type I and Type II error calculation, and model checking procedures see Appendix 4.

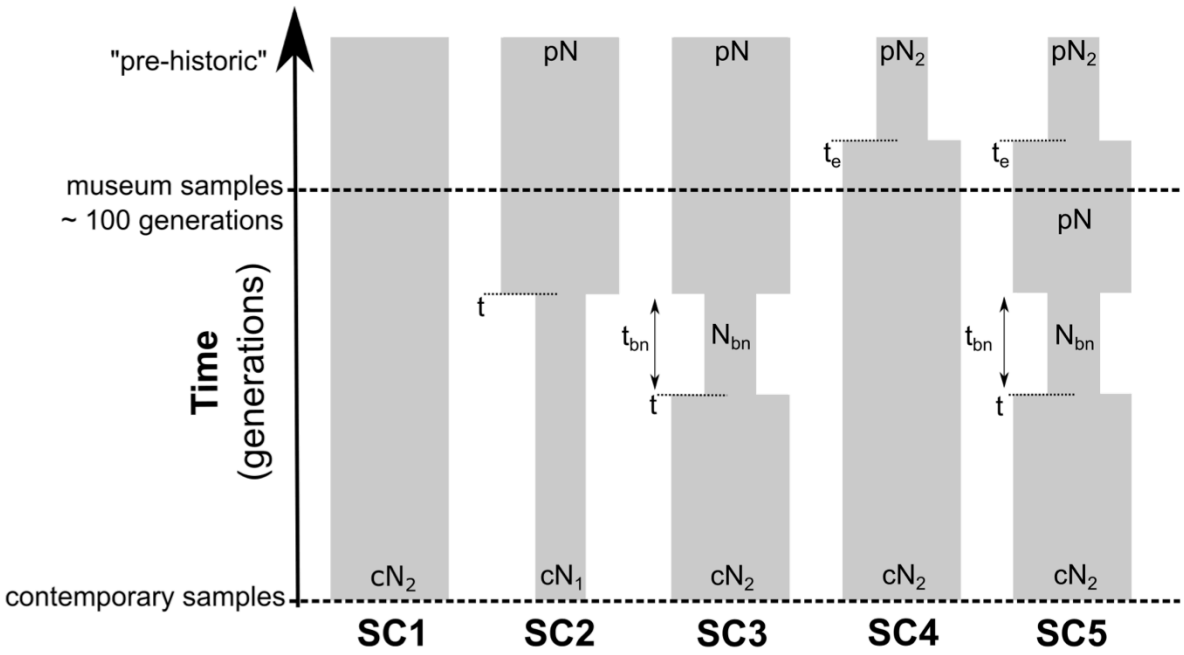

Figure 2: The five demographic histories tested on Gouldian finch data using Approximate Bayesian Computation in DIYABC, with notation used for demographic parameters tested. Where xN_x variables represent effective population sizes, and t_x variables represent the timing of the demographic events. Details for the prior distributions for each of these parameters is provided in text and in Table A11, and the posterior probability for each of the competing scenarios is provided in Table A12.

Contemporary Effective Population Size

Contemporary effective population size (N_e) is the N_e of the current (or most recent few) generations, and can be estimated ($\widehat{N}_e$) from genetic data using a single sample, or individuals sampled across a number of generations (Luikart et al., 2010; Waples, 2005). This is different from the N_e estimated from coalescent approaches (such as those from DIYABC) which represent the N_e over long-time scales (i.e. $4N_e$ generations (Hare et al., 2011). From a plethora of available methods, recent simulation studies have identified temporal and single sample estimators that are the most robust to deviation from simplifying assumptions such as a single isolated population, population bottlenecks and overlapping generations (Gilbert and Whitlock, 2015; Wang, 2016). Two of the most reliable single-sample estimators (Linkage Disequilibrium and Sibship Frequency (Wang, 2009; Waples, 2006)) and the most reliable temporal method (Maximum Likelihood (Wang and Whitlock, 2003)), were used to estimate N_e of the Gouldian finch sampled over a number of years at multiple sites using 16 microsatellites (Bolton et al., 2016). The samples used for each estimator depends on the requirements and precision of each estimator, and are described in detail for each estimator below. Adult age information is not available for these Gouldian finch samples, it is, therefore, not possible to utilise bias corrections for $\widehat{N}_e$(e.g. Waples & Yokota 2007), or methods that explicitly utilise age structure in the calculations (e.g. Wang *et al.* 2010; Waples *et al.* 2011). N_e was not estimated in historical samples because the total sample set was taken over a period of 30 years which is a gross violation of the assumptions, and in combination with the low number of successfully amplified loci, sampling a single year (or even the entire data set) did not yield sufficient precision to be included (i.e. all estimates were infinite).

All N_e estimators used here assume samples derive from a single cohort in a species with non-overlapping generations. When these assumptions are met, single sample estimators tend to measure the number of effective breeders in the parental population (N_b), which is reflective of the inbreeding effective size (N_{eI}). N_b accounts for variation in reproductive success and unequal sex ratios, but cannot predict genetic change within a generation like N_e

(Wang, 2009). If the sample includes multiple generations, then $\widehat{N}_e$ may reflect per generation N_e, or a composite of N_b that produced each generation in the sample, and will depend on the organismal life-history and what genetic properties the estimators measure (Wang, 2016, 2009, Waples et al., 2014, 2013; Waples and Do, 2010).

The linkage disequilibrium (LD) method is a single sample estimator (Hill, 1981; Waples, 2006; Waples and Do, 2010), and was implemented using NeEstimator v.2.01 (Do et al., 2014). Although this method has low accuracy and precision for populations >200, this method can reliably identify N_e in small populations (Waples and Do, 2010), and will enable the exclusion of an N_e <200 in the Gouldian finch. Microsatellite $\widehat{N}_e$were generated for each sampling locality for each year, and for each year with samples pooled across sampling localities (Table S2 for sample sizes). In addition, 3839 SNPs previously described in Bolton et al, (2016) were used to estimate N_e in 2008 and 2009 from adults pooled across sampling localities (Table A2). The LD method can account for lifetime monogamy or random mating systems (Waples and Do, 2010; Weir and Hill, 1980); random mating was assumed in the Gouldian finch because previous work has shown that they are not purely monogamous (Bolton et al., 2017). To reduce bias from low frequency alleles in the LD analysis, only results from analyses including alleles with a frequency greater than 0.02 were included (Waples and Do, 2010).

The sibship assignment frequency method is another single sample estimator (Wang, 2009), and was implemented in Colony v2.0.6.2 (Jones and Wang, 2010). Estimates were generated for each sampling locality in each year, and for each year pooled across sampling localities. There were insufficient individuals in each year to provide a reliable estimate using the SNP data (Table S2). The sibship frequency method allows for correction for non-random mating or random mating according to degree of deviation from Hardy-Weinberg equilibrium (Jones and Wang, 2010; Wang, 2009); here the assumption of random mating was used because none of the contemporary samples deviated from Hardy-Weinberg equilibrium and there was no evidence of assortative mating according to head-colour morph (Bolton et al.,

2017, 2016). The effects of sibship size prior on $\widehat{N}_e$ from the sibship frequency method were evaluated by comparing results using the "weak" sibship prior, and a "Known N_e" prior. The sibship sizes were calculated as the mean number of offspring per male and per female from previous data on Gouldian finch breeding at Wyndham, where mean paternal sibship size was 3.0 and maternal sibship size was 3.6 (Bolton et al., 2017). The "Known N_e" prior includes an estimate of sex ratio (1.26 (Bolton et al., 2017)) and an estimate of 1000 for N_e was used based on previous work (Garnett et al., 2011).

Temporal estimators (using samples separated by generations) estimate the variance effective population size (N_{eV}), which reflects the amount of genetic drift in a population (Baalsrud et al., 2014; Wang et al., 2016; Waples, 2010). These produce an estimate of the harmonic mean of Ne over the sampled generations, but overlapping generations can cause bias in these estimates (Waples and Yokota, 2007). The temporal method of N_e estimation was implemented using the Maximum likelihood approach implemented MLNe v1.0 (Wang and Whitlock, 2003), because ML methods are much more accurate than moment methods when the number of generations between samples is small (Wang et al., 2016; Waples and Yokota, 2007). A sample of 145 birds from the Australian Wildlife Conservancy's Mornington Sanctuary was compared against a sample of 260 birds pooled across sampling localities caught in 2008. Another estimate was provided by comparison of allele frequencies in same 2006 sample against a pooled sample of birds caught in 2009 (n=134). A third estimate was produced by measuring change between samples in 2006, 2009 and a sample of 22 individuals in Wyndham caught in 2013. SNP data was not used here because there were too few individuals sufficiently spaced across generations. The Gouldian finch generation time in these analyses was considered to be one year. Populations were assumed to be not in migration-drift equilibrium and the maximum N_e was set to 20,000.

Results

All 104 historical samples yielded at least one microsatellite genotype, but after removing loci with more than 30% missing data, and individuals with data missing from more

than 50% of loci there were genotypes for 101 of 104 individuals. Across the five remaining loci, the frequency of missing data was 2.5%, see Appendix 2 for more details.

Historical diversity and structure

Summary statistics for genetic diversity per locus in the entire historical sample are presented in Table 1, and per sampling state and region (category within state, see Figure 1) in Tables S5-6. Microsatellite data showed no evidence for geographic genetic structure according to state or sampling region in the historical dataset based on the results of the AMOVA or pairwise F_{ST} (Tables S7-S10). Similarly, the clustering analysis in STRUCTURE showed no evidence of genetic structure. The optimal ΔK was K=2, but the highest log probability of the data (Ln P(Data)) was at K=1 (Figure A1a,b). Inspection of the q-plots showed all individuals were equally admixed (Figure A1c), and therefore a K=1 was determined to be the most appropriate model of population structure in the historical samples.

Summary diversity for mitochondrial control region data in the historical population is presented in Table 2, with sampling state and region presented in Tables A5-6. The mtDNA data showed no evidence of genetic differentiation by state according to pairwise nucleotide differences (Table S8-9), but there was statistically non-significant evidence for genetic differentiation according to haplotype frequency (AMOVA: Among populations 2.07%, Within Populations 97.93; FST=0.02 p=0.054), possibly driven by haplotype frequency differences between Queensland and the Northern Territory (Table A8-9). However, at the sampling level region (within states) there was no evidence of genetic differentiation for any measure (Table S8 and S10). Median-joining network describing differences between states are presented in Figure 3.

Table 1: Summary of microsatellite diversity statistics for each locus, measuring number of alleles or allelic richness (N_A), the number of alleles rarefied to the minimum sample size in the historical dataset for that locus (N_{Ar}), number of alleles private to each time period (N_{PA}), observed and expected heterozygosity (H_O, H_E), and the ratio of number of alleles to size range (M). For each statistic the mean and standard deviation across the nine loci is presented.

	Parameter	Ego52	BF18	Tgu11	Pca7	Pco2	Mean (± SD)
Historical	n	98	101	99	100	94	
	N_A	9	11	10	26	5	12.20 (± 8.04)
	N_{PA}	0	0	2	1	1	0.80 (±0.84)
	H_O	0.63	0.72	0.66	0.96	0.60	0.71 (± 0.15)
	H_E	0.71	0.74	0.67	0.94	0.61	0.73 (± 0.12)
	M	0.64	0.42	0.48	0.43	0.56	0.50 (± 0.09)
Contemporary	n	251	251	251	251	251	
	N_A	11	16	11	28	4	14.00 (± 8.92)
	N_{Ar}	9.41	13.35	8.38	24.66	3.92	11.94 (± 7.86)
	N_{PA}	2	5	3	3	0	2.6 (± 1.86)
	H_O	0.74	0.65	0.63	0.92	0.56	0.70 (± 0.14)
	H_E	0.77	0.78	0.62	0.93	0.59	0.74 (± 0.14)
	M	0.69	0.12	0.46	0.44	0.57	0.46 (± 0.21)

Table 2: Summary of diversity indices (± sampling standard deviation) for 221bp mitochondrial control region data in the historical and contemporary samples. For each time point the table describes, the number of samples (N), the number of segregating sites (S), the (uncorrected) number of haplotypes (H), number of private haplotypes (H_P), haplotype diversity (*h*), nucleotide diversity (π), and Fu's F and Tajima's D neutrality tests, where bolded values are statistically significant ($p<0.02$).

	N	S	H	H_P	*h*	$\pi \times 10^2$	Tajima's D	Fu's F
Historical	104	10	23	11	0.80 (± 0.03)	0.84 (± 0.07)	-0.60	**-19.28**
Contemporary	152	13	20	9	0.76 (± 0.03)	0.71 (± 0.05)	-0.86	**-11.54**

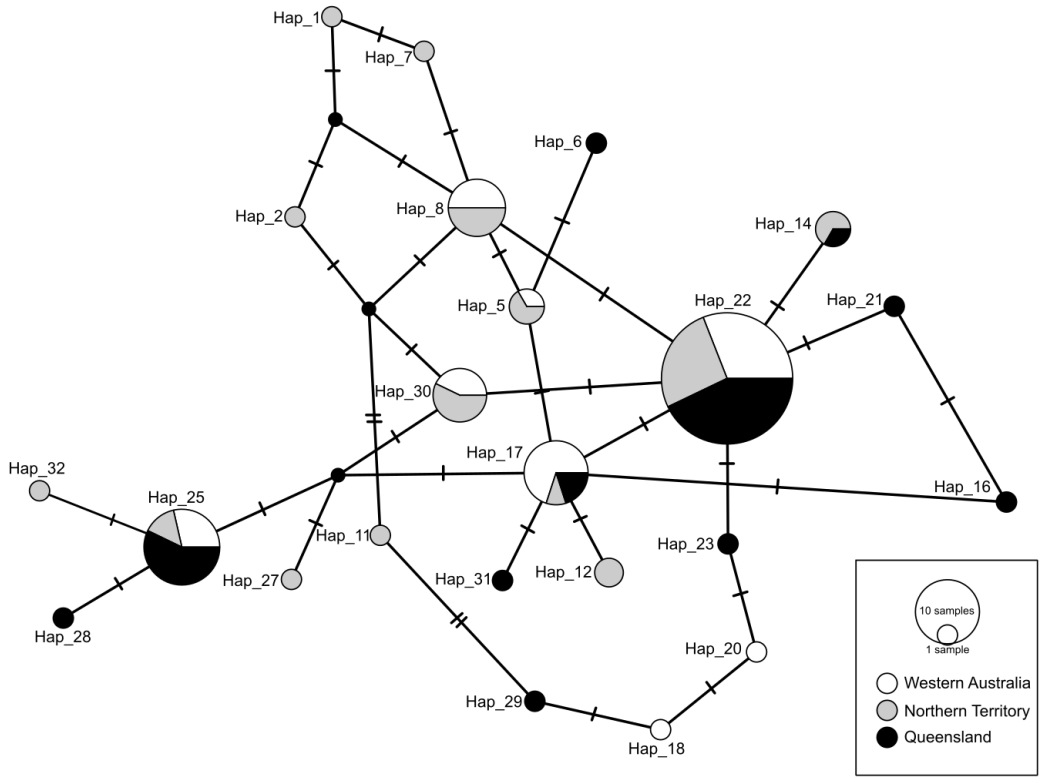

Figure 3: Median-joining network for mitochondrial control region haplotypes in the historical sample of the Gouldian finch. Colours represent Australian states, which is a proxy for eastern, central and western sections of the Gouldian finch distribution. Number of strokes joining the nodes (haplotypes) indicates the number of mutations between haplotypes.

Diversity through time

The allelic richness and heterozygosity for each locus in the historical and contemporary populations are presented in Table 1. Wilcoxon signed-rank tests between historical and contemporary microsatellite data showed no evidence for a reduction in allelic richness in the microsatellite dataset (V=9, p=0.81) (Figure 4a), nor in observed (V=10, p=0.59) or expected (V=7, p=0.1) heterozygosity. AMOVA showed no evidence of differentiation between historical and contemporary populations (Within Individuals 96.05%, Among Individuals 3.92%, Among populations 0.03%; F_{IS}=0.04 p=0.001; F_{ST}=0.0003 p=0.93; F_{IT}=0.04 p=0.001), with evidence of a reduction in heterozygosity in the contemporary population (Historical F_{IS}=0.005 p=0.44, Contemporary F_{IS}=0.05 p=0.0003). Similarly, clustering analysis in STRUCTURE found no evidence of differentiation (Figure A2).

Summary diversity statistics for the mtDNA data are presented in Table 2. Haplotype richness was reduced by 29.6% in the contemporary population, which is significantly lower than the historical richness based on the resampling test from 1000 resamples (p<0.000) (Figure 2b). The specific haplotypes lost are presented graphically in Figure 5. There was a significant reduction in haplotype diversity (t=10.48, df=221.45, $p<1x10^{-16}$), and nucleotide diversity (t=16.31, df=173.08, $p<1x10^{-16}$) in the contemporary population. In contrast, there was no evidence for differentiation between historical and contemporary samples according to haplotype frequencies (Percent variation between historical and contemporary populations: 0.41%, Within Populations: 99.59%, F_{ST}=0.0041, p=0.16), or according to pairwise sequence differences (Percent variation between historical and contemporary populations: 0%, Within Populations: 100%, ϕ_{ST} =-0.0021, p=0.52).

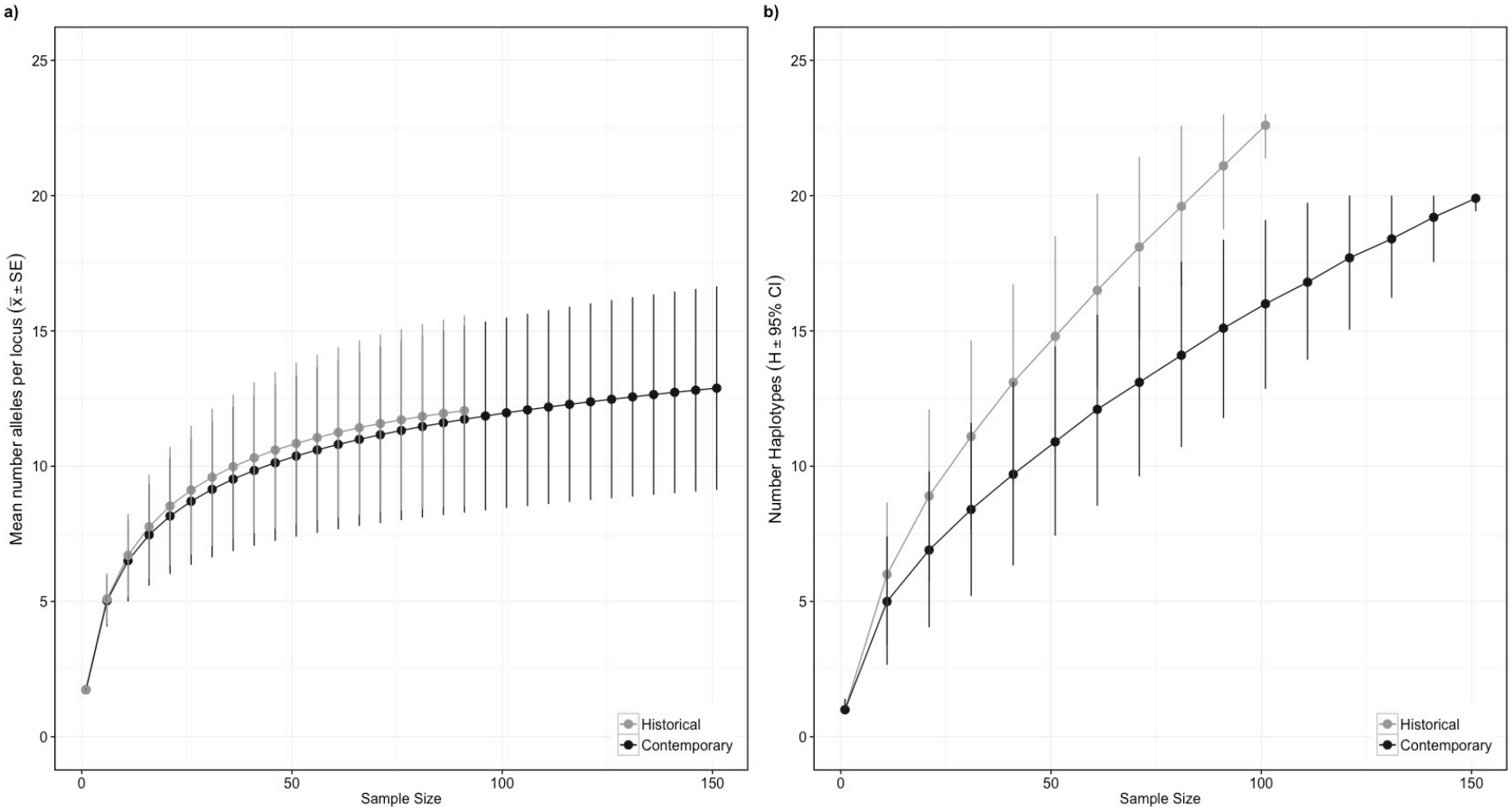

Figure 4: Rarefaction curves comparing richness between historical and contemporary time periods, where plot a) shows microsatellite data as the mean number of alleles per locus (± standard error), and b) shows number of mitochondrial haplotypes (± 95% rarefaction confidence intervals.

