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Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird

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Abstract

Many songbirds are socially monogamous but genetically polyandrous, mating with individuals outside their pair bonds. Extra-pair paternity (EPP) varies within and across species, but reasons for this variation remain unclear. One possible source of variation is population genetic diversity, which has been shown in interspecific meta-analyses to correlate with EPP but which has limited support from intraspecific tests. Using eight populations of the genetically polyandrous red-winged blackbird (*Agelaius phoeniceus*), including an island population, we investigated whether population-level differences in genetic diversity led to differences in EPP. We first measured genetic diversity over 10 microsatellite loci and found, as predicted, low genetic diversity in the island population. Additional structure analyses with multilocus genotypes and mtDNA showed the island population to be distinct from the continental populations. However, the island population's EPP rate fell in the middle of the continental populations' distribution, whereas the continental populations themselves showed significant variation in EPP. This result suggests that genetic diversity by itself is not a predictor of EPP rate. We discuss reasons for the departure from previous results, including hypotheses for EPP that do not solely implicate female-driven behaviour.

Introduction

Extra-pair paternity (EPP), in which males and females mate outside of their social pair bonds, has been extensively documented in songbirds. Nearly 90% of all species surveyed have been found to engage in EPP, with the frequency of EPP varying widely both within and across species (Petrie & Kempenaers, 1998; Griffith *et al.*, 2002). Research on EPP has sought to identify why this variation exists and what factors cause it, with the aim of understanding the selective forces driving the evolution of animal mating systems.

One popular hypothesis is that EPP confers indirect genetic benefits to offspring (Jennions & Petrie, 2000). Females obtain these benefits in one of two ways: by mating with genetically superior extra-pair mates to acquire additive genetic benefits (i.e. good genes; Kempenaers *et al.*, 1997; Sheldon *et al.*, 1997; Johnsen *et al.*, 2000), or by mating with genetically compatible extra-pair mates to acquire nonadditive genetic benefits (Tregenza & Wedell, 2000; Neff & Pitcher, 2005; Pryke *et al.*, 2010; Løvlie *et al.*, 2013). Despite inconclusive empirical support within and across species (e.g. Kleven *et al.*, 2006; Wilk *et al.*, 2008; Bollmer *et al.*, 2012), indirect benefits for EPP remain the dominant hypothesis for this behaviour, although alternatives have been advanced that suggest EPP is costly to females, is not explicitly adaptive to females or is not strictly female-driven (Westneat & Stewart, 2003; Arnqvist & Kirkpatrick, 2005; Forstmeier *et al.*, 2011, 2014).

If obtaining indirect benefits is indeed the primary function of EPP for females, then a possible source of

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variation in EPP could be the genetic diversity of a population from which females select mates. Hypotheses for the function of EPP share the assumption that benefits to offspring are conferred when females mate with males that are genetically distinct from their social mate (Petrie & Lipsitch, 1994). As a population becomes more diverse, given random spatial distribution, the probability that a female will encounter a suitable extra-pair mate should increase. Therefore, a prediction of all indirect benefit models is that EPP should be more common in populations with greater genetic diversity. In a population with low diversity, females face low odds and high costs – such as the time and energetic demands of searching, exposure to pathogens, and male retaliation or withholding of parental care – of finding a suitable extra-pair mate (Weatherhead *et al.*, 1994; Westneat & Rambo, 2000; Valera *et al.*, 2003). Conversely, females in a genetically diverse population should experience a more favourable cost–benefit ratio. By altering the predicted benefits, genetic diversity could be a demographic trait that influences female mating strategies and thereby drives variation in EPP.

To date, two correlational studies and one experimental study have examined the relationship between genetic diversity and EPP rate. The correlational studies (Petrie *et al.*, 1998; Gohli *et al.*, 2013) reported a positive relationship between EPP rates and genetic diversity (measured as allozyme and nucleotide diversity) across multiple species. Separately, the experimental study (Ockendon *et al.*, 2009) focused on a single species (house sparrow, *Passer domesticus*) to test for changes in EPP after individuals from a continental population were deliberately introduced to an island population with low microsatellite diversity and EPP rate (Griffith *et al.*, 1999). Unexpectedly, the study found that although EPP rose immediately after the introduction, this increase was due to island females mating not with continental males, as expected if females had sought to increase offspring genetic diversity, but with island males. Thus, although the positive relationship between EPP and genetic diversity followed the prediction, the pattern of mating that led to its occurrence did not. Aside from dominance or familiarity conferring an advantage to island males (Ockendon *et al.*, 2009), an unexplored hypothesis is that selection could have favoured assortative mating over population admixture to maintain locally adapted profiles, especially if gene flow had been reduced before the experimental introduction. Population structure analyses would enable identification of genetically distinct populations where different reproductive trends may be expected to occur.

