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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

Peri E. Bolton¹, Lee Ann Rollins^{1,2}, James Brazill-Boast^{1,*}, Kang-Wook Kim³, Terry Burke³, Simon C. Griffith¹

¹ *Department of Biological Sciences, Macquarie University, Sydney, New South Wales, Australia, 2109*

² *Deakin University, Geelong, Australia. School of Life and Environmental Sciences, Centre for Integrative Ecology, Locked Bag 20000, Geelong, Victoria, 3220, Australia*

**Current address for James Brazill-Boast: Ecosystems and Threatened Species Unit, Regional Operations Division, NSW Office of Environment and Heritage, Sydney South, NSW 1232, Australia*

³ *Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*

Corresponding Author: Peri Elizabeth Bolton (peri.bolton@mq.edu.au, Ph: +61298501304)

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Running head: Extra-pair paternity & sex ratio in the wild Gouldian finch

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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

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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, 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 (Thornycroft, 1966; Houtman & Falls, 1994). Assortative white-throated sparrow pairs would lack necessary parental co-ordination 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, while 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). In order 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 behavior, 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 modeling (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 (Environmental Protection and Biodiversity and 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 to 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 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 non-territorial and they forage in small flocks (Brazill-Boast & 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.

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 nestboxes 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 nestboxes 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 nestboxes 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 (<50µL), 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

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observations of a colour-banded adult visiting a nest to feed offspring. In addition, every one to two weeks, birds congregating around waterholes were mist-netted and blood-sampled, in order to gain a broader sample of the adult population, including individuals that were deemed to be non-breeding (although it is possible that they were breeding in undetected nests either inside or outside the study area).

Only nestlings from nestboxes 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 nestboxes see Supplement 1.

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 labeled dyes and a 3bp 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 Supplement 1). 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 Supplement 2.

For each year in our dataset, 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 dataset, 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 (Supplement 2).

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 (out of ten). If an offspring mismatched both social parents at two or

more loci (not attributable to dropout), then intra-specific 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 dataset 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 Supplement 3. 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 a) it was more directly comparable with the captive experiments and b) 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 was 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 intra-specific brood parasitism, and further explore our broader findings of intra-specific brood parasitism in relation to other Estrildid finches (Supplement 3).

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 ($Z^R W$) x black male ($Z^r Z^r$) = 4; black female ($Z^r W$) x red male ($Z^R Z^r$) = 8), and same-morph pairs (total families = 36; black female x black male = 34; red female x 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 dataset from Figure 1 in Pryke *et al.* (2010) and tested whether there was a difference in the occurrence of extra-pair paternity (see Supplement 3 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 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 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 dataset.

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 H_0 with the observed effect size, 3) power to reject H_0 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 Supplement 4 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 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 2009) (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; b) and “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 1980's (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, thus the degree of isolation from the wild populations is difficult to ascertain.

In order 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 dataset 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 dataset 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 ($\ln P(D)$) 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 $\text{LnP(D)}_{\text{max}}=1$.

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 non-exclusion probability for these datasets 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). Out 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 re-nested 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 (out 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 out 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 out of 57), representing 8.6% of offspring produced by the social mother (20 out 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 intra-specific 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 quasi-parasitism, 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 Supplement 3 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 extra-pair paternity, and extra-pair sires were identified breeding themselves and nesting up to 4.28km away from the nest in which they gained paternity (other males were 0.33 and 0.54km 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 Supplement 3.

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, Figure 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 pairs in the and same-morph pairs, though 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, Supplement 3 (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; Figure 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 test: $W=8$, $p=0.45$), nor was there a difference when accounting for brood size (GLM: $\chi^2=0.073$, $df=1$ $p=0.79$) (Figure 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 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 (Figure 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 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). In nests where we would predict there to be a reduction in heterozygote red males, there was no significant effect (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 pairing types on brood sex-ratio (GLM: $\chi^2=1.52$, $df=1$, $p=0.29$), nor when considering all four pairing types observed (GLM: $\chi^2= 2.65$ $df=3$ $p=0.45$) (Figure 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 versus 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 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, $t = -2.823$, $df = 9$, $p = 0.02$), but no significant difference between expected heterozygosity (two-tailed paired t -test, $t=-1.95$, $df=9$, $p=0.08$) or observed heterozygosity ($t=-1.81$, $df=9$, $p=0.10$).