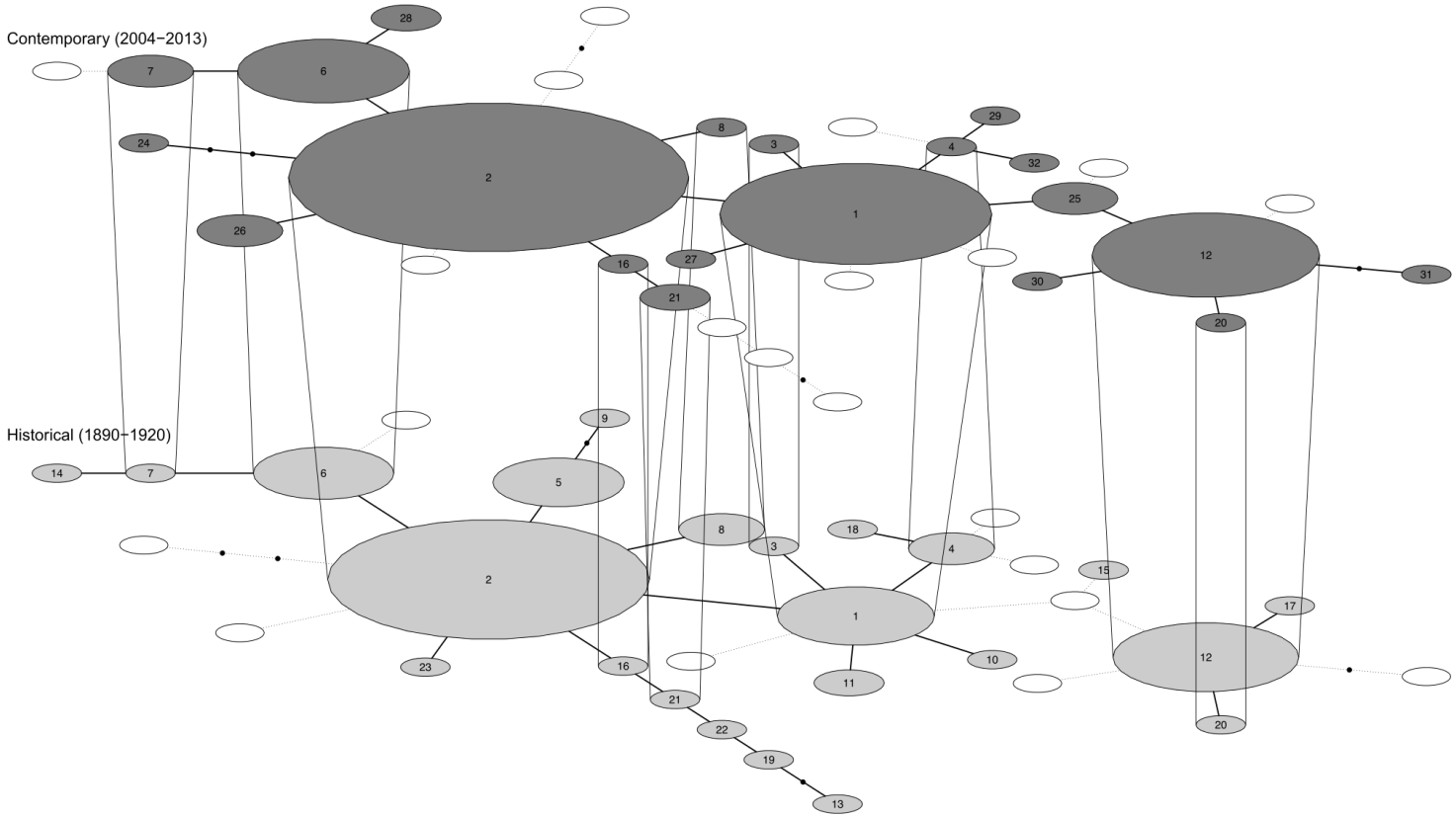

Figure 5: Temporal parsimony network showing haplotypes shared between the historical and contemporary sampling periods produced using R script TempNet (Prost and Anderson, 2011). Ellipse size represents the abundance of a particular haplotype (as in Figure 3), and empty ellipses represent haplotypes that are absent in a particular time point. Dotted lines represent haplotypes separated by a single, un-sampled mutation. Black dots represent each additional mutation that two haplotypes are separated by (e.g. one black dot indicates two haplotypes are separated by two mutations).

Demographic history

Using the BOTTLENECK program, there was no evidence of a heterozygote excess caused by a genetic bottleneck in the contemporary population (p=0.69), or in the historical population (p=0.31). Conversely, measures of the M-ratio (number alleles/allele size range) suggested that both historical and contemporary populations had undergone a genetic bottleneck (M<0.68; Table 1).

Estimates of Tajima's D found no evidence of a deviation from neutrality in the mtDNA data (Table 2). However, Fu's F was significantly negative in both historical and contemporary datasets (Table 2), which is indicative of expansion. Mismatch distribution tests conducted on the mtDNA data found no evidence for a sudden demographic expansion in the contemporary sample (sum of squared deviation (SSD)=0.0038, p=0.57), or in the historical sample (SSD=0.0071, p=0.70). Similarly, mismatch distributions did not support a spatial expansion in the contemporary (SSD=0.0041, p=0.47), or historical population (SSD=0.008, p=0.59).

Different demographic scenarios were tested explicitly using Approximate Bayesian Computation. Both approaches used to calculate posterior probability of demographic scenarios found highest support for the two scenarios that did not include demographic change in the twentieth century, but was unable to differentiate between them (SC1 and SC4, Table A12). Although posterior probabilities were low for SC1 and SC4 (Direct: 0.43-0.47, Logistic: 0.49-43), their 95% confidence intervals did not overlap with any of the other scenarios using the logistic method (Table A12). The same result was found when the mitochondrial data was modeled separately (data not shown). Error rate testing showed that SC1 and SC4 were consistently selected in both approaches even when posterior data were simulated under other scenarios (SC2-3 & SC5). Consequently, type I error rates for SC1 and SC4 were high in both the logistic and direct approaches (SC1: 76.71%, 58.45%; SC4: 20.34%, 36.86%) (Table A13). Type II error rates were similarly high in both the logistic and direct approaches (SC1: 16.37%, 33.81%; SC4: 68.18%, 56.44%) (Table A13). Both chosen scenarios provided similar estimates for the N_e of the contemporary population (cN_2) as

somewhere between 3560 and 7790, with central tendencies between 5700 and 6130 (Table 3). In SC4, estimates of the timing (t_e) and size (pN_2) of the ancient population were much less precise (Table 3), where the past population size in particular was bimodal and occupied the length of the prior distribution (Figure A4c).

Table 3: Estimates from the posterior distribution for demographic parameters from the most supported scenarios in the Approximate Bayesian Computation analysis. The most supported scenarios were SC1, which modeled no demographic change, and SC4, which modeled an ancient demographic change, with no contemporary demographic change. Notation cN_2 represents the contemporary effective population size; t_e is the estimate of the timing of the ancient demographic event (in number of generations which is equal to years in the Gouldian finch), while pN2 represents the ancient effective population size (before t_e). See Figure 3 for more information on modeled scenarios and parameter notation.

Parameter	Mean	Median	Mode	95% HDPI
(SC1) cN_2	5960	5700	6040	[3560-7740]
(SC4) cN_2	5900	5920	6130	[3870-7790]
(SC4) t_e	208000	184000	22900	[17600-479000]
(SC4) pN_2	510	514	630	[368-975]

Contemporary Effective Population Size

Across years and sites $\widehat{N}_e$ from the LD mehod varied between negative values and infinity, with the lowest 95% confidence boundary at 63.8 (Figure A5 and Table A14). The LD method has poor accuracy for N_e>200, and the negative and infinite estimates suggest that sampling error is larger than any signal of LD and drift (Waples and Do, 2010). In such cases, the lower 95% CI should be used to infer a minimum possible population size (Waples and Do, 2010). Estimates from samples pooled across localities in 2008 and 2009 using

microsatellites and the LD method were between 762.7 and Infinite (Table 4a). The much larger SNP dataset achieved better precision with estimates between 2243 and 3258.9 (Table 4a). Because this is a mixed age sample of a species with overlapping generations, the LD estimate reflects the N_e per generation if the species is short-lived, otherwise it is a composite of N_b that produced each generation sampled (Waples et al., 2014; Waples and Do, 2010).

$\widehat{N}_e$ from the sibship frequency method using the "Known N_e" prior was significantly larger than using the weak sibship prior (t=5.03, df=19.022, $p=7.4x10^{-5}$), and across years and localities estimates ranged between 13 and 930 (with confidence intervals between six and the program's maximum N_e value, Figure A6 and Table A15). The estimates from 2008 and 2009 pooled across sampling localities were between 134 and 963 (95% CI 104-1413) (Table 4b). The degree of accuracy and precision of this estimator when used on a mixed-age sample remains to be quantified, but it represents a composite of the N_b for each of the cohorts represented in the sample (Wang, 2016, 2009).

Results from the maximum likelihood temporal estimator show $\widehat{N}_{eV}$ as between 399 and 19989 (Table 4c). Temporal methods on mixed age samples perform better when the samples are spaced multiple generations apart (Waples and Yokota, 2007), which may explain why the two two-sample estimates (2006-2008 and 2006-2009) have upper confidence limits that were the maximum N_e set by the user (20,000).

Table 4: Summary of results for analyses of contemporary estimates of effective population size ($\widehat{N}_e$) with 95% confidence limits in square brackets. Estimates presented here are from samples pooled across sampling localities, where sample sizes for each year are presented in curly brackets. Part a) shows the estimates from linkage-disequilibrium method from 16 microsatellites and 3839 SNPs. Part b) shows the sibship frequency method with the "Known N_e" and weak sibship prior, using microsatellite data only. Part c) shows the maximum likelihood temporal method for each time period using

a) Linkage Disequilibrium	Microsatellites		SNPs	
(Microsatellites + SNPs)	2008 {n=260}	2009 {n=134}	2008 {n=76}	2009 {n=69}
	12698.3	1631.4	2804.8	2554
	[2275.4-Infinite]	[762.7-Infinite]	[2461.1-3258.9]	[2243.4-2963.6]
b) Sibship Frequency	"Known N_e" prior		Weak sibship prior	
(Microsatellites only)	2008 {n=260}	2009 {n=134}	2008 {n=260}	2009 {n=134}
	663 [553-789]	963 [711-1413]	195 [159-246]	134[104-174]
c) Temporal	2006{n=145}	2006 {n=145}	2006 {n=145} – 2009 {n=134}	
(Microsatellites only)	- 2008 {n=260}	- 2009 {n=134}	- 2013 {n=22}	
	1481 [682-20000]	19989 [1379-20000]	399 [253-811]	

Discussion

To test a putative decline of between 40-87% in Gouldian finch populations over the twentieth Century (Franklin, 1999; Franklin et al., 2005; Tidemann, 1996), we compared levels of genetic diversity, population connectivity and effective population size from samples derived from before and after the reported decline (historical: 1890-1920; contemporary 2004-2013). Previous work was unable to reliably identify the level of connectivity between birds in the west and Queensland because of the paucity of contemporary samples available (Bolton et al., 2016), but comprehensive historical samples from Queensland in this study demonstrates that these individuals were part of one large population including those from the western part of the species' range. This result is unusual because the Gulf of Carpentaria and the Selwyn ranges in western Queensland constitutes a significant biogeographic break for a number of species and allo-species distributed across the monsoon tropics (Bowman et al., 2010; Ford, 1987; Pepper et al., 2016; Toon et al., 2010), and may be indicative of large dispersive capacity of Gouldian finches (Bolton et al., 2016; Woinarski and Tidemann, 1992). Furthermore, given that the historical sample showed no evidence of geographic genetic structure, human impacts have had no detectable effect on patterns of gene-flow (Bolton et al., 2016). Finally, microsatellite and mtDNA data suggest that genetic diversity in the historical population was comparable with the contemporary population.

The most striking result from these analyses is that ABC found no evidence for a strong reduction in the population size in the Gouldian finch in the twentieth Century (reported to be up to 87%), corroborated by no decline in microsatellite diversity. Yet, there was evidence of a reduction in mtDNA diversity across time. One explanation is that the population did not decline as severely as was parameterized in the ABC model (87%, N_{bn} 10-1000), and the less severe declines documented using museum records (40-50% declines) may be more realistic (Franklin, 1999; Franklin et al., 2005). Indeed, the 87% estimate may be more spatially biased than museum records if finch trappers had high site fidelity (Graham et al., 2004; Tidemann, 1996). Other studies on birds that have purportedly experienced strong historical population declines were also unable to detect population genetic signatures for

decline (Dussex et al., 2015; Welch et al., 2012). In these species it is possible that declines were not as severe as thought, or that overlapping generations and long-lifespans can harbor genetic diversity and mask such signatures (Welch et al., 2012).

Simulation studies have shown that detecting moderate bottlenecks with recovery (~50% reduction over hundreds of generations) using summary statistics (such as gene diversity) has very low power unless sampling before and directly after the bottleneck using thousands of markers (Mourier et al., 2012; Ramakrishnan et al., 2005). Meta-analytic and simulation studies have shown that bottlenecks of >200 individuals, irrespective of the percent decline, are virtually undetectable through changes in genetic diversity (Hoban et al., 2014; Jackson et al., 2013). The DIYABC program simulates an instantaneous demographic event (Cornuet et al., 2014, 2008), but further simulation studies have demonstrated that the type of decline (gradual or instantaneous) and the rate of recovery over the scale of a few generations can dramatically influence the magnitude of change in summary statistics and hence the power of detection (Hoban et al., 2014, 2013). That a decline in diversity was observed only in the mitochondrial data may be because of the smaller N_e of mitochondrial DNA; this may result in the loss of alleles at a greater rate than in nuclear DNA. Comparison of the long-term N_e derived from DIYABC with contemporary estimators may be the best approach to discern recovery in the Gouldian finch population (Hare et al., 2011). Contemporary $\widehat{N}_e$ suggests that the current N_e may be lower than the long-term N_e but the confidence intervals for some methods do overlap with the estimate from DIYABC. Although these data cannot provide quantitative estimates as to the severity of the decline and recovery in the Gouldian finch, our analyses suggest that the decline was not as severe as the upper estimate from finch trapping data (<87%, N_e>1000) (Tidemann, 1996).

Interestingly, although some tests showed no signal for bottlenecks or expansion, the M-ratio test (on microsatellites) and Fu's D (on mtDNA) provided evidence of bottlenecks and expansions in both historical and contemporary samples. That these were detected in both samples may be indicative of an ancient demographic event, which was also supported by the

ABC analysis in Scenario 4. This may be a signal of demographic events associated with glacial cycles in the Pleistocene, which have structured genetic diversity in other monsoon tropical species in Australia (Catullo et al., 2014; Kearns et al., 2014). The other explanation for these results is recurrent population fluctuations, and may be another factor obscuring the ability to detect a single bottleneck in the twentieth Century. Indeed, census data for Gouldian finches at Yinberrie Hills and catches at Mornington Sanctuary and Wyndham show some population fluctuations over a few years (Bolton et al., 2016; Legge et al., 2015; O'Malley, 2006). However, populations that fluctuate generally have very low effective population size to census size (N_e/N) ratios (Hung et al., 2014; Vucetich et al., 1997). $\widehat{N}_e$ minima for the contemporary Gouldian finch populations are quite variable across years, but these estimates are similar or larger than the current estimated adult Gouldian finch population size (1000-2500 individuals, Garnett et al., 2011). A more extensive genetic sampling scheme over the twentieth Century, with knowledge of census sizes would be the most appropriate way to resolve the recent demographic history of the Gouldian finch, but such samples are not available.

Our estimates of N_e and effective number of breeders (N_b) can be used to infer whether the population is currently of conservation concern. The three different methods of contemporary N_e estimation employed here reflect the different aspects of generational N_e, variance N_e, and effective number of breeders (N_b). Because the generation time is short in the Gouldian finch, the LD estimates probably reflect generational N_e (Waples et al., 2014; Waples and Do, 2010), which is approximately 2,000 individuals and roughly consistent with estimates of adult population size (1000-2500 breeding adults, Garnett et al., 2011). In contrast, the sibship method likely reflects a composite of the N_b resulting in the present mixed-age sample (Wang, 2016, 2009). Here, the estimates were consistently a few hundred individuals, which is roughly consistent with the observation that known breeding localities do not contain more than a hundred or so individuals (Brazill-Boast et al., 2013; Tidemann et al., 1999). Indeed, the minimum bound of many of these N_e estimates are within the range to

be considered of conservation concern using the 50/500 or more conservative 100/1000 rule, that guides the minimum population sizes to guard against inbreeding and loss of evolutionary potential through genetic drift (Frankham et al., 2014; Franklin, 1980).

The ratio between effective population size and census size (N_c) is generally thought to be low (0.1) (Frankham, 1995; Palstra and Fraser, 2012), and if the results presented here are taken at face value then the Gouldian finch N_c ought to be much larger than the N_e. In the Gouldian finch samples $\widehat{N}_b < \widehat{N}_e$, which conforms to the idea that $N_b \leq N_e$ in species with non-overlapping generations (Wang, 2009; Waples et al., 2013). However, the Gouldian finch does live beyond a single reproductive year (Bolton et al., 2016; Legge et al., 2015), and the N_e estimators used are highly sensitive to generation time and degree of overlap in mixed age samples (Wang, 2009; Waples et al., 2014; Waples and Do, 2010). Indeed, recent work has shown that the relationship between N_e/N_c and N_e/N_b is highly dependent on a few key demographic parameters: sexual maturity, lifetime fecundity and lifespan (Lee et al., 2011; Ruzzante et al., 2016; Waples et al., 2013; Waples and Antao, 2014). In other birds, on average N_b was larger than N_e (1.35), and N_b and N_e were much closer to parity with N_c (0.86-0.65 (Waples et al., 2013)). Banding estimates of some of these parameters in the Gouldian finch suggest extremely high mortality rates (99-81%) and short life expectancies which resembles a type III survivorship curve (Woinarski and Tidemann, 1992). However, it is impossible to differentiate the effects of high juvenile dispersal from mortality due to low regional banding efforts (Bolton et al., 2016; Woinarski and Tidemann, 1992). Subsequent work at Australian Wildlife Conservancy's Mornington Sanctuary and Wyndham on wild Gouldian finches show that adults have been recaptured at the same site up to three and six years apart (Bolton et al., 2016; Legge et al., 2015). At Wyndham, a minimum of 2-13% of an adult sample will be from a previous generation with parents and their offspring breeding in subsequent years (Bolton et al., 2016). However, it is impossible to tell whether the remainder of the population is made up of first or multi-generation migrants. Therefore, understanding the lifespan and survivorship curve of the Gouldian finch in the wild is critical to providing a

more accurate assessment of N_e, N_b and N_c estimates. That work will be challenging because it is likely that individuals move between sites over their life.