The present study investigates the population structure and the relationship between levels of genetic diversity and EPP in seven continental and one island (Bahamas) population of red-winged blackbird (*Agelaius phoeniceus*). Rates of EPP in this species are well documented and known to vary across continental populations (reviewed in Searcy & Yasukawa, 1995; Yasukawa, 2013), but EPP in the island population has not been previously studied. Genetic diversity across all populations is also unknown, although the island population is predicted to have lower genetic diversity due to founder effects and greater effects of drift (Frankham, 1997). Lastly, although continental populations of blackbirds do not show population differentiation (Ball *et al.*, 1988), it is unclear whether this lack of structure extends to island populations.

Here, we measure EPP and allelic diversity across both previously and newly surveyed populations to examine whether population structure and genetic diversity predict rates of EPP. We use multilocus genotypes from microsatellites to compare differences in genetic diversity with differences in mating patterns. As in Ockendon *et al.* (2009), a first approximation of genetic diversity can be measured using neutral markers. Although the use of microsatellites does not explicitly test for diversity at loci of adaptive interest, it avoids the issue of conflation between drift and selection that is likely to occur with the use of coding regions. Furthermore, the high mutation rates of microsatellites suggest that any population structure found to limit genetic diversity in microsatellites is likely to be limiting at other, potentially adaptive, loci (Wenzel *et al.*, 2012).

Materials and methods

Study system

Red-winged blackbirds are a widespread species in North America, occurring from Canada to Costa Rica on the continent (Yasukawa & Searcy, 1995). In the Caribbean, blackbirds are known to occur in the Bahamas, with a large population on Grand Bahama Island (Jaramillo & Burke, 1999). Red-winged blackbirds are socially polygynous, with males establishing harems of typically one to four females (Orians, 1969), but are genetically polyandrous (Bray *et al.*, 1975; Westneat, 1993b; Searcy & Yasukawa, 1995). Females frequently raise broods (continental mean: 3.3 eggs, Yasukawa & Searcy, 1995; Bahamas mean: 2.7 eggs, I. A. Liu, personal observation) of mixed paternity, and EPP occurs despite male countermeasures such as repeated copulation and mate guarding (Westneat, 1993a, 1994).

Sampling

We analysed data from seven continental populations and one island population of red-winged blackbirds (Table 1). This data set comprised all populations for which red-winged blackbird EPP estimates were available, plus three new study populations. For three

populations, we used published EPP rates (New York, Westneat, 1993b; Washington, Gray, 1996; Kentucky, Westneat & Mays, 2005). For two other populations, we used EPP rates from subsets of data shared by researchers (Ontario, P. Weatherhead, personal communication; Wisconsin, K. Yasukawa, unpublished). For the remaining three populations (Pennsylvania, Michigan, Bahamas), we report the first estimates of EPP. Michigan samples were collected at Michigan State University's Kellogg Biological Station in south-western Michigan in 2004 and 2005. Pennsylvania samples were collected at Conneaut Marsh in north-west Pennsylvania in 2009. Lastly, Bahamas samples were collected in 2009 and 2011 at sites around Freeport, Grand Bahama Island (90 km east of Florida). The Bahamas population is thought to be resident (Jaramillo & Burke, 1999) and philopatric, with banded individuals observed at the same sites across breeding seasons (I. A. Liu, personal observation).

Most blood samples for genetic diversity were collected at the same time that EPP was measured. The exceptions were samples from Ontario, Washington and Wisconsin (Table 1). Although it is ideal to measure EPP and genetic diversity from the same samples to avoid potential confounding results from temporal variation (Petrie & Kempenaers, 1998), the constraints of seasonal field collection required us to use available EPP data from past studies.

Adults were captured using mist nets, grain-baited walk-in traps or walk-in traps placed over nests, and then bled from the brachial vein using sterile 26G × ½ in. BD PrecisionGlide needles. Blood was collected in capillary tubes or onto Whatman FTA bloodstain cards treated with 1 M EDTA. Adults were colour-banded for individual identification. Chicks were bled between 0 and 7 days post-hatch from the tarsal or brachial vein.

Territory owners were assigned by behaviours such as location of song perches and defence against intruders. Females in each harem were identified by their association with the territorial male. Occasionally, territory assignments of females and chicks were unclear, especially when nests of females were on territory boundaries and were defended by multiple males when approached. Because determination of EPP hinges on reliable identification of the social male, these individuals were discarded from our analysis.

DNA extraction and amplification

DNA from samples collected before 2000 were extracted using the methods reported in their respective studies (Table 1; Yasukawa *et al.*, 2009). DNA from samples collected after 2000 was extracted with a Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) and evaluated for concentration and purity using a Nanodrop spectrophotometer. Samples with poor concentrations (< 4.0 ng μL^{-1}) were re-extracted.