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 dataset (AMOVA F_{ST} = -0.0038, p =1), or the full dataset (AMOVA F_{ST} = 0.0040, p =0.31). In the domesticated population the clustering analysis indicated a ΔK_{\max} = 2, and LnP(D)_{\max} was also $K = 2$ (Figure 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 $K=4$ (Figure S5) and $K=2$ (Figure 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 Figure 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). Intra-specific brood parasitism observed in the Gouldian finch is within the range observed in these same Estrildid finches (0.51-10% of offspring) (Supplement 3). In the wild population, females appeared not to be constrained by nesting synchrony or density in their

ability to gain extra-pair paternity (Supplement 3), and some offspring were sired by extra-pair males breeding up to 4.2km 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). Although our relatively small sample sizes, particularly of the key mixed-morph pairs in the wild reduced the power of our tests. However, the biological effect size seen in earlier studies of 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 (~300km 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 minimise 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 sub-structuring 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 are inconsistent with predictions about inter-morph 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 inter-morph conflict. The time is ripe to investigate these questions, as the rapid influx of genomic resources are 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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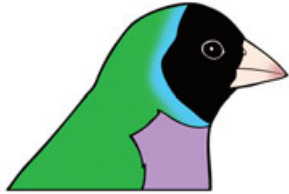
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Box 1: a) Explanation of the alleles and sex linkage of the red/black polymorphism in the Gouldian finch. b) Explanation of the key predictions used in this paper that are derived from the laboratory studies on domesticated Gouldian finches.

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

Table 1: Summary of the extra-pair paternity/offspring (EPP/O) and intra-specific brood parasitism (IBP) data in the Gouldian finch from 2008 & 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
$Z^R W \times Z^r Z^r$	4	6	14	5	1	66.7	26.3	36.1 (± 15.5)	2	33.3	9.52
$Z^r W \times Z^R Z^r$	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
$Z^r W \times Z^r Z^r$	34	38	153	13	3	18.4	7.8	42.8 (± 8.5)	7	13.2	4.05
$Z^R W \times Z^R Z^r$	2	2	8	0	-	0	0	0	0	0.0	0.00

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

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	Offspring Genotype						p	h	power
					Z ^r W	Z ^R W	Z ^r Z ^r	Z ^R Z ^r	Z ^R Z ^R			
Z ^r W	Z ^r Z ^r	34	141	Expected	0.5	-	0.5	-	-	0.01	0.22	0.75
				Observed	86	-	55	-	-			
Z ^r W	Z ^R Z ^r	8	31	Expected	0.25	0.25	0.25	0.25	-	0.55	0.26	0.20
				Observed	6	8	6	11				
Z ^R W	Z ^r Z ^r	4	11	Expected	0.5	-	-	0.5	-	0.55	0.27	0.15
				Observed	7			4	-			
Z ^R W	Z ^R Z ^r	2	7	Expected	0.25	0.25	-	0.25	0.25	0.48	0.62	0.25
				Observed	3	3		0	2			

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 \pm standard deviation; H_E is the expected heterozygosity \pm 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 (\pm 5.80)	0.72 (\pm 0.14)	0.75 (\pm 0.14)	0.04
<i>Red</i>	16	6.26 (\pm 3.78)	0.69 (\pm 0.15)	0.74 (\pm 0.13)	0.06
<i>Black</i>	32	7.50 (\pm 4.10)	0.73 (\pm 0.14)	0.73 (\pm 0.16)	-0.01
Wild	48	11.86 (\pm 7.66)	0.75 (\pm 0.16)	0.77 (\pm 0.15)	0.01
<i>Red</i>	16	8.76 (\pm 5.43)	0.74 (\pm 0.20)	0.74 (\pm 0.20)	0.01
<i>Black</i>	32	8.60 (\pm 4.91)	0.75 (\pm 0.14)	0.77 (\pm 0.16)	0.01