Conclusions and management implications

Comparison of genetic data from historical and contemporary samples in the Gouldian finch was unable to detect a severe bottleneck occurring over the twentieth Century. The population bottleneck intensity was probably much less (40-50%, N_e>1000), but less intense declines are nearly impossible to detect even with temporal population genetic data (Hoban et al., 2014; Mourier et al., 2012). Quantification of the bottleneck will likely remain unresolved unless large a number of markers are applied in a time series that includes samples proximal to the supposed bottleneck time.

This work has identified two key areas relevant to current conservation management of the Gouldian finch that ought to be addressed. The first is to clarify the status of the current Gouldian finch population in Queensland. We have demonstrated that previously the Gouldian finch was a single population across the entire distribution. However, the Gouldian finch is currently much less common across Queensland (Garnett et al., 2011), and previous work had insufficient contemporary samples to draw robust conclusions about connectivity between Queensland and the western range (Bolton et al., 2016). The reduced densities and/or reduction in suitable habitat may substantially alter movement patterns and hence gene flow. Therefore, even preliminary banding and genetic surveys in western Queensland and Cape York will yield important results.

The second priority required for improved conservation management decisions is the more accurate estimation of demographic parameters such as generation time and adult life-expectancy. Data from contemporary populations of the Gouldian finch suggest that the current global N_e is probably around a thousand individuals, but N_b may be as low as a few hundred. However, an understanding of the demographic parameters in the wild will enable better sampling designs, and the implementation of N_e estimation methods that account for demography (Wang et al., 2010; Waples et al., 2011; Waples and Yokota, 2007), will enable

better predictions about how this relates to census size (N_c). Accurate estimates for generation time and adult lifespan are currently unavailable because the Gouldian finch return rates between years are very low to nil, which makes it impossible to differentiate mortality from dispersal and determine the age of individuals (Bolton et al., 2016; Legge et al., 2015; Woinarski and Tidemann, 1992). Current survey methods are systematic in surveying the same sites (water holes) across years, but there is annual variation in the availability of drinking water in the wider environment, therefore current mark recapture methods may be more effective if considering a larger set of water holes contemporaneously. An alternative, realistically available for birds of this size in the next few years, could be to use RFID, GPS or satellite tracking of cohorts of birds (Bonter and Bridge, 2011; Bouten et al., 2013). This can potentially provide an average life-expectancy, and provide much needed information on dispersal distances in this species and help predict movement of birds in and out of Queensland (Bolton et al., 2016).

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Table A1: Historical specimens and their voucher numbers. Museum abbreviations are: AMNH is the American Museum of Natural History, MV is Museum Victoria and SAM is the South Australia Museum. State abbreviations are: NT is Northern Territory, QLD is Queensland and WA is Western Australia. "Region" refers to the geographic region assigned to the sample in Figure 1 of the main text, where E and W are abbreviated for East and West, and “Location” was detail described on the voucher.

Sample ID	Voucher	Museum	Date	State	Region	Location	Latitude	Longitude
16739	721686	AMNH	1911	NT	Top End	Anson Bay	-13.36	130.27
16740	721687	AMNH	1911	NT	Top End	Anson Bay	-13.36	130.27
16741	721688	AMNH	1895	NT	Top End	Mary River	-12.61	131.67
16742	721689	AMNH	1903	NT	Top End	Eureka	-13.49	132.02
16743	721690	AMNH	1903	NT	Top End	South Alligator River	-12.43	132.41
16744	721694	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16745	721695	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16746	721696	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16747	721697	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16748	721700	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16749	721701	AMNH	1905	NT	Top End	Port Darwin	-12.42	130.91
16738	155716	AMNH	1917	QLD	NA	NA	NA	NA
16750	721704	AMNH	1914	QLD	Western QLD	Normanton	-17.83	141.03
16751	721705	AMNH	1914	QLD	W QLD	Normanton	-17.83	141.03
16752	721706	AMNH	1913	QLD	W QLD	Normanton	-17.83	141.03
16753	721707	AMNH	1913	QLD	W QLD	Normanton	-17.83	141.03
16754	721708	AMNH	1892	QLD	W QLD	Gulf of Carpentaria	NA	NA
16755	721709	AMNH	1892	QLD	W QLD	Gulf of Carpentaria	NA	NA
16756	721710	AMNH	1893	QLD	NA	NA	NA	NA
16757	721711	AMNH	1893	QLD	NA	NA	NA	NA

Sample ID	Voucher	Museum	Date	State	Region	Location	Latitude	Longitude
16758	721712	AMNH	1893	QLD	NA	NA	NA	NA
16759	721713	AMNH	1893	QLD	NA	NA	NA	NA
16760	721714	AMNH	1893	QLD	NA	NA	NA	NA
16761	721715	AMNH	1893	QLD	NA	NA	NA	NA
16762	721716	AMNH	1892	QLD	NA	NA	NA	NA
16763	721717	AMNH	1892	QLD	NA	NA	NA	NA
16764	721718	AMNH	1892	QLD	NA	NA	NA	NA
16765	721719	AMNH	1890	QLD	NA	NA	NA	NA
16766	721720	AMNH	1890	QLD	NA	NA	NA	NA
16767	721721	AMNH	1893	QLD	NA	NA	NA	NA
16768	721722	AMNH	1893	QLD	NA	NA	NA	NA
16769	721726	AMNH	1899	QLD	NA	NA	NA	NA
16797	721782	AMNH	1914	QLD	NA	Auburn	NA	NA
16770	721727	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16771	721728	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16772	721729	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16773	721730	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16774	721731	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16775	721732	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16776	721733	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16777	721734	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16778	721735	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16779	721736	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16780	721737	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16781	721738	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16782	721739	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16783	721740	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16784	721741	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18

16785	721742	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16786	721743	AMNH	1910	WA	E Kimberley	Napier	-13.95	127.18
16787	721744	AMNH	1909	WA	E Kimberley	Napier	-13.95	127.18
16788	721749	AMNH	1900	WA	W Kimberley	Derby	-17.35	123.66
16789	721750	AMNH	1900	WA	W Kimberley	Derby	-17.35	123.66
16790	721751	AMNH	1900	WA	W Kimberley	Derby	-17.35	123.66
16791	721752	AMNH	1900	WA	W Kimberley	Derby	-17.35	123.66
16792	721753	AMNH	1908	WA	E Kimberley	Parry’s Creek	-15.37	128.13
16793	721754	AMNH	1908	WA	E Kimberley	Parry’s Creek	-15.37	128.13
16794	721755	AMNH	1909	WA	E Kimberley	Parry’s Creek	-15.37	128.13
16795	721756	AMNH	1902	WA	E Kimberley	Thompson's Springs	-15.6	128.7
16796	721757	AMNH	1902	WA	E Kimberley	Thompson's Springs	-15.6	128.7
16637	R615	MV	1905	NT	Top End	Darwin	-12.42	130.91
16638	R616	MV	1905	NT	Top End	Darwin	-12.42	130.91
16639	R617	MV	1905	NT	Top End	Darwin	-12.42	130.91
16640	R627	MV	1905	NT	Top End	Darwin	-12.42	130.91
16641	R628	MV	1905	NT	Top End	Darwin	-12.42	130.91
16642	R629	MV	1905	NT	Top End	Darwin	-12.42	130.91
16643	R630	MV	1905	NT	Top End	Darwin	-12.42	130.91
16644	R631	MV	1905	NT	Top End	Darwin	-12.42	130.91
16645	R632	MV	1905	NT	Top End	Darwin	-12.42	130.91
16647	R14580	MV	1905	NT	Top End	Darwin	-12.42	130.91
16648	R14582	MV	1905	NT	Top End	Darwin	-12.42	130.91
16649	R14583	MV	1905	NT	Top End	Darwin	-12.42	130.91
16650	R14584	MV	1905	NT	Top End	Darwin	-12.42	130.91
16660	R618	MV	1905	NT	Top End	Darwin	-12.42	130.91
16661	R619	MV	1905	NT	Top End	Darwin	-12.42	130.91
16662	R620	MV	1905	NT	Top End	Darwin	-12.42	130.91
16663	R621	MV	1905	NT	Top End	Darwin	-12.42	130.91

Sample ID	Voucher	Museum	Date	State	Region	Location	Latitude	Longitude
16664	R622	MV	1905	NT	Top End	Darwin	-12.42	130.91
16665	R623	MV	1905	NT	Top End	Darwin	-12.42	130.91
16666	R624	MV	1905	NT	Top End	Darwin	-12.42	130.91
16667	R625	MV	1905	NT	Top End	Darwin	-12.42	130.91
16668	R626	MV	1905	NT	Top End	Darwin	-12.42	130.91
B14585	B14585	MV	1905	NT	Top End	Darwin	-12.42	130.91
16646	R11496	MV	1897	QLD	NA	NA	NA	NA
16651	R17435	MV	1896	QLD	W QLD	Bourketown	-17.75	139.55
16652	R17436	MV	1896	QLD	W QLD	Bourketown	-17.75	139.55
HLW4249	HLW4249	MV	1911	QLD	W QLD	Burketown	-17.75	139.55
HLW4258	HLW4258	MV	1905	QLD	W QLD	Normanton	-17.67	141.07
HLW4211	HLW4211	MV	1910	WA	E Kimberley	Napier	-14.28	126.63
HLW4213	HLW4213	MV	1904	WA	W Kimberley	Derby	-17.32	123.63
16630	B21297	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16631	B21294	SAM	1914	QLD	Cape York	North Queensland	-13.27	141.88
16632	B21296	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16634	B1602	SAM	1914	QLD	Cape York	Stuart River	-14.1167	143.5333
16635	B54904	SAM	1896	QLD	W QLD	Burketown	-17.75	139.55
16636	B21295	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16654	B21292	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16655	B21293	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16659	B21291	SAM	1914	QLD	Cape York	Cape York	-13.27	141.88
16633	B870	SAM	1912	WA	W Kimberley	Fitzroy River	-17.8	123.6667
16653	B1962	SAM	1916	WA	W Kimberley	Kings Sound	-16.7333	123.3
16656	B9218	SAM	1896	WA	E Kimberley	Pine Creek	-17.3	127.45
16657	B1961	SAM	1917	WA	W Kimberley	Kings Sound	-16.7333	123.3
16658	B1960	SAM	1917	WA	W Kimberley	Kings Sound	-16.7333	123.3

Table A2: Total number of adults individuals with microsatellite genetic data from different sampling years at the five major sampling populations from the contemporary sample, a subset of which was used in (Bolton *et al.*, 2016). In brackets is the number of individuals by year for the SNP data. There were two individuals that were sampled in multiple years (2008-2009 and 2012-2013) in Wyndham and for simplicity were excluded from all analyses and this table.

	2005	2006	2007	2008	2009	2010	2011	2012	2013
Mornington	39 (16)	145 (14)	48 (9)	14 (2)	42 (11)	0	0	0	0
Wyndham	0	0	0	165 (19)	38(5)	17 (3)	33 (6)	32 (6)	22 (6)
Bradshaw	0	0	16 (13)	31 (26)	5 (9)	0	0	0	0
Delamere	0	0	6 (22)	24 (12)	20 (19)	0	0	0	0
Yinberrie Hills	0	0	4 (6)	26 (17)	29 (25)	0	0	0	0
Total	39 (16)	145 (14)	74 (50)	260 (76)	134 (69)	17 (3)	33 (6)	32 (6)	22 (6)

Appendix 2: Microsatellite Reliability

For each locus in the historical dataset we counted how many times a genotype was scored, whether or not the locus genotype was changed from the first time it was scored (C), and whether there was evidence of null alleles (one of the scored genotypes was homozygous, others were heterozygous). We also estimated null alleles on the finalised dataset using CERVUS v3.0.6 (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007). Deviation from Hardy-Weinberg equilibrium and FIS was calculated in ARLEQUIN v.3.5 (Excoffier & Lischer, 2010) using an exact test with a Markov Chain length of 1,000,000 and 100,000 dememorization steps. Table S3 shows the results of the reliability analysis.

Linkage disequilibrium was calculated for each pair of loci in the historical dataset in GENEPOP v4.2 (Raymond & Rousset, 1995). No pair of loci was in linkage disequilibrium after Bonferroni correction for multiple testing (Table S4).

Table A3: summary showing the reliability of each microsatellite locus in the historical dataset. N refers to the number of individuals in which a locus was genotyped, R is the number of individuals that were genotyped at least twice (≥2), and %C is the percentage of times a repeated (R) locus was changed after the first genotype was scored. %Nulls were calculated from the repeated genotype data, and is presented against estimates from Cervus. Hardy-Weinberg equilibrium deviation p-value is from exact test in Arlequin, and the Bonferroni corrected significance level is 0.006. Those loci marked with an asterisk had high levels of null-alleles, likely driving deviation from Hardy-Weinberg Equilibrium. These were excluded from the final analysis.

Locus	N	R(%)	%Missing	%Nulls	%Nulls (Cervus)	%C	HWE P-value	F_{IS}
Ego26*	90	31	10.89	32.26	8.79	12.90	0.0009	0.17
Ego31*	79	23	21.78	17.39	24.39	8.70	0.0000	0.40
Ego49*	97	44	3.96	15.91	8.52	6.82	0.0000	0.16
Ego52	98	32	2.97	12.50	4.82	9.38	0.11	0.11
Ase24*	85	25	15.84	20.00	25.69	16.00	0.0000	0.41
BF18	101	50	0.00	0.00	0.58	0.00	0.07	0.03
Tgu11	99	38	1.98	26.32	-0.38	23.68	0.04	0.02
Pca7	100	54	0.99	5.56	-1.62	1.85	0.45	-0.03
Pco2	94	33	6.93	9.09	0.47	9.09	0.37	0.03

Table A4: Results from linkage disequilibrium analysis conducted in GenePop, bolded p-values are those significant at $p<0.05$, but no comparisons were significant after Bonferroni correction for multiple testing ($p< 0.0013889$).

Locus 1	Locus 2	P-Value
Ego26	Ego31	0.32048
Ego26	Ego49	0.249485
Ego31	Ego49	0.650475
Ego26	Ego52	0.072602
Ego31	Ego52	0.255063
Ego49	Ego52	0.419356
Ego26	Ase24	0.60624
Ego31	Ase24	0.67868
Ego49	Ase24	0.719406
Ego52	Ase24	0.604816
Ego26	BF18	0.881
Ego31	BF18	0.273648
Ego49	BF18	0.024305
Ego52	BF18	0.998254
Ase24	BF18	0.409158
Ego26	Tgu11	0.414234
Ego31	Tgu11	0.635029
Ego49	Tgu11	0.228492
Ego52	Tgu11	0.015243
Ase24	Tgu11	0.208477
BF18	Tgu11	0.201164
Ego26	Pca7	0.758769
Ego31	Pca7	0.072857
Ego49	Pca7	0.104167
Ego52	Pca7	0.16108
Ase24	Pca7	0.592792
BF18	Pca7	0.438313
Tgu11	Pca7	0.935057
Ego26	Pco2	0.555773
Ego31	Pco2	0.023616
Ego49	Pco2	0.282489
Ego52	Pco2	0.330617
Ase24	Pco2	0.983115
BF18	Pco2	0.151639
Tgu11	Pco2	0.508686
Pca7	Pco2	0.600099

Appendix 3: Historical geographic genetic diversity & differentiation

Table A5: Genetic diversity indices for each state sampled in the historical dataset in the microsatellite and mitochondrial datasets. Microsatellite diversity is represented as, average number of alleles (allelic richness) (N_A), average number of private alleles (N_{PA}), observed and expected heterozygosity (H_O, H_E). Inbreeding coefficient (FIS) is derived from AMOVA, where no values were significant at $p<0.05$. Diversity measures specific to the mitochondrial data are: number of segregating sites (S), raw number of haplotypes (H), private haplotypes (H_P), haplotype diversity (*h*), and nucleotide diversity (π).

	Western Australia	Northern Territory	Queensland
a) Microsatellites			
n	31	34	36
N_A	9.60 (± 6.12)	9.80 (± 6.50)	9.40 (± 4.72)
N_{PA}	0.60 (± 0.89)	1.2 (± 1.64)	0.80 (± 0.84)
H_O	0.75 (± 0.17)	0.72 (± 0.16)	0.68 (± 0.16)
H_E	0.74 (± 0.13)	0.73 (± 0.13)	0.74 (± 0.11)
F_{IS}	-0.03	-0.01	0.06
b) Mitochondrial control region			
n	34	34	36
S	10	10	10
H	8	12	11
H_P	2	8	7
h	0.81 (± 0.06)	0.89 (± 0.03)	0.70 (± 0.08)
π x10^2	0.88 (± 0.13)	0.88 (± 0.10)	0.80 (± 0.12)

Table A6: Genetic diversity indices for each region (see Figure 1) sampled in the historical dataset in the microsatellite and mitochondrial datasets. Microsatellite diversity is represented as, average number of alleles (allelic richness) (N_A), average number of private alleles (N_{PA}), observed and expected heterozygosity (H_O, H_E). Inbreeding coefficient (FIS) is derived from AMOVA, where no value was significant at $p<0.05$. Diversity measures specific to the mitochondrial data are: number of segregating sites (S), raw number of haplotypes (H), private haplotypes (H_P), haplotype diversity (*h*), and nucleotide diversity

	Western Australia		Northern Territory		Queensland
	West Kimberley	East Kimberley	Top End	Western QLD	Cape York
a) Microsatellites					
n	7	24	34	11	8
N_A	5.60 (± 0.40)	8.80 (± 5.40)	9.80 (± 6.50)	7.20 (± 3.42)	5.60 (± 2.70)
N_{PA}	0.20 (± 0.45)	0.40 (± 0.89)	1.2 (± 1.64)	0	0.40 (± 0.55)
H_O	0.63 (± 0.38)	0.78 (± 0.14)	0.72 (± 0.16)	0.79 (± 0.13)	0.71 (± 0.12)
H_E	0.72 (± 0.17)	0.74 (± 0.12)	0.73 (± 0.13)	0.74 (± 0.13)	0.75 (± 0.11)
F_{IS}	0.08	-0.06	-0.01	-0.11	0.02
b) Mitochondrial control region					
n	9	25	34	11	8
S	8	6	10	8	5
H	7	9	12	5	5
H_P	0	2	8	3	4
h	0.94 (± 0.07)	0.76 (± 0.08)	0.89 (± 0.03)	0.71 (± 0.14)	0.79 (± 0.02)
$\pi*10^2$	1.1 (± 0.24)	0.79 (± 0.15)	0.88 (± 0.10)	1.0 (± 0.26)	0.70 (± 0.21)

Table A7: Summary of AMOVA results for microsatellite data for sampling localities in the historical dataset. AMOVA uses *a priori* population groupings, which were based on sampling state and regions (within states), see Figure 1 in the main text.

	States	Regions
Within Individuals	99.63%	103.31%
Among Individuals	0.61%	-2.94%
Among Populations	-0.24%	-0.37%
F_{IS}	0.006	-0.03
F_{ST}	-0.002	-0.004
F_{IT}	0.45	-0.03

Table A8: Summary of AMOVA results for haplotype frequencies and pairwise nucleotide differences in mitochondrial control region data for sampling localities in the historical dataset. AMOVA uses *a priori* population groupings, which were based on sampling state and regions (within states), see Figure 1 in the main text.

	States		Regions	
	Haplotype frequency	Nucleotide differences	Haplotype frequency	Nucleotide differences
Among Populations	2.07%	-0.74%	0.15%	-0.08%
Within Populations	97.93%	100.74%	99.85%	100.08%
F_{ST}	0.02	-0.007	0.002	-0.0008

Table A9: Pairwise differentiation statistics for each sampled state in the historical dataset for microsatellites and mitochondrial DNA. Bolded are values that are significant at $p<0.05$, but none were significant after Bonferroni correction for multiple testing ($p<0.0167$)

Population Comparison	Microsatellite F_{ST}	Haplotype Frequency F_{ST}	Haplotype Sequence ϕ_{ST}
Western Australia vs. Northern Territory	-0.004	0.003	-0.018
Western Australia vs. Queensland	0.0009	0.023	-0.008
Northern Territory vs. Queensland	-0.004	**0.036**	0.003

Table A10: Pairwise differentiation statistics for each sampled region in the historical dataset for microsatellites and mitochondrial DNA.

Population Comparison	Microsatellite F_{ST}	Haplotype Frequency F_{ST}	Haplotype Sequence ϕ_{ST}
West Kimberley vs. East Kimberley	0.005	0.006	0.057
West Kimberley vs. Top End	0.005	0.037	0.021
West Kimberley vs. Western Queensland	-0.023	0.060	0.073
West Kimberley vs. Cape York	-0.013	-0.045	-0.112
East Kimberley vs. Top End	-0.005	-0.010	-0.021
East Kimberley vs. Western Queensland	-0.013	0.004	-0.025
East Kimberley vs. Cape York	0.010	-0.023	0.022
Top End vs. Western Queensland	-0.011	0.017	-0.004
Top End vs. Cape York	-0.003	-0.008	-0.013
Western Queensland vs. Cape York	-0.008	-0.048	0.028

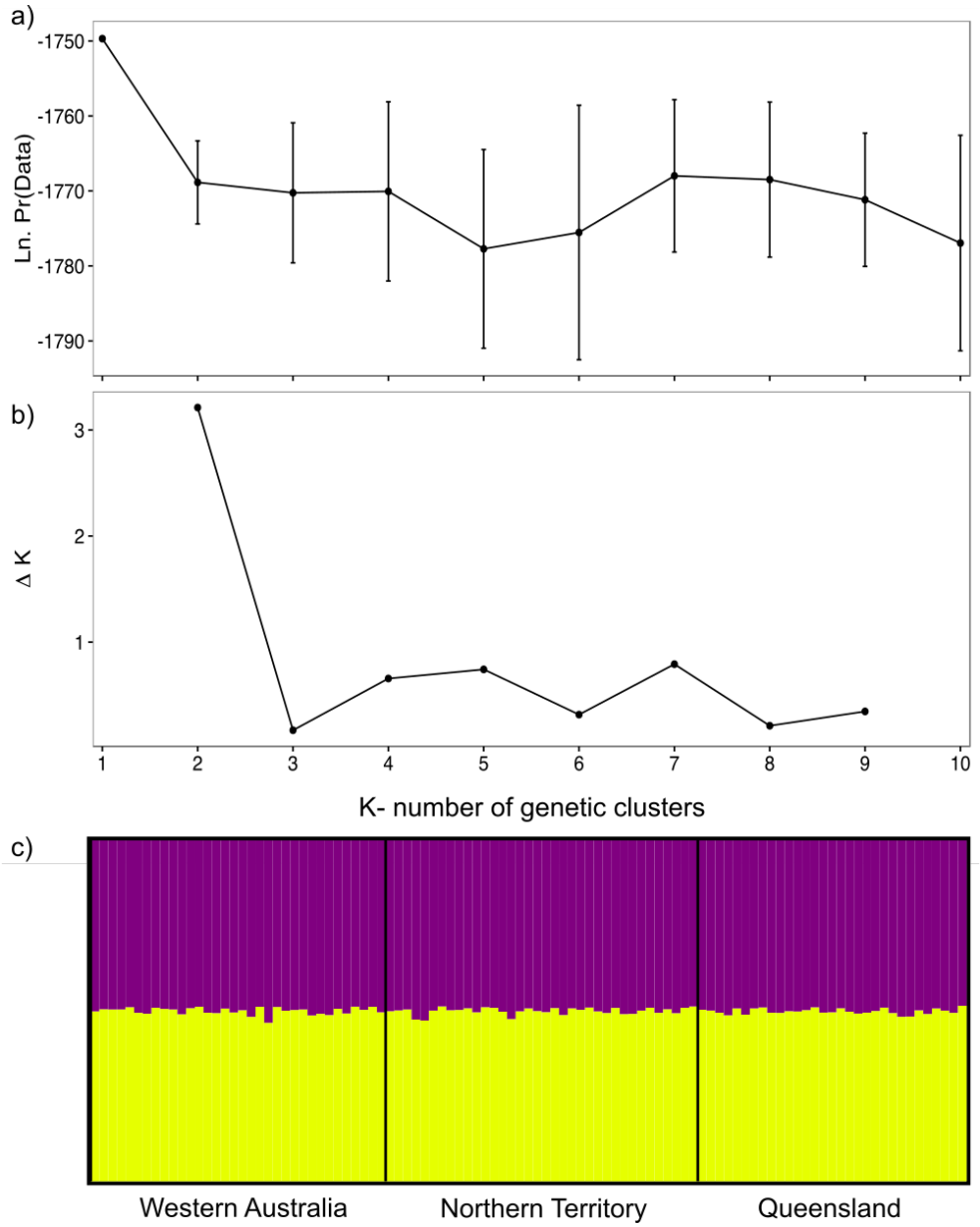

Figure A1: Results from the clustering analysis in STRUCTURE comparing regions within the historical dataset, where a) is shows the LnP(Data) plot, showing that the most likely configurations of clusters is one, b) the Evanno method gives a ΔK=2, but is unable to assess K=1, c) shows the q-plot of admixture proportions between historical and contemporary samples, showing that there is no genetic difference between these two time periods

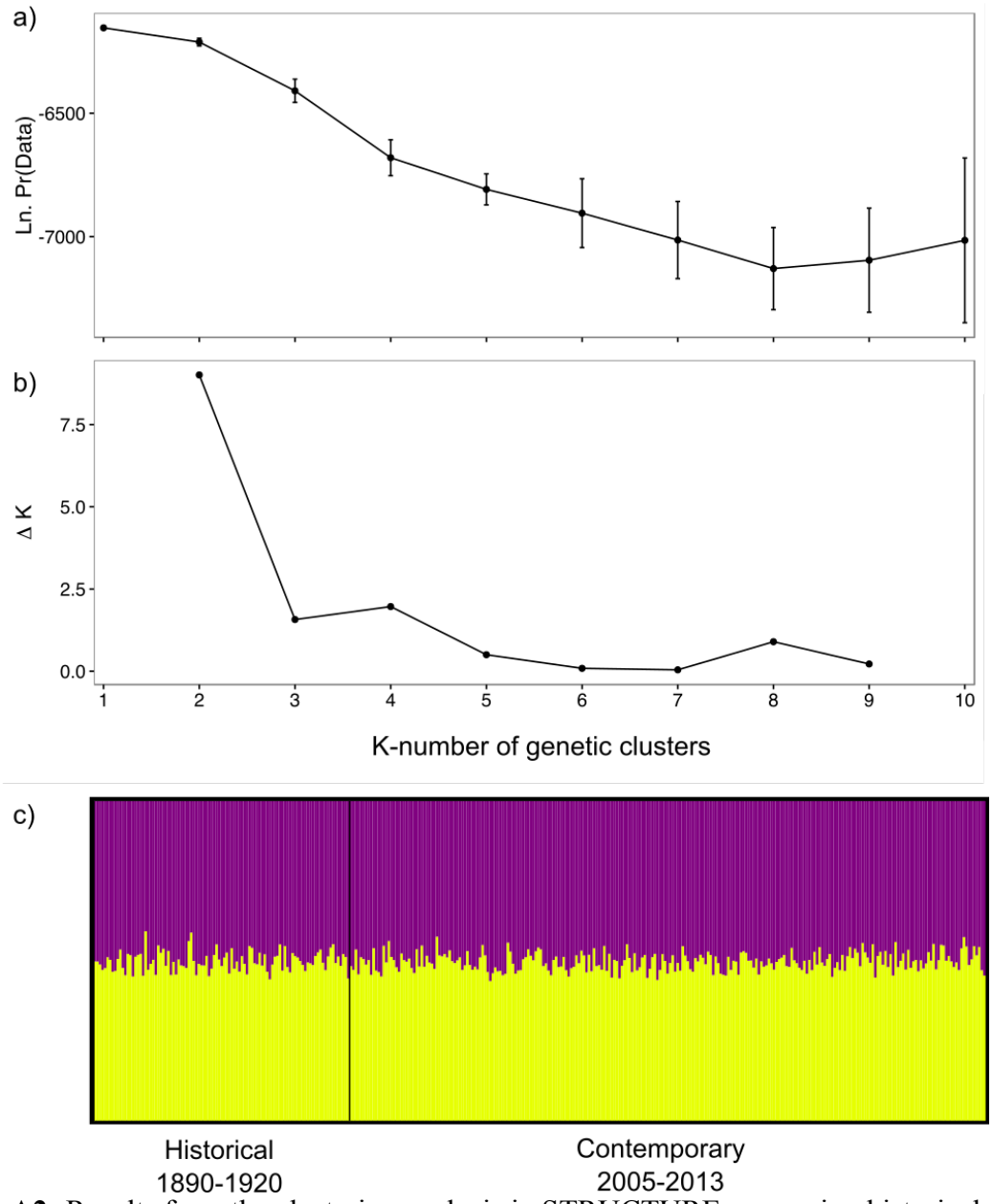

Figure A2: Results from the clustering analysis in STRUCTURE comparing historical and contemporary populations, where a) is shows the LnP(Data) plot, showing that the most likely configurations of clusters is one, b) the Evanno method gives a ΔK=2, but is unable to assess K=1, c) shows the q-plot of admixture proportions between historical and contemporary samples, showing that there is no genetic difference between these two time periods

Appendix 4: Demographic inference with Approximate Bayesian Computation in DIYABC

This supplement includes extra material that is relevant to Approximate Bayesian Computation (ABC), as per recommendations in the user manual and associated papers (Cornuet *et al.*, 2010, 2014).

ABC was conducted on the historical dataset and contemporary dataset (5 microsatellite loci + mtDNA). Table A11 shows demographic and mutational models to generate the simulated datasets for the ABC analysis. Microsatellite mutational model follows a Generalized Stepwise Mutation Model, a geometric distribution describes (shape determined by parameter P) the increase and decrease in microsatellite length with each mutation. Mitochondrial mutational model, % invariant sites and shape of the gamma distribution was derived from the best model chosen by Bayesian Information Criterion in jModelTest 2.1.4 (Darriba *et al.*, 2012). For microsatellite data we compared summary statistics: mean number of alleles (N_A), heterozygosity (H_E), M-ratio (M), and differentiation (F_{ST}) between historical and contemporary samples. Mitochondrial data we compared summary statistics: number of haplotypes (H), number of segregating sites (P_S), Mean pairwise differences (π), Tajima's D, private segregating sites (S_P), and differentiation between sampling time periods (F_{ST}).

From the datasets simulated under different demographic hypotheses, the ABC approach takes a number of simulated datasets that resemble the observed data's summary statistics. These selected datasets are then used to approximate the posterior probability of scenarios, their parameters, and the confidence in scenario choices.

The most likely scenario was chosen by comparison of results from the direct-estimate approach and from the logistic regression (Table A12), which found Scenario 1 and 4 to be the most probable. Confidence in scenario choice was estimated based on a direct and logistic regression estimates (as above) using 500 pseudo-observed datasets derived from the posterior distributions for each scenario and type I and type II errors were estimated from the frequency

Table A11: a) Priors for variables used in demographic simulations in DIYABC. Each prior name corresponds to variable names outlined in scenarios 1-5 in Figure 2. Effective population size (N) parameters are expressed in number of individuals, and time (t) parameters are expressed in generations=years. b) Mutational model priors for microsatellite markers, where μ is mutation rate, P is the shape of the geometric distribution describing the size of microsatellite mutations, and SNI refers to the rate of single nucleotide insertions/deletions in the microsatellite. c) Mutational model priors for mitochondrial control region, where K is the transition/transversion ratio.

Prior	Distribution	Conditions
a) demographic parameter priors		
cN_1	Uniform~[200-8000]	$cN_1<pN$
cN_2	Uniform~[200-8000]	
N_{bn}	Uniform~[10-1000]	$N_{bn}< cN_2$
pN	U~[1,000-100,000]	
pN_2	U~[10-1000]	$pN_2<cN_2$
t	U~[10-90]	
t_{bn}	U~[1-50]	
t_e	U~[8,000-500,000]	
b) Microsatellite mutation priors		
Mean-μ	U~[$1x10^{-4}$ – $1x10^{-3}$]	
Individual locus-μ	G~[$1x10^{-5}$ – $1x10^{-2}$]	
Mean-P	U~[0.1-0.3]	
Individual locus-P	G~[0.01-0.9]	
Mean-SNI	LogU~[$1x10^{-8}$-$1x10^{-5}$]	
Individual locus-SNI	G~[$1x10^{-9}$-$1x10^{-4}$]	
c) Mitochondrial control region		
Mutation model	Kimura 2-Parameter	
% Invariant Sites	82.3	
Gamma Shape	0.632	
Mean-μ	Uniform~[$1x10^{-9}$-$1x10^{-5}$]	
Individual locus-μ	Gamma~[$1x10^{-9}$-$1x10^{-3}$]	
Mean K	Uniform~[0.05-20]	
Individual K	Gamma~[0.05-20]	

that the scenario chosen by the previous procedure was found to be the highest probability in the pseudo-observed data (Table A13). Additionally, the chosen model was checked for

'goodness-of-fit' by simulating 1,000 datasets from the posterior distribution of parameters. This confirms that the posterior of the scenario is similar to the observed dataset, which may occur due to mis-specification of demographic or mutational parameters. The model checking datasets were simulated using all summary statistics available in DIYABC. Visualisation of the model checking procedure by principal component analysis of summary statistics is presented in Figure A3.

The posteriors of model parameters were estimated using local linear regression of 1% of simulated datasets and a logit transformation. Posterior distributions of parameters were visualized using the *locfit* function in R (Figure A4).

Table A12: Posterior probabilities and [95% confidence interval] for each of the simulated demographic scenarios using the direct approach from 500 of the simulated datasets closest to the observed, and the logistic regression approach using 1% of the data closest to the observed dataset.

	SC1	SC2	SC3	SC4	SC5
Direct approach	0.43 [0.00,0.86]	0.04 [0.00,0.21]	0.04 [0.00,0.21]	0.47 [0.03,0.90]	0.03 [0.00,0.18]
Logistic approach	0.49 [0.45,0.54]	0.07 [0.02,0.12]	0.00 [0.00,0.05]	0.43 [0.38,0.48]	0.00 [0.00,0.05]

Table A13: Error rates for the competing scenarios selected by a) the logistic regression method and the b) direct approach method, based on the simulation of based on simulation of 500 pseudo-observed datasets (PODs – the "True Scenario") derived from the posterior distribution. The total number of PODS for a given scenario is represented in brackets () beside the scenario number. For each "True Scenario" the percent of times another scenario was assigned the highest posterior probability by the logistic regression approach is presented. Type I error is the percent of times the true scenario was not correctly assigned, and the type II error is the percent of times a scenario was chosen when it was not the correct scenario.

a) Logistic approach	Chosen scenario					
True Scenario	1	2	3	4	5	Type I Error
1 (219)	-	0.91%	0.00%	74.89%	0.91%	76.71%
2 (17)	5.88%	-	5.88%	29.41%	35.29%	76.47%
3 (15)	0.00%	0.00%	-	53.33%	33.33%	86.67%
4 (236)	18.64%	1.69%	0.00%	-	0.00%	20.34%
5 (13)	7.69%	15.38%	15.38%	23.08%	-	61.54%
Type II Error	16.37%	1.66%	0.62%	68.18%	2.67%	
b) Direct approach	Chosen scenario					
True Scenario	1	2	3	4	5	Type I Error
1 (219)	-	0.00%	0.46%	57.53%	0.46%	58.45%
2 (17)	23.53%	-	5.88%	58.82%	11.76%	100.00%
3 (15)	20.00%	0.00%	-	66.67%	6.67%	93.33%
4 (236)	35.17%	0.85%	0.00%	-	0.85%	36.86%
5 (13)	38.46%	0.00%	15.38%	23.08%	-	76.92%
Type II Error	33.81%	0.41%	0.82%	56.44%	1.23%	

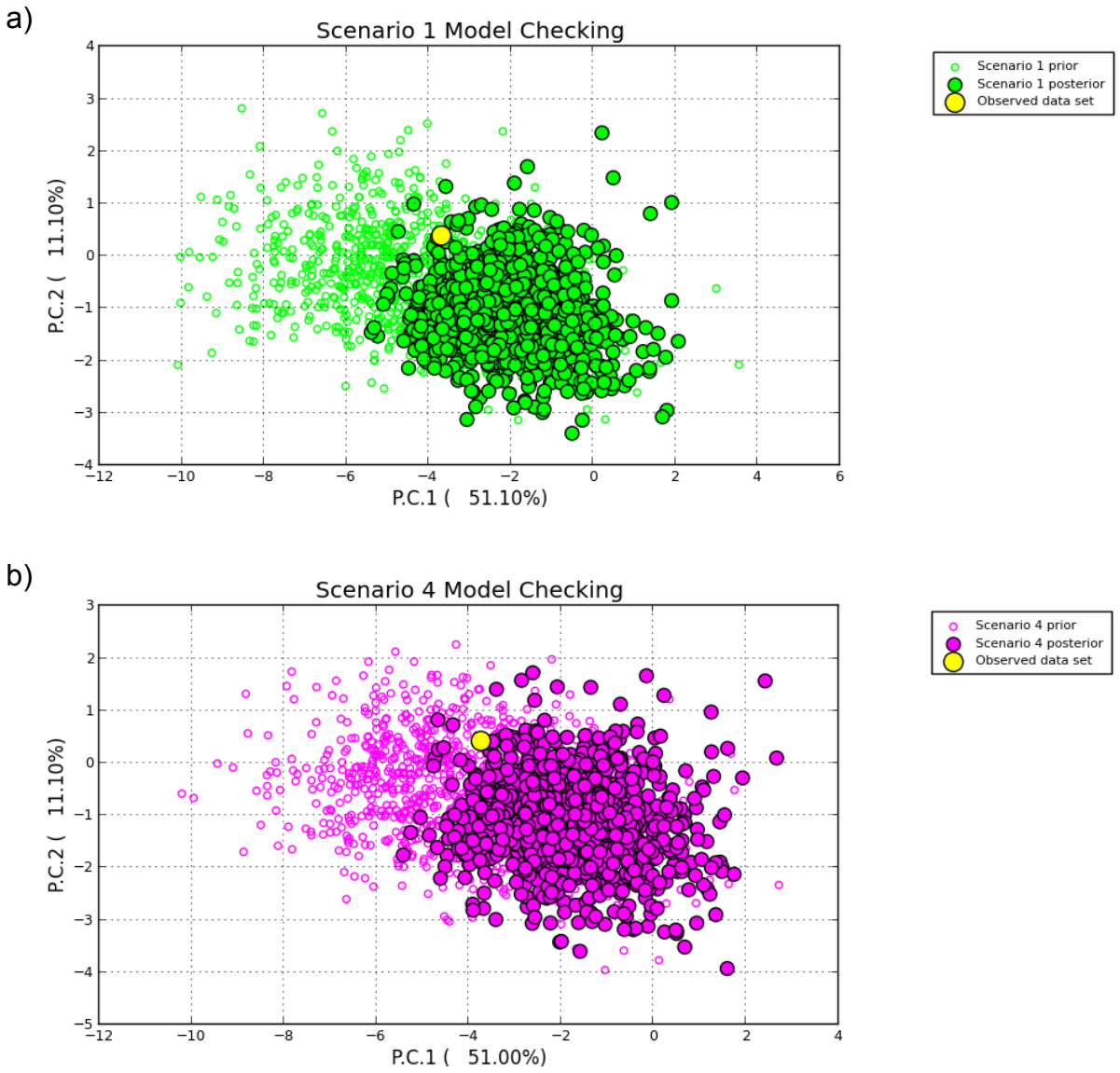

Figure A3: Principal Components Analysis for model checking on scenarios 1 and 4 (a and b) using all available summary statistics. Where empty circles represent data simulated from the prior distribution, filled circles are data simulated from the posterior distribution.

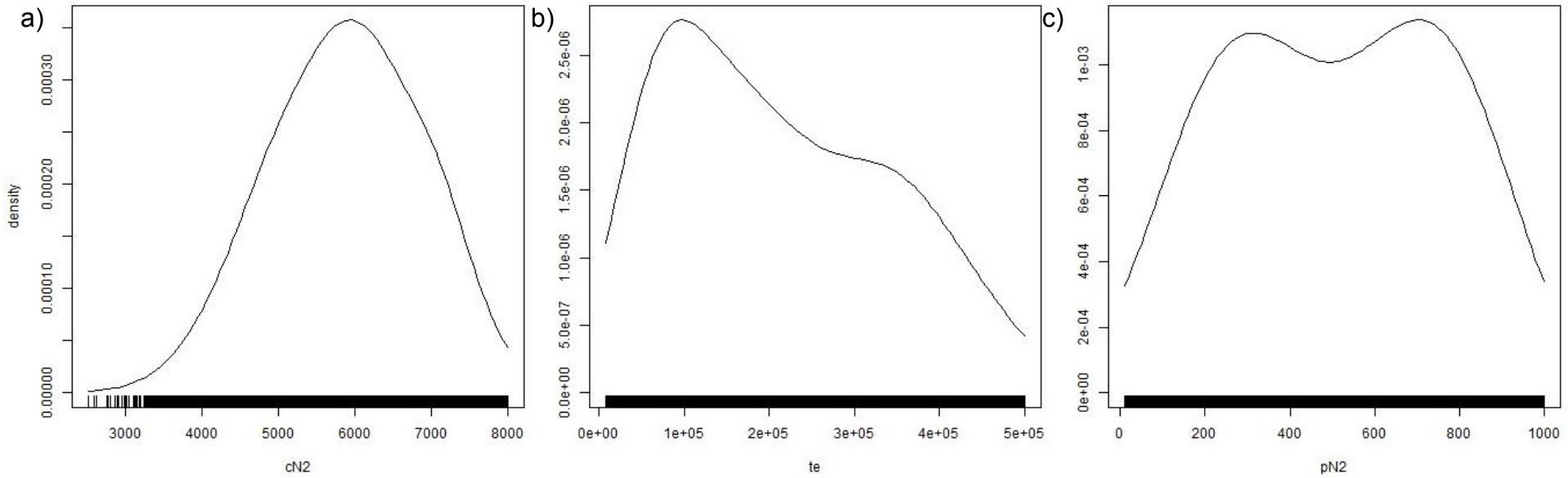

Figure A4: Posterior probability density distributions for scenario 4 parameters output from the *locfit* function in R. Plot a) shows contemporary effective population size (cN); b) shows timing of the ancient bottleneck (te), and c) shows the ancient effective population size (pN_2). See Table 3 in the main manuscript for the descriptive statistics of these distributions. The results for the posterior of the cN2 parameter in Scenario 1 are not shown because the probability density function gave very similar results to Scenario 4

Appendix 5: Estimation of contemporary effective population size

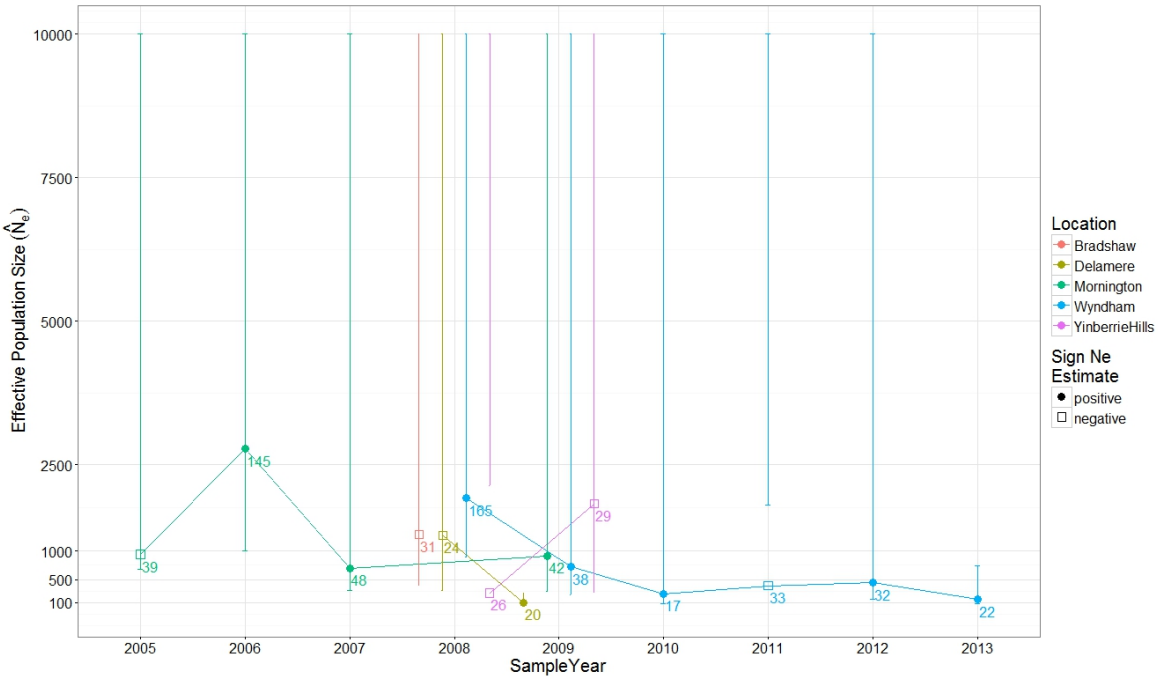

Figure A5: Estimates of effective population size ($\widehat{N}_e$) using the linkage disequilibrium method on adults from each sampling locality in each year using 16 microsatellite loci. Open squares indicate negative $\widehat{N}_e$ that have been corrected by taking the absolute value; negative values, like "infinite" estimates arise when sampling error is larger than any genetic drift signal (Waples & Do, 2010). Error bars are 95% confidence intervals from the parametric estimation method, where "infinite" estimates have been corrected to an arbitrary value of 10,000. Uncorrected estimates are presented in Table A14.

Table A14: Uncorrected $\widehat{N}_e$ and parametric confidence intervals from the linkage disequilibrium method for each sampling locality and year. Negative estimates are presented here, but occur when genetic data can be explained entirely by sampling error, and are estimated separately from the confidence intervals, which can provide positive integers as a lower bound. Due to inaccuracy at N_e>200 and sampling error, negative estimates and "infinite" estimates have the same biological interpretation of a "very large" population (Waples & Do, 2010).

	Mornington	Wyndham	Bradshaw	Delamere	Yinberrie Hills
2005	-940.1 [673.2-Inf]	-	-	-	-
2006	2776.1 [1008.1-Inf]	-	-	-	-
2007	701.9 [307.3-Inf]	-	-142.7 [Inf-Inf]	-	-
2008	Inf [Inf-Inf]	1917.5 [900.3-Inf]	-1289.8 [399.1-Inf]	-1274.4 [311.1-Inf]	-267.1 [2141-Inf]
2009	913.4 [303.4-Inf]	719.5 [242.4-Inf]	-	104.5 [63.8-261.9]	-1819.6 [274.3-Inf]
2010	-	251.4 [81.2-Inf]	-	-	-
2011	-	-392.3 [1804.3-Inf]	-	-	-
2012	-	447.6 [155.5-Inf]	-	-	-
2013	-	158.5 [86.4-739.6]	-	-	

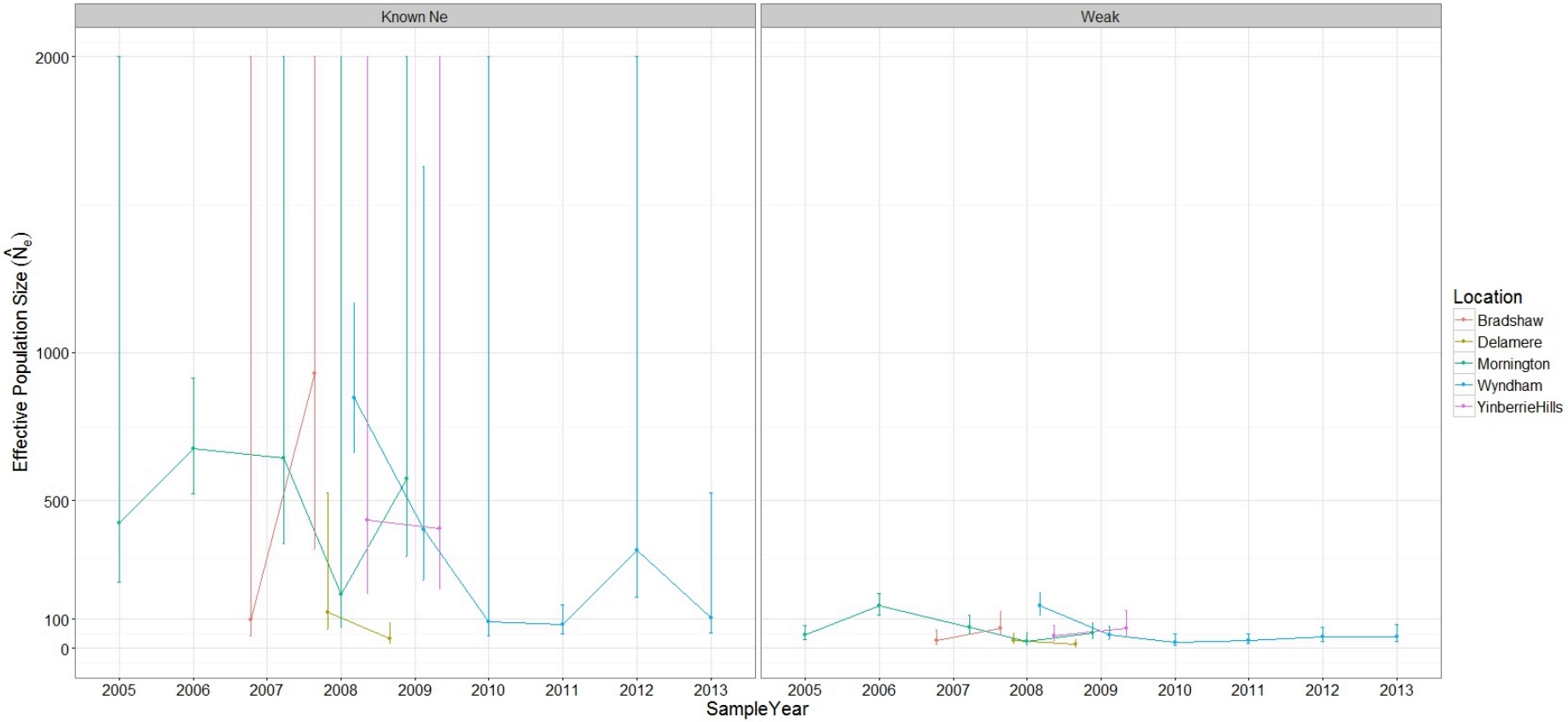

Figure A6: Estimates of effective population size ($\widehat{N}_e$) using the sibship frequency method on adults from each sampling locality in each year using 16 microsatellite markers. Error bars are 95% confidence intervals, where values >2,000 have been corrected to 2,000 to ease visualisation (Uncorrected values are in Table A15)

Table A15: $\widehat{N}_e$ from sibship frequency analysis with uncorrected confidence intervals in square brackets, where a) is the result from the known prior (Sex ratio=1.26, N_e=1000), and b) are the results from the weak sibship size prior.

a) Known N_e	Mornington	Wyndham	Bradshaw	Delamere	Yinberrie Hills
2005	423 [225-2926]	-	-	-	-
2006	674 [522-915]	-	-	-	-
2007	645 [355-2637]	-	96 [44-2147483647]	-	-
2008	182 [67-2147483647]	846 [662-1169]	930 [334-2147483647]	123 [64-525]	433 [187-2147483647]
2009	574 [309-2920]	402 [229-1628]	-	32 [17-89]	406 [201-2147483647]
2010	-	91 [44-11237]	-	-	-
2011	-	81 [49-147]	-	-	-
2012	-	331 [174-2820]	-	-	-
2013	-	103 [52-525]	-	-	-
b) Weak sibship prior	Mornington	Wyndham	Bradshaw	Delamere	Yinberrie Hills
2005	46 [29-77]	-	-	-	-
2006	144 [113-187]	-	-	-	-
2007	73 [49-114]	-	27 [14-62]	-	-
2008	24 [12-57]	144 [113-188]	69 [42-126]	28 [15-52]	42 [25-79]
2009	54 [34-86]	47 [29-76]	-	13 [6-30]	68 [43-128]
2010	-	21 [11-49]	-	-	-
2011	-	28 [16-50]	-	-	-
2012	-	39 [23-72]	-	-	-
2013	-	40 [23-80]	-	-	-

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Chapter Seven

General Discussion

Photo: Peri Bolton

Effective conservation management programs for endangered species require detailed knowledge of the target species. The Gouldian finch (*Erythrura gouldiae*) is a species with a history of population declines, and its colourful charisma has led it to become a flagship for the conservation of monsoonal savanna species. Yet, there remains much uncertainty about this species' fundamental biology in the wild. The species was down-listed by the IUCN from 'Endangered' to 'Near Threatened' on the basis of localised population surveys and *ad hoc* bird watcher observations (Garnett *et al.* 2011; Birdlife International 2013), which is insufficient evidence to demonstrate that the population has stabilised or recovered. Furthermore, experiments on domesticated Gouldian finches revealed that there is an incompatibility between the red and black head colour morphs, such that when they interbreed more than 30% of their offspring die (Pryke & Griffith 2009a). This genetic incompatibility has the potential to be a hindrance to the recovery of this species, as it may further slow population growth rates. In this thesis, I have used population genetic methods to infer effective population size, patterns of geographic connectivity, and the extent of incompatibility in the wild. Because my thesis has two major foci, conservation and evolutionary biology, I will first discuss how my results influence future conservation research and management of the Gouldian finch; then continue by synthesizing my findings on incompatibility and population genetics in the wild, and outlining possible future research on the evolution of incompatibility via domestication.

Conservation management and future directions

The simplest conservation consequence of my work is that the genetic incompatibility itself is unlikely to be a threatening process in the wild. While this is good news for *in situ* recovery of the Gouldian finch, the differences between domesticated and wild populations have consequences for *ex situ* (captive breeding) recovery programs. Indeed, since 1999 there have been active efforts in the Mareeba Wetlands Reserve (Eastern Cape York, Queensland) to breed and release domesticated Gouldian finches (Esparza-Salas 2007; Wildlife

Conservancy of Tropical Queensland 2017). In Chapter Three, I identified genetic differences between domesticated (from the laboratory studies) and wild Gouldian finches, and found genetic differentiation between red and black morphs, in line with our predictions about incompatibility (Table 7.1). Therefore, reintroduction risks broader consequences of hybridization between captive and domesticated stocks (such as gene-expression differences (Waples 1991; Roberge *et al.* 2008)), and the introduction of incompatible alleles into the wild (if they are not already present at low frequency). However, the risks associated will depend on the gene flow from the domesticated to wild population, and the selection pressures and effective population size of the recipient (wild) population. Some theoretical modelling suggests gene-flow may erode genetic incompatibilities when the selection driving incompatibilities is weak (Lemmon & Kirkpatrick 2006). Indeed, the spread of incompatible or "domesticated" alleles throughout the Gouldian finch population will depend on the effective population size of the recipient (wild) populations, and on the rate of gene-flow out of the wetlands and from Queensland into the western part of the Gouldian finch range.

There is no evidence for population differentiation across the full historical range and in the western (core) range of the contemporary Gouldian finch (Chapters Five and Six). Although there were insufficient contemporary Queensland samples to draw conclusions about contemporary gene flow between the west and Queensland. The relevance to management of the results from the western range will depend on the definition of population connectivity relevant to the conservation goal at hand, and definitions (if any) in relevant legislative instruments (Fallon 2007). The methods applied in this thesis measure the effective-number of migrants per generation (N_em), and applies to the consequences on gene-frequencies between sub-populations; whereas demographic connectivity is defined by the dependence of sub-population growth-rates on migration (m) (Waples & Gaggiotti 2006; Lowe & Allendorf 2010). Lowe and Allendorf (2010, Table 1 and 2) highlight some conservation management-relevant definitions of connectivity thresholds (e.g. Adaptive connectivity to spread adaptive alleles (N_em>0.1) vs harvest connectivity to maintain fisheries

yields (large *m*)). Based on the results in Chapter Five, the Gouldian finch appears to have sufficient gene flow to maintain similar gene-frequencies between subpopulations. In an island model at mutation-drift equilibrium it is possible to calculate the effective number of migrants from indices of genetic differentiation (where $F_{ST} \approx 1/(1+4N_em)$ (Wright 1943)). If I use the mean value of the effective migration rate ($2N_em$) inferred from the pairwise F_{ST} analyses in Chapter Five, and the effective population size estimated by SNPs in 2008 (Chapter Six Table 4, N_e=2554) the migration rate is ≈ 0.074, which means populations are potentially demographically independent (Hastings 1993; Waples & Gaggiotti 2006). However, the migration rate is dependent on size of N_e and some of the estimates in Chapter Six suggest that the Gouldian finch sampling localities are demographically dependent (m>0.1 (Hastings 1993)).

A continuing theme throughout this thesis has been the limitation of population based connectivity estimates in high-gene flow situations (Waples 1998; Palsbøll *et al.* 2007; Samarasin *et al.* 2016), and this is further confounded by the imprecision (and inaccuracy) of the N_e estimates. As explained in Chapter Six the N_e estimates may not be accurate as many of the underlying assumptions of these estimators were violated, due to limitations in our knowledge of the species' life-history and inherent limitations in the sensitivity of estimators above a certain N_e (Waples & Do 2010). Therefore there is still considerable uncertainty surrounding the connectivity and N_e in the Gouldian finch. Ultimately, it is up to the conservation managers to decide what level of uncertainty is acceptable to make decisions (McDonald-Madden *et al.* 2008; Polasky *et al.* 2011), and weigh the 'genetic health' against immediate anthropogenic threatening processes (Jamieson & Allendorf 2012). Indeed, the Threatened Species Committee sat in 2016 to revise the conservation status of the Gouldian finch under the Australian Commonwealth Environment Protection and Biodiversity Conservation Act (1999). Partly as a result of my submission to the committee discussing the population genetic uncertainty (Appendix I), the committee decided that there was insufficient evidence to remove the Gouldian finch from the 'Endangered' category (Department of

Environment and Energy 2016), as had been done by the IUCN (Birdlife International 2013). Below I will identify the key questions in future research that will be fruitful in resolving some of these uncertainties.

I) The status of Queensland: Aside from being a pertinent question in Australian state politics, informed conservation of the Gouldian finch requires baseline information about the populations in Queensland. Although there are anecdotal reports of large flocks in Cape York (anecdotal verbal reports from aviculturists to myself), and Gouldian finch sightings are reported in Western Queensland and Cape York on databases such as eBird and Atlas of Living Australia, to my knowledge there is currently no systematic assessment of Gouldian finch populations in Queensland. List-length analysis could preliminarily be used to compare abundances between Queensland and the west, using lists sourced amateur bird watchers and BirdLife Australia surveys (Franklin 1999; Szabo *et al.* 2010). However, to be comparable with existing genetics work done here, genetic samples ought to be taken to compare effective population size and connectivity estimates. Future work should endeavour to collect genetic samples from sites in the eastern part of the Northern Territory and western Queensland (e.g. Arnhem land, McArthur River Region, Chidna, and Lawn Hill National Park) and Cape York (Mareeba Wetlands and Lakefield National Park). The suggestions for these localities were based on Gouldian finch observations in the online Atlas of Living Australia between 2000 and 2017. A concerted effort to collect genetic samples in any of these regions may yield a sufficient sample size to yield small confidence intervals on allelic frequencies (Waples 1998; Hale *et al.* 2012; Fung & Keenan 2014), and therefore resolve the level of genetic connectivity with the western range. Further, the inclusion of wild and captive Mareeba Wetlands birds will facilitate the identification of admixture between wild and domesticated Gouldian finches.

II) Migration: Migration in humans is currently relevant in both international and domestic politics, but there is still uncertainty around estimates of demographic connectivity and migration rates in the Gouldian finch. I have identified that in an island model under

migration-drift equilibrium the Gouldian finch is effectively a randomly mating population, but the population based methods applied here a) may be biased by deviation from these basic assumptions and fail to capture very recent decreases in connectivity and b) are inherently limited in their scope to precisely infer migration rates in situations of high gene-flow (Waples 1998; Lowe & Allendorf 2010; Samarasin *et al.* 2016).

One way to explore this question would be to conduct a programme of genetic tagging and use kinship-based methods (detection of relatives within and between populations) to infer recent migration, as they are a promising approach to infer recent migration rates in high-gene flow situations (Palsbøll *et al.* 2010; Feutry *et al.* 2016). Although these methods are being increasingly used in these situations (Telfer *et al.* 2003; Peery *et al.* 2010; Bravington *et al.* 2016), they are still relatively new and have not been extensively validated in terms of the appropriate individual and genetic marker sampling schemes (Wang 2006), and expectations of demographic and population genetic theory (Palsbøll *et al.* 2010; Reid *et al.* 2016). However, these methods are extremely promising and make up for the logistical and analytical difficulties on traditional mark-recapture techniques in dispersive species in remote inaccessible areas (Kool *et al.* 2013). Indeed, much of the debate about the conservation status of the Gouldian finch has surrounded whether the Gouldian finch population has stabilised or recovered (Garnett 2011; Here). To resolve this, I recommend a consistent long term programme of using genetic tagging to monitor changes in population size and migration (Palsbøll 1999; Schwartz *et al.* 2007; Palsbøll *et al.* 2010). As an exploratory exercise I have estimated the number of full-siblings and half-siblings within, and between, sampling localities for the 2008 and 2009 datasets (Appendix II). This preliminary work shows that relatives are equally frequent within and between localities, even over extremely long distances, which is suggestive of the large dispersal capacities of the Gouldian finch.

III) Life-history parameters and N_e estimation: The final priority for conservation management of this species is to obtain information on key life history parameters (age at

breeding and average lifespan) to improve estimation of N_e. The relationship of these parameters to N_e is described in Chapter Six, and will not be repeated here. Moreover, information gleaned from priority II and III research objectives are important parameters to use in population viability models to provide reliable quantitative estimates of extinction risk (Brook *et al.* 2000; Coulson *et al.* 2001). The suggested mark-recapture, radio- or satellite-tracking methods will also be complementary for estimation of individual dispersal. However, these methods have proven nearly impossible to conduct on the necessary scales in Gouldian finch habitat (Woinarski & Tidemann 1992; Tidemann *et al.* 1999; Chapter Five), and GPS-satellite tracking is currently unavailable for birds of this size. Another method worth investigating would be to use telomere length as a molecular marker for the age of birds caught in the field, but would require extensive validation (Dunshea *et al.* 2011).

Colour polymorphism and incompatibility

The main findings of this thesis is that the Gouldian finch in the wild shows no evidence for (severe) genetic incompatibility (Chapters Three & Four), and across the sampled range supports a large (Chapter Six), genetically homogenous population (Chapter Five). Table 7.1 describes the observational predictions derived from experiments on domesticated Gouldian finches, and the outcomes from work myself, and others, addressing these predictions. For example, the domesticated Gouldian finches use of extra-pair paternity with a compatible male to ameliorate individual fitness losses, and is a mechanism of reinforcement (Servedio & Noor 2003; Griffith 2010; Pryke *et al.* 2010). In my thesis, I have explored almost every piece of observational evidence possible at this time, resulting in the most conservative conclusion is that the incompatibility is very weak (or absent) in the wild. A prediction that was discussed in Chapter Two and Three was that incompatibilities ought to be associated with genetic differentiation between morphs. The degree of differentiation and accumulation of incompatible elements will be contingent on a) the strength of disruptive correlational selection and reinforcement between morphs and b) the number of effective

migrants between morphs (N_em). The evidence presented in table 7.1 suggests that incompatibility is weak (or absent) in the wild, but does not discount that there is disruptive selection on morphs. Weak selection for incompatible elements themselves may be swamped by high gene-flow (Lemmon & Kirkpatrick 2006).With regard to gene-flow, the head-colour morphs may have initially evolved as a result of isolation in Pleistocene refugia (Bowman *et al.* 2010), but while there is evidence for a Pleistocene bottleneck and expansion scenario (Esparza-Salas 2007; Chapter Six), any evidence of subdivision between morphs or regions has been lost (Kim 2011; Chapter Five). The lack of genetic differentiation in the wild suggests there will be more than ample gene-flow to prevent drift and the random fixation of incompatible elements in the Gouldian finch genome (Wright 1943; Waples 1998). Indeed, the work of Kim (2011), and myself (Appendix III) found that there was no genome wide differentiation except in a small region of LD surrounding the SNPs associated with head-colour on the Z-chromosome (Kim 2011).

In line with our predictions, however, there is genetic differentiation between morphs in the domesticated birds (Chapter Three). The domestication process itself may have been responsible for the evolution (or intensification) of the incompatibility between morphs, which was discussed at length in Chapter Three. The domestication process (population bottlenecks, artificial selection and breeding morphs separately) may have expanded an area of linkage around the colour locus itself, enabling pre-existing incompatible variants lurking at low frequencies to hitchhike along with red and black colouration (Barton 2000; Montgomery *et al.* 2010; Marsden *et al.* 2015). High coverage whole genome re-sequencing of red and black, wild and domesticated birds will enable the changes in linkage and allele frequencies on the Z-chromosome to be quantified. Further experimental crosses and transcriptome sequencing on hybrid and pure offspring may elucidate the underlying molecular mechanisms of incompatibility (Pavey *et al.* 2010; Davidson & Balakrishnan 2016). Furthermore, even simpler work can be done to quantify other differences between the wild and domesticated populations, for example, increases in colouration intensity as a marker

for artificial selection for colouration (Cieslak *et al.* 2011; Hoffman *et al.* 2014), and the separate breeding of morphs and incompatibility should lead to an enrichment of homozygote red males in the domesticated birds.

In chapter two of this thesis I explored possible ways in which processes involved in the origin and maintenance of colour polymorphism may be a threatening process, particularly after an initial population perturbation. The remainder of my thesis focused on the incompatibility as a threatening process in the Gouldian finch, for which there was no substantial evidence. However, the evidence accumulated in Table 7.1 does not directly link to the disruptive correlational selection that might be occurring between morphs. Therefore, the behavioural conflicts between morphs, and the genetic architecture and nature of other correlated traits may still be relevant to population dynamics in the Gouldian finch (Kokko *et al.* 2014; Chapter Two). The lower bound of some of the N_e estimates in Chapter six does suggest that the Gouldian finch may be subject to genetic drift, and therefore to variation in head-colour ratio, which may exacerbate any conflicts between morphs (Chapter Two). However, most estimates of N_e were >1000, and the demographic analyses suggested that N_e did not drop below 1000 in the 20th Century (Chapter Six), so whether other favourable properties of colour polymorphism (e.g. niche partitioning) helped buffer the decline in N_e remains to be seen (Forsman *et al.* 2008; Chapter Two). Indeed, while trait correlations between morphs have been well characterised in the domesticated population (Pryke & Griffith 2006; Pryke *et al.* 2007), relatively little is known about head-colour morphs in the wild Gouldian finch (Brazill-Boast *et al.* 2013). The evidence so far suggests that there are fundamental differences in the biology of the wild and domesticated birds, so future research on this charismatic species should endeavour to compare the nature and strength of selection on head-colour morphs in the domesticated and wild Gouldian finches.

Table 7.1: Summary of the observational evidence that would be in support of genetic incompatibility between Gouldian finch head-colour morphs. Column two indicates whether the particular hypothesis has been tested (and the reference), and the outcome

Observation based hypothesis: evidence for incompatibility	Completed?	Outcome/comment
Survival (Pryke & Griffith 2009a)		
Offspring from incompatible pairs will have lower survival to fledging	Brazill-Boast *et al.* 2013	Red males paired assortatively increased survival to fledging, but no difference in offspring survival between red males in disassortative pairs, and black males in assortative, or disassortative pairs.
Offspring from incompatible pairs will have lower lifetime survival probabilities, particularly females	Chapter Four	Not adequately tested: Chapter Four found male sex-ratio bias in the adults, is unable account for whether these were the offspring of compatible or incompatible parents.
Heterozygous male offspring will have reduced lifetime survival	Chapter Four	No differences between hatchling, juvenile and adult age classes in frequency of heterozygote males.
Deviation from Mendelian expectations in offspring	Chapter Three	The head colour genotypes of offspring did not deviate from Mendelian expectations in mixed morph pairs.

Mate-choice and differential allocation (Pryke & Griffith 2009a; b, 2010; Pryke *et al*. 2010)		
Mixed morph pairs will have higher rates of extra-pair paternity	Chapter Three	No difference between pairing types in presence of extra-pair offspring, nor in the percent clutch sired by an extra-pair male.
Extra-pair offspring will be sired by compatible male	Chapter Three	No evidence that extra-pair offspring were preferentially sired by a male with a compatible head-colour, but caveat that this dataset has a small sample size.
There will be assortative mating with respect to head-colour	Pryke & Griffith 2007; Chapter Three	Pryke & Griffith (2007) possibly pseudoreplicated pairs, but found pattern of assortative mating at Mornington. Chapter Three used individually banded pairs (no pseudoreplication) and found pairs mated randomly with respect to head-colour.
Male biased offspring sex ratio	Chapter Three	No evidence for a male bias in offspring of mixed or same morph pairs.

Observation based hypothesis: evidence for incompatibility	Completed?	Outcome/comment
Incompatible pairs will show lower rates of in provisioning behaviours	Brazill-Boast *et al.* 2013	No differences in provisioning rates between morphs or mixed and same morph pairs.
Incompatible pairs will have smaller clutch sizes	Brazill-Boast *et al.* 2013	No differences in clutch size between morphs or mixed and same morph pairs.
Incompatible pairs will have longer latency to lay	Brazill-Boast *et al.* 2013	No differences in lay-date latency between morphs or mixed and same morph pairs.
Differences in egg mass/volume	No	Not tested
Condition & genetic differentiation (Kim 2011; Griffith *et al.* 2011; Chapter Three)		

Offspring from mixed morph pairs will have lower body condition	Chapter Four	No differences in nestling body condition whether they were from mixed or same-morph parents. This cannot be tested for adults in the population, as we do not know the identity of the parents.
Offspring from mixed morph pairs will have high baseline and peak stress responses (corticosterone)	No	Not tested. Could only be tested on nestlings and adults with known pedigree.
Heterozygous males will have reduced body condition	Chapter Four	Body condition in hatchlings and adults is equivalent across genotypes.
Mixed-morph pairs will have lower body condition	Chapter Four	No differences in body condition between mixed morph and same morph pairs.
Mixed morph pairs will have higher baseline and peak stress responses (corticosterone)	Chapter Four	Not tested
Genetic differentiation between morphs	Kim 2011; Chapter Three	No differentiation in the wild (Kim 2011), but differentiation in sample of domesticated birds (Chapter Three).

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Appendix I

Population Genetics of the Gouldian Finch (Erythrura gouldiae)

Submission to the Threatened Species Committee for review of threat status under the Environmental Protection and Biodiversity Conservation Act 1999

Population Genetics of the Gouldian Finch (Erythrura gouldiae)

Submission to the Threatened Species Committee for review of threat status under the Environmental Protection and Biodiversity Conservation Act 1999

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Key Findings:

Genetic Connectivity: One large, highly connected population.

Genetic effective population size: Preliminary estimates of Ne between 478 and 20,000.

1. Aims

The main aims of my PhD that are relevant to the conservation status of the Gouldian Finch are to:

- Assess the genetic effective population size (Ne)
- Assess population connectivity using genetic methods

This report submitted to the Department of Environment provides a summary of the relevant information with regards to these main aims. These data are, as yet, unpublished, ought to be treated confidentially and cited as pers comm. We will keep the Department updated with publication of these data which will include much more thorough discussion.

2. Sampling contemporary populations

Previous work on the conservation genetics Gouldian finch only included two contemporary wild populations (Yinberrie Hills and Mornington: Esparza-Salas 2007). Our study has expanded the sampling to include 5 major populations in the west, and one minor sample (n=5) from Chidna in Queensland (Figure 1). Blood samples collected from these localities were included in connectivity and effective population size analyses using three different molecular datasets.

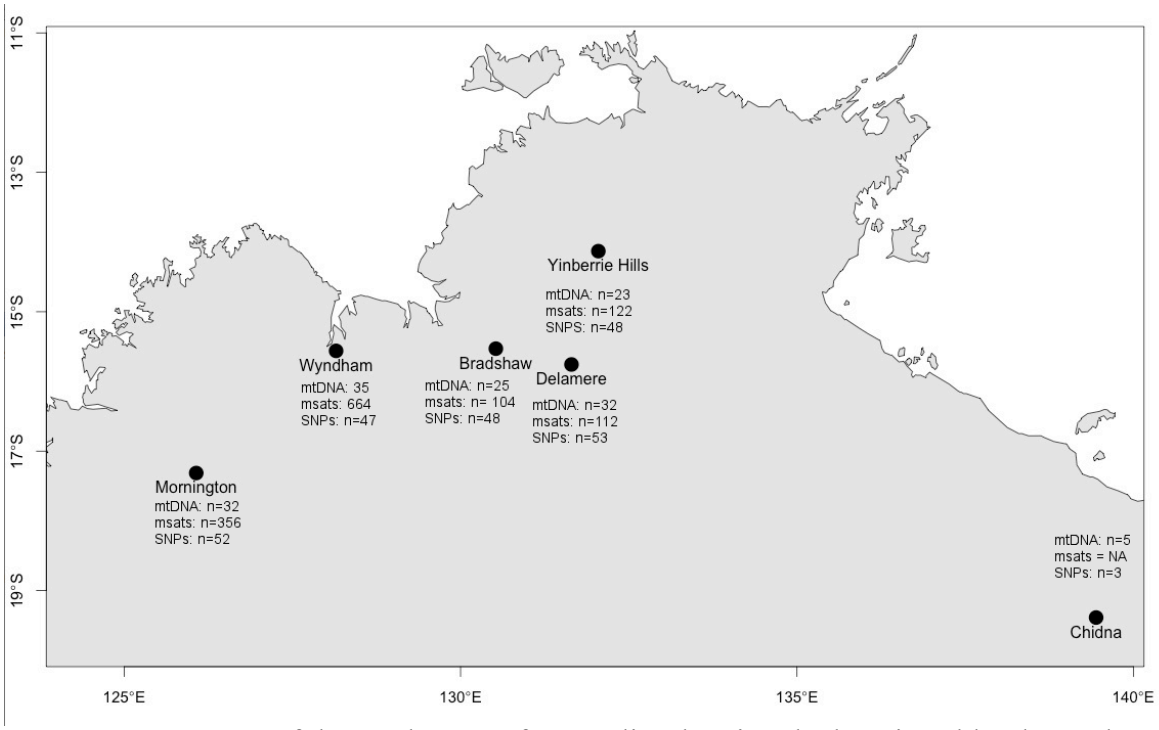

Figure 1: Map of the north-west of Australia, showing the locations blood samples were collected between 2005–2013. Below each location is the sample size for each molecular dataset.

3. Genetic Connectivity

Previous work on connectivity found that there was very high migration rates between Mornington and Yinberrie Hills, and was unable to differentiate delineate separate populations (Esparza-Salas 2007). However, this study had a low number of molecular markers (six microsatellites), which may obscure fine-scale genetic differentiation. With additional regional sampling and better genetic markers we hope to get a more realistic idea of the structure and movement of Gouldian finch populations.

We assessed genetic connectivity between our 5 major populations in using three molecular datasets. We will describe the main results for each data type separately, then come to a conclusion. The full methods and results are available on request and will be published in a scientific journal.

3.1 mtDNA

We sequenced the mitochondrial control region for all 6 populations. Like previous results we found no evidence for population structure in the west (Monington to Yinberrie), and were unable to draw statistically valid conclusions about the connectivity of the Queensland sample.

3.2 Microsatellites

We have results from 10 microsatellite markers (Pryke *et al.* 2010), and conducted a number of population genetic analyses to assess the population structure in the western part of the range. Like previous work, we found no evidence of population genetic structure or Isolation-by-Distance using common population genetic statistical methods (F_{ST}, Bayesian Clustering and Discriminant Analysis of Principal Components (Pritchard *et al.* 2000; Excoffier & Lischer 2010; Jombart *et al.* 2010).

Population genetic methods can inform about the evolutionary differences between populations, but populations with high genetic connectivity can be demographically independent. Demographic independence and estimation of contemporary migration rates is difficult to estimate with genetic methods (Waples & Gaggiotti 2006).We used two individual pairwise distance methods to give a qualitative estimate of whether there is much exchange between populations on ecological timescale, pairwise relatedness and spatial autocorrelation (Smouse & Peakall 1999). The prediction for both of these methods is that if there is limited movement on the contemporary timescale there should be higher relatedness or spatial autocorrelation measure (*r*) within populations. Neither analysis found any evidence of restricted movement in the Gouldian finch, as is demonstrated in Figure 2.

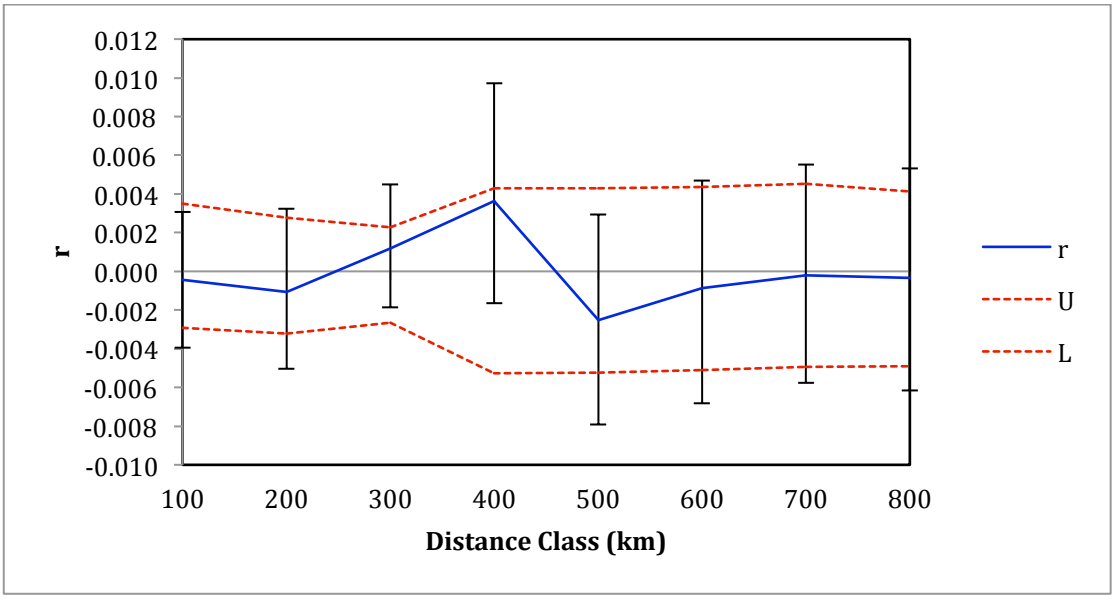

Figure 2: Correlogram from spatial autocorrelation analysis, showing no effect restricted movement within and between populations at different distance classes (between Yinberrie and Mornington). Blue line (r) represents the autocorrelation coefficient from the comparison between genetic and geographic distance matrices, and error bars are the bootstrap 95% confidence intervals about the *r* estimate for that distance class. The red U and L lines represent the upper and lower 95% confidence intervals for the null hypothesis of no spatial structure generated by permutation of samples across the distance classes. Population codes: MO=Mornington, WY=Wyndham, BA=Bradshaw, DE=Delamere, YH=Yinberrie Hills, CH=Chidna.

3.2 SNPs

For a subsample of each population we used a next generation sequencing (NGS) method (Genotyping-by-Sequencing (Elshire *et al.* 2011)) to take a random subsample of the genome, which produces a larger number of molecular markers than has been used in the Gouldian finch previously. Although the power of individual loci are low, NGS technologies allow the amplification of thousands of loci simultaneously and far exceed the power of standard microsatellite datasets, and have been used to delineate very weak genetic differentiation (Waples 1998; Funk *et al.* 2012). After data cleaning, we obtained 3,839 variable SNP loci for analysis.

We subjected these data to the same statistical testing as the microsatellite dataset and the results were also indicative of no population genetic structure between the western regions. We found weak evidence of separation between Queensland and the West using the ordination method Discriminant Analysis of Principal Components (Jombart *et al.* 2010), but should be treated with caution owing to the small population sample (Figure 3)

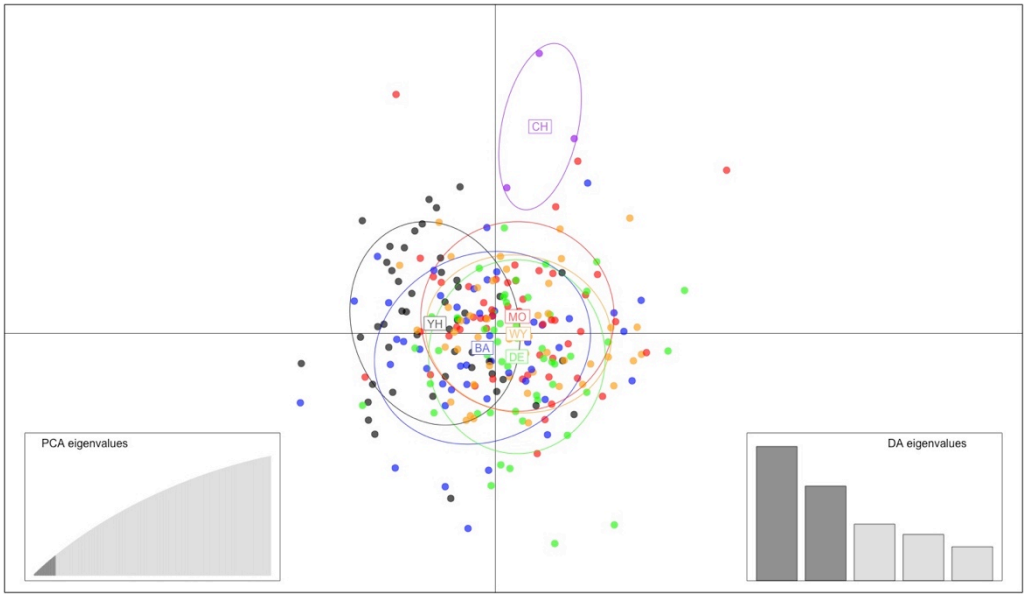

Figure 3: Results from the Discriminant Analysis of Principal Components analysis in the microsatellite and SNP datasets. We used *adegenet* to find the optimal number of clusters in each dataset, and ran discriminant analysis on these groupings, as well as on sampling locality. Results from the clustering analysis on the SNP dataset found only one cluster. However, when sampling locality is run in a discriminant analysis c) the scatter plot shows no differences between the western populations, with some separation of the Chidna population.

3.3 Concluding remarks

The results from these population genetic analyses confirm what has been previously found in the Gouldian finch, that there is one continuous genetic population in the Western core range. Our spatial autocorrelation analyses and pairwise relatedness showed no local genetic structure within sampling localities, which either suggests very large local population sizes, or that there is a great deal of movement in and out of these sampling localities. Local census sizes are mostly a few hundred individuals (Sullivan *et al.* 2009; Garnett *et al.* 2011), and studies of breeding birds at Wyndham, Newry and Yinberrie show the number of breeding individuals to be less than census sizes (Tidemann *et al.* 1999; Brazill-Boast *et al.* 2013). We believe it more likely these animals are highly mobile, perhaps more mobile than the 10-20km movement radius found in banding recoveries and radio-tracking data (ABBBS summary data; Kimberley Maute, pers comm.). Indeed, anecodotal reports suggest birds moved between Wyndham and Newry (130km displacement) within the space of a few weeks (S. Pryke pers comm.), but we do not know whether this scale of movement is a regular occurrence for the Gouldian finch. Further, our results only suggest very high migration over a long period of time, and do not allow us to distinguish between very high between neighbouring regions, or whether there is capacity to move beyond neighbouring regions. We also do not know whether this high connectivity results from the movement entire flocks, or individuals. However, we can say that the lack of spatially and temporally systematic census surveys cross the range of the Gouldian finch should be considered very cautiously and potentially subject to pseudoreplication.

4. Effective Population size (preliminary data)

As we have previously conveyed, we believe that census size survey methods are flawed, and may not provide robust estimates of the population size. There are a number of ways in which the genetic 'effective' population size may be much smaller than the census size in the Gouldian finch, which we have explained in previous correspondences.

The 'effective' population size (Ne) is an abstract genetic concept that describes the size of an "ideal" genetic population. The effective population size determines how susceptible a population is to inbreeding and to future loss of genetic diversity, both of which are important for future population persistence. It can be conceptualised as the number of individuals that contribute genes to subsequent generations, but does not directly equate to the actual number of breeding individuals as effective population size is affected by variance in sex ratio, offspring survival & reproduction and mating system. The census size (Nc) of adults is typically larger than the effective population size, but there is debate about how much larger, and what factors predict the size difference (Frankham 1995; Palstra & Fraser 2012). IUCN threat status categories are based loosely on published ratios between effective population size and census size (Mace *et al.* 2008). Recently, Frankham *et al.* (2014) recommended that these original criteria be expanded to include more variation in Ne/Nc ratio and updated information about preventing inbreeding and loss of genetic diversity over longer timescales.

There are a number of different ways to measure the genetic effective population size which use demographic or genetic data. What I will present in this section represents preliminary data, which will form part of a much larger paper that will include many more estimators and simulations that explore deviations from assumptions that might affect the accuracy of the Ne estimates. I have focussed preliminary estimates on genetic estimator methods that are most robust to violation of assumption under a number of different population migration models (Gilbert & Whitlock 2015).

4.1 Linkage disequilibrium method:

Linkage disequilibrium describes non-random associations of genetic markers; disequilibria can arise from physical linkage of markers (e.g. on the same chromosome), selection, migration, or changes in population size. This latter property is utilised to infer Ne from a single sample, implemented in NeEstimatorV2 (Do *et al.* 2014). This method can be thought of as representing number of effective breeders in the parental generation, reflecting a property "inbreeding" effective size, which reflects the degree of inbreeding. In species with overlapping generations, however, it doesn't strictly represent the previous generation because all "offspring" are different ages (Waples 2005).

I used my SNP data set of 3,839 loci and separately for the microsatellite dataset of 10 loci, to infer the Ne in the years with the largest sample sizes. I also combined all three years into a single "population" in a separate estimate of Ne. Other years are not presented because small individual sample sizes can lead to very large sampling variance and cause estimates of negative or undefined Ne (Do *et al.* 2014). Because our previous results suggest one homogenous genetic population, all individuals from our five western sampling localities were included in this analysis.

There are two versions of this method that assume Random Mating or Monogamy (Weir *et al.* 1980; Do *et al.* 2014). Random mating assumes all offspring arise from different combinations of males and females, but monogamy assumes life-long pair bonds: neither of which are good approximations for the Gouldian finch. The Gouldian finch will occasionally form new partnerships within and between breeding seasons, and 35% of nests (7.7% of total offspring) at Wyndham had offspring sired by a different male (Bolton, Unpublished). Because of this, we have presented results from both analyses in Figure 4.

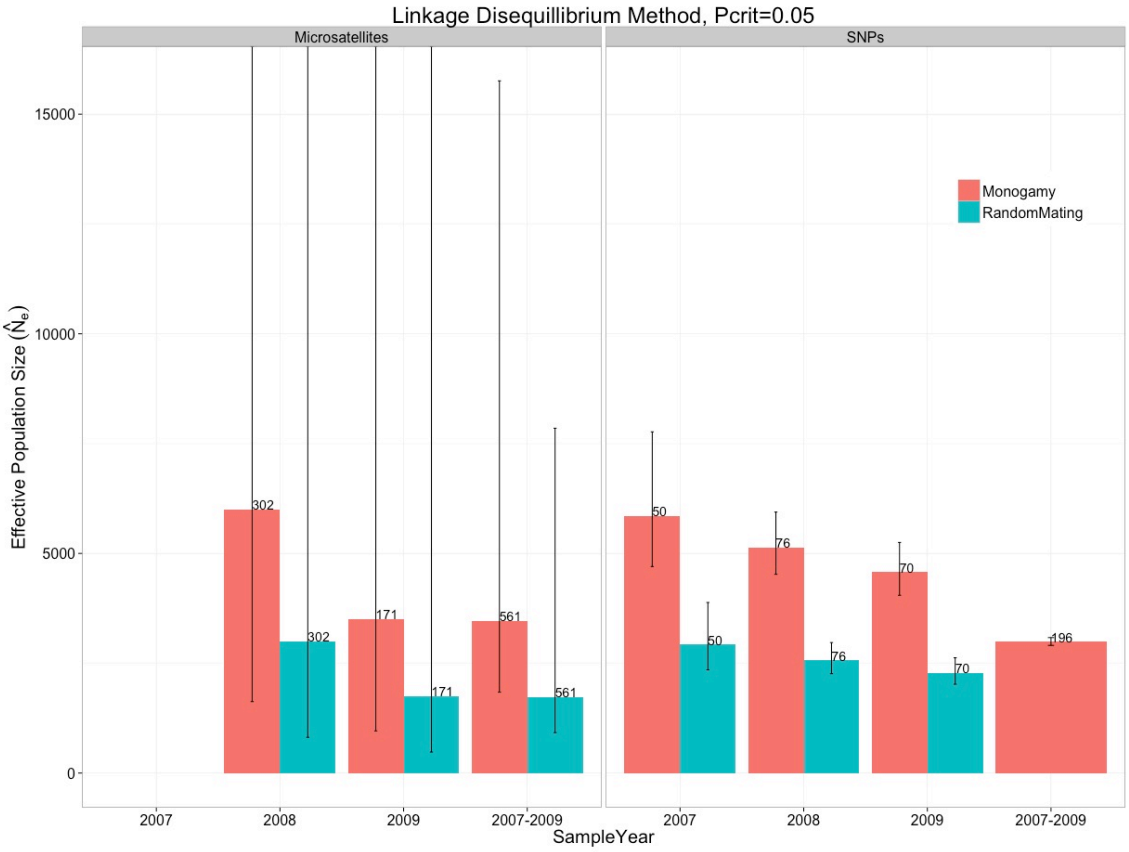

Figure 4: Results from linkage disequilibrium estimation of Ne in NeEstimator V2. Colours represent the different mating system corrections, error bars are 95% parametric confidence intervals, numbers beside the estimate are the number of individuals in the sample. Missing data from particular categories meant that the programme gave an "undefined" Ne estimate.

Ne estimates from this method generally represent the population size at the parental generation, but may also be sensitive to population sizes in the recent past (Waples 2005, 2006; Waples & Do 2008). What constitutes the "recent past" will depend on the generation time of a species, and the size of past population size changes. Simulations have shown that between five and eight (discrete) generations need to pass after population expansion before the linkage disequllibrium method can detect the true Ne (Waples 2005), but this would take longer for species with overlapping generations. In the case of the Gouldian finch it is commonly assumed that the generation time (and lifespan) is approximately one year based on age of first breeding and very poor return rates of individuals between years. However, this is only an "average" generation time and some individuals have been recaptured six years later (at Mornington Sanctuary). This is a violation of one of the assumptions that can cause inaccurate estimates of Ne.

Like many of these genetic estimators of Ne, there are a number of assumptions that underlie them that are not necessarily applicable to most natural populations. This method assumes that all loci are physically unlinked, not under selection, and the organism has discrete generations, and a single closed population (Waples 2006). In a best attempt to satisfy this last requirement we sampled from the entire western part of the range, but when there is very high migration rates (m>0.1) and Ne>500 this method will produce undefined or inaccurate estimates (Gilbert & Whitlock 2015). This is potentially the situation in the Gouldian finch population. In these cases, the use of another estimation method (MLNe, discussed below) is recommended, but often the estimates just reflect the Ne of the entire metapopulation (Wang & Whitlock 2003; Gilbert & Whitlock 2015). Additionally, to my knowledge there has been no work that specifically looks at the effects of number of individuals sampled in cases with thousands of markers on estimate accuracy, and only been evaluated in microsatellites using much smaller values of Ne (England *et al.* 2006; Waples 2006). Therefore, I suggest these estimates be treated as preliminary and with caution, particularly because 95% confidence intervals for many estimates are as low as Ne=478. However, we can probably confidently conclude that this species is not at any risk of inbreeding (as also confirmed by the heterozygosity analysis).

4.2 Temporal methods

Another programme that estimates effective population size uses samples spaced out over a few generations, and uses the change in allele frequencies owing to drift to measure the harmonic mean of Ne over that time period (Wang & Whitlock 2003; Waples 2005). There are a number of different estimators(Do *et al.* 2014), but for a preliminary glimpse we chose the most accurate estimator: MLNe (Wang & Whitlock 2003; Gilbert & Whitlock 2015). This method most reflects the variance effective population size, which represents the effects of genetic drift between generations.

For a first estimate, we used individuals sampled from 2005, 2009 & 2013 and an equivalent sample from 2007 & 2008 & 2009, in a closed population. The results are presented in Table 1.

The closer spaced sample from 2007-2009 shows a considerably higher Ne than the more distantly spaced estimate. This is likely because of two the combined effects of overlapping generations obscuring the effects of genetic drift, and the short timescale over which it was sampled. These estimates are suggestive that the population is not at risk of strong effects of genetic drift in the short-term, but our lower estimates indicate that they might be at risk of loss of evolutionary potential in the longer term (Frankham *et al.* 2014).

Table 1: Results from MLNe temporal method of Ne estimation, representing the harmonic mean of the variance effective population size.

	2005 & 2009 & 2013	**2007 & 2008 & 2009**
$\widehat{N}$e	1675	19994
Lower 95% CI	611.2	1146.3
Upper 95% CI	20000	20000

4.3 Concluding remarks

Given that these estimates are very preliminary and confidence intervals include effective population sizes that are potentially at risk, we do not think it is appropriate to weight this data to highly in decisions about threat status.

5. Important knowledge gaps:

The limitations of much of these methods is that they cannot directly capture the movement patterns of real birds on a timescale that is ecologically relevant. Indeed, we have presented very strong evidence for a genetically homogenous population, but may still mean they are demographically independent. However, given the effective population sizes we have observed (preliminarily), it is quite likely that migration rates are above the threshold typically considered for demographic independence (m=0.1) (Waples & Gaggiotti 2006). However, our Ne estimates are not without flaws, and can only describe the genetic effective population size, and demographic estimates of effective population size can be very different (e.g Arden & Kapuscinski 2003). Therefore, it would be valuable to track the birds across the landscape using GPS trackers (technology small enough will soon be available), which would have a much larger range than radio-tracking. These data could provide information about:

- Demographic connectivity

- Individual and flock movement behaviour
- Habitat and space use on local and regional scales.

Further, previous work from our lab has identified that on the local scale (at Wyndham) appropriate nesting sites are limiting for the Gouldian finch (Brazill-Boast *et al.* 2010, 2011). To my knowledge no attempt has been made at extrapolating these specific nest requirements across the entire range. Indeed, this might be particularly useful for targeting regions for population recovery in Queensland.

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Appendix II

Estimation of migration rates

Estimates of pairwise migration rates from pairwise FST estimates from ARLEQUIN analyses in Chapter Five are presented in Table A1.

Siblings and half-sibling frequencies in different populations can give an indication of how far individuals (or their parents) move (Feutry et al., 2016). In Chapter Six, I used the frequency of sibling relationships in the population to infer effective population size in COLONY (Jones and Wang, 2010). This program also estimates the probability that a given dyad is a half-sibling or a full-sibling, which I now use to estimate the frequency of siblings in different sampling localities.

Sibling and half-siblings were estimated across all five sampling localities in 2008 and 2009 separately for the 16 microsatellite dataset. Given this species has overlapping generations: I also calculated the sibling probabilities for the 2008 and 2009 years combined. I used the Weak sibship prior to weight assignment of siblings as described in Chapter Six. I chose a probability of ≥0.98 as evidence that a dyad were half- or full-siblings. Table A2 shows for each sampling locality the number of full-siblings, half-siblings and siblings sampled across 2008-2009. Equivalent of siblings and half-siblings were found within the same sampling locality as there were between sampling localities (7 within, 7 between).

References:

Feutry, P., Berry, O., Kyne, P.M., Pillans, R.D., Hillary, R., Peter, M., Marthick, J.R., Johnson, G., Gunasekera, R.M., Bax, N.J., Bravington, M., 2016. Inferring contemporary and historical genetic connectivity from juveniles. Mol. Ecol. doi:10.1111/mec.13929

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Table A1: Estimates of effective migration rate ($2N_em$) derived from Arlequin analyses in Chapter Five. Below the diagonal is the estimated effective migration rate using the 16 microsatellite loci, and above the diagonal is calculated from the dataset of 3839 SNPs.

	Mornington	**Wyndham**	**Bradshaw**	**Delamere**	**Yinberrie Hills**	**Chidna**
Mornington		Inf	Inf	Inf	Inf	66.06
Wyndham	899.28		Inf	Inf	Inf	43.21
Bradshaw	119.61	282.82		Inf	Inf	95.68
Delamere	255.81	433.26	175.08		Inf	54.23
Yinberrie Hills	212.36	1759.07	Inf	Inf		99.9
Chidna	35.58	42.80	123.59	535.42	66.80	

Table A2: The number of full-siblings (FS), half-siblings (HS) assigned within and between major sampling localities in 2008 and 2009. Xyear denotes siblings and half siblings that were assigned when the 2008 and 2009 datasets were combined. All FS and HS relationships were inferred at ≥0.98 probability.

	Mornington			**Wyndham**			**Bradshaw**			**Delamere**			**Yinberrie Hills**		
	FS	**HS**	**Xyear**	**FS**	**HS**	**Xyear**	**FS**	**HS**	**Xyear**	**FS**	**HS**	**Xyear**	**FS**	**HS**	**Xyear**
Mornington	0	0	0												
Wyndham	0	0	0	2	4	1									
Bradshaw	0	0	1	0	1	0	0	0	0						
Delamere	0	0	0	0	0	0	0	0	0	0	0	0			
Yinberrie Hills	0	1	0	0	0	3	0	1	0	0	0	1	0	0	0

Appendix III

A colourful genomic landscape: patterns of gene flow in an Australian colour polymorphic finch

Poster for European Society of Evolutionary Biology Conference, Lausanne Switzerland, 2015

Genomic landscape of colour polymorphism

Peri E. Bolton[1*], Adam P. Cardilini[2], Lee Ann Rollins[2], James Brazill-Boast[1], Sarah Legge[3], Kimberly L Maute[4], Kang-Wook Kim[5], Simon C Griffith[1]

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[3]Australian Wildlife Conservancy, Australia
[4]School of Biological Sciences, University of Wollongong, Australia
[5]Department of Plant and Animal Sciences, University of Sheffield, United Kingdom

Introduction: colour polymorphism and speciation?

The Gouldian finch has a sympatric colour polymorphism. Morphs have a distinct behavioural and physiological phenotype, possibly maintained by frequency-dependent disruptive selection. Head-colour is a sex-linked Mendelian trait expressed in both sexes, and was recently mapped to the Z-chromosome[1].

Experiments in captivity have detected **pre- and post-zygotic incompatibilities** between morphs. Despite this, gene flow between morphs is expected; up to 30% of wild breeding pairs are mixed-morph.

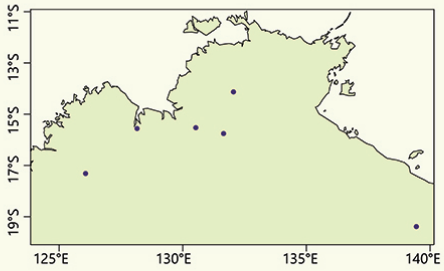
Questions

- How does colour polymorphism and incompatibility affect gene flow between morphs?

- What is the genetic architecture of colour polymorphism? Are secondary loci affiliated with the broader behavioural and physiological phenotype?

Materials and methods

We analysed 193 black-headed, and 66 red-headed wild caught birds across the geographic range (below).



DNA was sequenced using Genotyping-By-Sequencing, and were assembled *de novo*. The final dataset included 3,839 SNPs, with an average site depth of 1.6x per individual.

The SNPs were analysed for evidence of genetic structure (i.e. restricted gene flow) and scanned for signatures of selection on (or near) SNPs.

Conclusions and future directions

- Any genetic incompatibility is insufficient to restrict gene-flow between morphs.

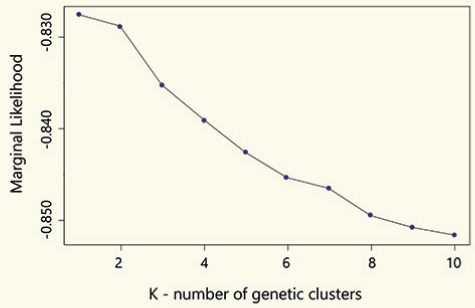
- Corroborates role of few loci of large effect generating polymorphism.

- Extensive gene flow across the geographic range.

Future work with these data will be used in conservation genetics and population demographic history, including the origin of morphs.

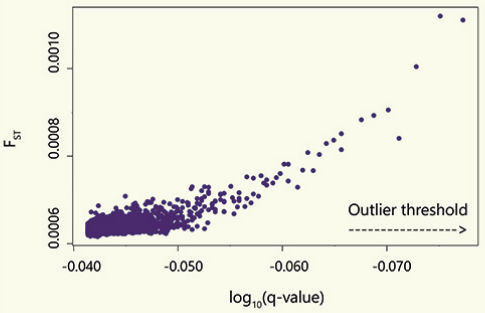
Results: ample gene-flow between morphs

Genome-wide differentiation between morphs was negligible, F_{ST} = 0.0002.



Above: marginal likelihoods for each genetic cluster. The highest marginal likelihood is the best model: one genetic cluster.

Results: no outlier loci



Above: q-value (a measure of statistical confidence) and F_{ST} for each SNP in the dataset. The critical q-value indicating an outlier SNP was -1.30, with a false discovery rate of 0.05.

Funding:
Australian Research Council Discovery Grant
Macquarie University Postgraduate Research Fund
Reference: [1] Kim *et al* 2015. Submitted

Appendix IV

Animal Ethics Approvals

Macquarie University Approvals:

Australian Wildife Conservancy

University of Wollongong

MACQUARIE
UNIVERSITY ~ SYDNEY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2007/037
Full Approval Duration: 15 December 2007 to 14 December 2010 (36 months)

To: A/Prof Simon Griffiths
CISAB
Macquarie University
Phone: (02) 9850 4186
Email: simon.griffith@mq.edu.au

Associate Investigator (s)
Dr Sarah Pryke Phone: 9850 4187
Ms Barbara Tschirren Phone: 9850 4187

Other people participating
Mr James Brazil-Boast Phone: 9850 4187
Ms Amanda Gilby Phone: 9850 4187
Mr Mike Fidler Phone: 4977 4006
Mr Robbie Miller Phone: 9876 5653

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: LIFE-HISTORY AND BIODIVERSITY IN FOUR ESTRILDID FINCHES: BEHAVIOUR, EVOLUTION AND CONSERVATION — IN CAPTIVITY

Type of animal research and description of project:
Evolutionary and behavioural research: The project will use captive finch species to investigate the evolutionary processes related to reproduction and life-history that underlie species diversity in birds. Housing will be in indoor cages under environmentally controlled conditions (min 1 bird, max 8 birds), box aviaries (min 2 birds, max 40 birds) or flight aviaries (min 2 birds, max 100 birds) of size and design as specified in approved protocol. Experimental processes include: breeding experiments, social experiments, social breeding experiments, parental-off-spring experiments and competition experiments. All experimental procedures to be conducted in accordance with details provided in the approved protocol.

Species of animal: Gouldian finch (*Erythrura gouldiae*); Long-tail finch (*Poephila acuticauda*); Black-throated finch (*Poephila cincta*); Zebra finch (*Taeniopygia guttata*)

Number: Gouldian finches: 720 per year (total 2160); Long-tail finches: 720 per year (total 2160); Black-throated finches: 720 per year (total 2160); Zebra finches: 720 per year (total 2160)

Location/s: (1) CISAB, Macquarie University, 209 Culloden Road, Marsfield, NSW 2122
(2) Save the Gouldian Finch, 9 Owens Road, Martinsville, NSW 2264

Amendments considered by the AEC during last period: N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions:

(This authority has been issued as the above condition (s) has been addressed to the satisfaction of the AEC)

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.

This authority remains in force from **15 December 2007** to **14 December 2008**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a PROGRESS report at the end of this period.**

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Date: 12-12-07

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY ~ SYDNEY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2007/038
Full Approval Duration: 01 January 2008 to 31 December 2010 (36 months)

To: A/Prof Simon Griffiths
CISAB
Macquarie University
Phone: (02) 9850 4186
Email: simon.griffith@mq.edu.au

Associate Investigator (s)
Dr Sarah Pryke Phone: 9850 4187
Ms Barbara Tschirren Phone: 9850 4187

Other people participating
Mr James Brazil-Boast Phone: 9850 4187
Ms Amanda Gilby Phone: 9850 4187

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: LIFE-HISTORY AND BIODIVERSITY IN FOUR ESTRILDID FINCHES: BEHAVIOUR, EVOLUTION AND CONSERVATION — IN THE WILD

Type of animal research and description of project:
Evolutionary and behavioural research - wildlife: The project will use wild finch species to investigate the evolutionary processes related to reproduction and life-history that underlie species diversity in birds. Research includes (1) mapping and characterizing variation of wild populations (individual phenotypes) (2) Breeding ecology and nest competition in the wild. Birds will be captured in the wild using mist nets. Experimental procedures include: banding (ABBBS); colour and morphological measurements; blood sampling; immunocompetence testing using PHA skin test; recording of parental care; sperm sampling; nest monitoring and observation; cross-fostering off-spring between nests. All experimental procedures to be conducted in accordance with details provided in the approved protocol.

Species of animal: Gouldian finch (*Erythrura gouldiae*); Long-tail finch (*Poephila acuticauda*); Black-throated finch (*Poephila cincta*); Zebra finch (*Taeniopygia guttata*)

Number: Gouldian finches: up to 400 per year (total 1200); Long-tail finches: up to 600 per year (total 1800); Black-throated finches: up to 600 per year (total 1800); Zebra finches: up to 1000 per year (total 3000)

Location/s: (1) Conservation land – Wyndham Shire, North Kimberley, WA 6740
(2) Fowler's Gap UNSW Arid Zone Research Station, via Broken Hill NSW 2880

Amendments considered by the AEC during last period: N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions:

(This authority has been issued as the above condition (s) has been addressed to the satisfaction of the AEC)

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.

This authority remains in force from **01 January 2008** to **31 December 2008**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a PROGRESS report at the end of this period.**

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Date: 12-12-07

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2010/053 **Date of expiry:** 31 December 2011

Full Approval Duration: *1 January 2011 to 31 December 2013 (36 months)*

Principal Investigator:
A/Prof Simon Griffith
Dept of Biological Sciences
Macquarie University NSW 2109
(02) 9850 1301
0425 746 674
simon.griffith@mq.edu.au

Associate Investigators:

Sarah Pryke	0431 746 276; 9850 1302
Dr Nina Svedin	9850 1302
Dr Paul McDonald	9850 1303
Ms Erica van Rooij	9850 1304
Ms Mylene Mariette	9850 1305
Mr Luke McCowan	9850 1305

In case of emergency, please contact:
Animal Welfare Officer Dr Sally Smith: 9850 7758 / 0439 497 383
or the Principal Investigator / Associate Investigator named above

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

Title of the project: The breeding ecology of four Australian finches

Type of animal research and aims of project:
Research (Wildlife) – This project aims to understand the evolutionary processes related to reproduction and life history that underlie the species diversity of birds.

Surgical Procedures category: 1,3 (Observation involving minor interference, Minor conscious intervention)

All procedures must be performed in accordance with the AEC approved documentation, unless Conditions of Approval state otherwise.

Numbers approved:

Species	Strain	Age/Sex/Weight	*Year 1*	*Year 2*	*Year 3*	Total	Supplier/Source
Gouldian Finch	Wild	0-3yrs/M and F/14g	600	600	600	**1800**	NA
Long-tail Finch	Wild	0-3yrs/M and F/15g	600	600	600	**1800**	NA
Masked Finch	Wild	0-3yrs/M and F/15g	200	200	200	**600**	NA
Zebra Finch	Wild	0-3yrs/M and F/11g	1000	1000	1000	**3000**	NA
		TOTAL	**2400**	**2400**	**2400**	**7200**	

** Some animals may be recaptured.*

Location of research:

Location	*Full street address*
Wyndham	Wyndham Shire, North Kimberley WA 6740
Fowler's Gap	Fowler's Gap UNSW Arid Zone Research Station via Broken Hill NSW 2880
Newry	Newry Cattle Station, Victoria River Highway NT

Amendments approved by the AEC since initial approval: N/A

Conditions of Approval:

1. All outstanding personnel details must be provided to the Research Office.
2. All relevant permits and licences needed for this work must be obtained prior to work commencing on this protocol.
3. Copies of all relevant permits and licences must be provided to the Research Office as soon as they become available.
4. Biosafety approval must be obtained.
5. All other information requested in the letter of approval received with the ARA must be provided to the Research Office before work commences.

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.

This authority remains in force from **1 January 2011 to 31 December 2011**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a PROGRESS REPORT before the end of this period.**

Prof Michael Gillings
Chair, Animal Ethics Committee

Date: *16 December 2010*

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

Simon Griffith

13 March 2015 at 12:05:55 PM AEDT

To: Peri Bolton

Fwd: Outcome of 11 December 2014 AEC Meeting - 2010/053 - Tissue Use

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Hi,

Here is their 'approval' of the tissue use.
I will locate and forward on the application that I submitted.

cheers

Simon

---------- Forwarded message ----------
From: **Animal Ethics** <animal.ethics@mq.edu.au>
Date: 18 December 2014 at 11:02
Subject: Outcome of 11 December 2014 AEC Meeting - 2010/053 - Tissue Use
To: Simon Griffith <simon.griffith@mq.edu.au>
Cc: Luke McCowan <luke.mccowan@mq.edu.au>

Dear Dr Griffith,

2010/053	***Dr Simon Griffith*** *(Biological Sciences)*	***The breeding ecology of four Australian finches***

Blood samples stored in ethanol

The tissue has already been collected and stored under a previously approved research protocol that is now expired. The tissue is to be collected and supplied under a current, approved research protocol.

Decision

The Committee noted your report on tissue use.

This email serves as official notification of the AEC decision. Please keep a copy for your records. Should you have any queries or require clarification, please contact the AEC Secretariat.

Regards,
Dr Karolyn White
Acting Chair, AEC

Animal Ethics
Office of the Deputy Vice Chancellor (Research)
Research Office
Level 3, Research Hub, Building C5C East
Macquarie University
NSW 2109 Australia
T: +61 2 9850 7758
F: +61 2 9850 4465
Email: animal.ethics@mq.edu.au
http://www.mq.edu.au/research

MACQUARIE University
SYDNEY·AUSTRALIA

Office Use Only Feb 05

Agenda Item No. 2.6

Department of Conservation and Land Management
Animal Ethics Committee

COVER SHEET
(To be completed by proponent, copy to be returned to proponent when approved)

1. **PROJECT TITLE:**
The conservation biology and ecology of finches in Australia's tropical savannas.

2. **CHIEF INVESTIGATOR:** Sarah Legge
current appointment: Biologist
contact address: Mornington Station. PMB 925, Derby. 6728
telephone number: 9191 4619 **mobile phone no:**
fax number: 9191 4619
email address: gouldian@australianwildlife.org

3. **EXPECTED DATE OF COMMENCEMENT AND DURATION OF PROJECT:** Ongoing

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COMMITTEE USE ONLY:

APPROVAL

The CALM AEC has considered this proposal and approves it for the period 25/2/05 to 16/12/05 subject to the following conditions:

1. Annual Report required by 16 / 12 / 05

2. Other comments _____

3. Chief Investigator to inform AEC Executive Officer immediately of any staff changes to this project.

AEC APPROVAL NUMBER: CAEC / 6 / 2005

CHAIR: _____ DATE: 25 / 2 / 05

Office Use Only:					
AEC Meeting Date:	*26/10/07*	*Signatures received:*	No ☐	Yes ☒	Incomplete ☐
Agenda Item No.:	*2.11*	*Competencies received:*	No ☐	Yes ☒	Incomplete ☐

Department Environment and Conservation
Animal Ethics Committee

COVER SHEET
(To be completed by proponent, copy to be returned to proponent when approved)

1. **PROJECT TITLE:**
   The conservation biology and ecology of finches in Australia's tropical savannas.

2. **CHIEF INVESTIGATOR:** Sarah Legge
   current appointment: Chief Ecologist, AWC
   contact address: Mornington Wildlife Sanctuary PMB 925, Derby. 6728
   telephone number: 08 9191 4619 **mobile phone no:**
   fax number: 08 6267 8065
   email address: sarah@australianwildlife.org

3. **EXPECTED DATE OF COMMENCEMENT AND DURATION OF PROJECT:** Ongoing

4. **PROJECTS INVOLVING THE COLLECTION OF VOUCHER SPECIMENS OR TARGETING AREAS POORLY SURVEYED ARE TO BE DISCUSSED WITH WA MUSEUM STAFF PRIOR TO COMMENCEMENT (IF RELEVANT):** No

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COMMITTEE USE ONLY:

APPROVAL

The DEC AEC has considered this proposal and approves it for the period 26/10/07 to 30/11/08 subject to the following conditions:

1. Annual Report required by 30/11/08
2. Other comments:
   1. CI to confirm the number of consecutive days trapping at each site.
3. Chief Investigator to inform AEC Executive Officer immediately of any staff changes to this project.

AEC APPROVAL NUMBER: **DEC AEC 43/2007**

CHAIR: _____ DATE: 26/10/07

DEC AEC Application for approval to undertake research involving vertebrate animals

Office Use Only:			
AEC Meeting Date:	27/08/2010	*1. Signatures received:*	No ☐ Yes ☒ Incomplete ☐
Agenda Item No.:	2.6	*2. Competencies received:*	No ☐ Yes ☒ Incomplete ☐
		3. Renewal Application (3yr):	No ☐ Yes ☒
		If yes, annual report submitted:	No ☐ Yes ☒ x3

Department of Environment and Conservation
Animal Ethics Committee

APPLICATION FORM COVER SHEET

To fill out this form you must refer to the DEC Standard Operating Procedures and the Disease Risk Management Guidelines. Answer all sections, indicating when there is no information available. Incomplete forms will result in delays in assessment or rejection of the nomination.

1. Project Title:

Monitoring the response of vertebrate fauna to management at AWC sanctuaries

2. Chief Investigator (CI):

CI's name:	Dr Sarah Legge	Telephone:	(08) 9191 4619
Agency/Company:	Australian Wildlife Conservancy	Mobile:	0438 983455
Current Position:	National Conservation and Science Manager	Fax:	(08) 9191 7403
Contact Address:	Mornington Wildlife Sanctuary, PMB 925, Derby, WA, 6728	Email:	sarah@australianwildlife.org

3. Expected date of commencement and duration of project (project to be renewed every 3 years):

Commencement date (d/m/y): November 2010 Completion date (d/m/y): ongoing

4. Is this project a 3 year renewal application? No ☐ Yes ☒

If yes, provide previous AEC approval number: DEC AEC: 43/2007 **and** 41/2007 **and** 42/2007

Note that our 3 existing permits all pertain to monitoring vertebrate taxa. The 3 projects were initiated at different times and new approvals were sought in each case. However, AWC's monitoring is a holistic program covering a range of key indicator species, and therefore we propose combining these permits into a single application that covers all the vertebrate taxa included in our monitoring program.

5. Projects involving the collection of voucher specimens or targeting areas poorly surveyed are to be discussed with WA museum staff prior to commencement:

No ☐ Yes ☒ N/A ☐

***COMMITTEE USE ONLY:* Approval Slip**

The DEC AEC has considered this proposal and approves it for the period 28/07/2010 to 28/07/2013 subject to, (i) the following conditions and (ii) submission and AEC approval of annual reports and renewal applications.

1. Annual Report required by 19/11/2010
2. Condition of approval/other comments:

   _____
   _____
   _____
3. Chief Investigator to inform AEC Executive Officer immediately of any staff changes to this project.

AEC APPROVAL NUMBER: DEC AEC: 2010 / 35

CHAIR: _____ DATE: 27/08/2010

University of Wollongong

THE ETHICS APPROVAL
THE FOLLOWING DATA WAS LOST

In reply please quote: BF:PP AE06/25
Further Information: Penny Poeman Ph: 4221 3350

Pro Vice-Chancellor (Research)
19 SEP 2006
University of Wollongong

15 September 2006

Professor L Astheimer
Vice Chancellor's Unit
Building 36

Dear Professor Astheimer

ANIMAL RESEARCH AUTHORITY

I am pleased to advise that the initial application dated 8 August 2006 has been finally approved by the University of Wollongong's Animal Ethics Committee and you can proceed with your research. The Animal Research Authority is for animal research being carried out in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes", 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.

Ethics Number:	AE06/25
Project Title:	Conservation management of seed-eating birds in the tropical savannahs
Name of Researchers:	Professor L Astheimer, Kimberly Maute, Sarah Legge, Stephen Murphy, Hayley Morgan, Sally Weeks, Jo Heathcote
The Authority remains in force from:	15 September 2006 to 14 September 2009 (unless suspended, cancelled or surrendered)
Numbers of Animals:	320 birds per site per year – maximum of 1920 birds a year

It will be necessary to inform the Committee of any changes to the research protocol and seek clearance in such an event. Please note that an Annual Progress Report is to be submitted to the Ethics Committee one year following the final approval date. A Final Report detailing whether the objectives of the research were met and the outcomes of the research should be forwarded to the Committee upon completion of the project. Please forward the completed 'Checklist for Animal House' and the "Animal House Planner' to the Animal House before commencing your research.

Yours sincerely

B. Ferry

A/Professor B Ferry
Chair, Animal Ethics Committee

cc A/Professor H Yeatman, School of Health Sciences
Dr T Maddocks, School of Biological Sciences

Research Services Office University of Wollongong NSW 2522 Australia
Telephone: +61 2 4221 3386 **Facsimile:** +61 2 4221 4338

TOTAL P.03