All DNA samples were genotyped at 10 microsatellite loci. We amplified loci that were already known to be polymorphic in red-winged blackbirds or polymorphic in other species and successfully tested in red-winged blackbirds (Table S1). For each individual, we ran three multiplex PCRs, the first two containing four primer pairs and the third containing two primer pairs. The forward primer in each pair was fluorescently labelled with 6-FAM, HEX (Sigma-Aldrich, St. Louis, MO, USA) or NED (Applied Biosystems, Waltham, MA, USA). Reactions consisted of 2.0 μL of DNA, 3.0 μL of Qiagen Type-It Multiplex PCR Master Mix, 1.6 μL RNAse-free water and 1.0 μL of 100 μM primer mix. PCR cycles were initiated at 95 °C for 5 min to activate the HotStarTaq Plus DNA polymerase, followed by 10 touchdown annealing cycles from 60 to 50 °C and 28 additional cycles at 50 °C. Each cycle consisted of denaturation at 95 °C for 30 s, annealing for 90 s and extension at 72 °C for 30 s. The final extension was at 68 °C for 10 min.

Plates were processed at the Duke Sequencing Facility using Applied Biosystem 3730xl DNA Analyzers, and genotypes were scored with GeneMarker v.1.8

Table 1 Summary of samples and populations used in the study.

Country/state/province	Site	Coordinates (decimal degrees)	Year samples collected for EPP analysis	Year samples collected for genetic diversity analysis	Reference for EPP
Bahamas	Grand Bahama Island	26.526, −78.751	2011	2009, 2011	Present study
Kentucky	Muhlenberg County	37.241, −87.047	1994–1997	1996	Westneat & Mays (2005)
Michigan	Kellogg Biological Station	42.410, −85.393	2004–2005	2004–2005	Present study
New York	Cornell University Experimental Ponds	42.504, −76.465	1988–1989	1991	Westneat (1993b)
Pennsylvania	Conneaut Marsh	41.591, −80.265	2009	2009	Present study
Ontario	Queen's University Biological Station	44.524, −76.374	1987–1989	2011	P. Weatherhead, personal communication
Washington	Columbia National Wildlife Refuge	46.913, −119.227	1990–1992	2010	Gray (1996)
Wisconsin	Newark Road Prairie	42.542, −89.141	1992	2009–2010	Present study

(SoftGenetics, State College, PA, USA) using size standard GS-500 to determine allele sizes. Homozygous loci were genotyped at least twice to account for the possibility of allelic dropout. We were unable to use MicroChecker (Van Oosterhout *et al.*, 2004) to scan for null alleles or dropout, because many loci had irregular alleles outside the base-pair lengths expected from the motif. These genotypes were verified with multiple (up to five) runs as genuine alleles and not artefacts of pull-up or stutter.

Calculation and comparison of genetic diversity

For the adults in each population (range = 13 in Ontario to 66 in the Bahamas), we calculated the mean raw number of alleles, effective number of alleles, observed and expected heterozygosity, Shannon diversity index and the inbreeding coefficient (F_{IS}) using GenAlEx v.6.501 (Peakall & Smouse, 2006, 2012). We then used the jackmsatpop function of the R package PopGenKit v.1.0 (Paquette, 2012) to generate a rarefaction curve of mean raw allelic diversity. This function measures the number of sampled alleles for a given constant increase in sample size for each population. Although measurements are not predictive past the sample size, the results can indicate whether sampling was sufficient to capture population allelic diversity. For each population, we ran 100 repetitions using a stepwise increase of one individual up to that population's sample size of adults. Analyses were performed in R v.3.1.1 (R Core Team, 2014).

To test the hypothesis that the Bahamas population had lower genetic diversity than the continental populations, we used the bootstrap to obtain estimates of uncertainty in three measures of diversity: population allelic diversity, the Shannon diversity index and expected heterozygosity for each of the eight populations. We used custom R scripts to take 1000 bootstrap resamples of size 9, 10 and 11 individuals from each population, across all populations, and calculate the three measures of genetic diversity for each resample. These resample sizes were chosen so that estimates of uncertainty could be generated for Ontario, which had the smallest sample size of 13 adults, thus requiring a bootstrap resample size of < 13 to obtain estimates of uncertainty. We chose three different sample sizes to ensure that the conclusion was robust to the choice of bootstrap sample size. We then excluded Ontario from the data set. The next smallest sample size was Wisconsin with 22 individuals; thus, removing Ontario allowed us to take larger bootstrap samples and thereby obtain more precise estimates of uncertainty in the genetic diversity measurements for the remaining seven populations. We chose bootstrap resample sizes of 15, 16 and 17 individuals, again to ensure that the conclusion was robust to the choice of bootstrap resample size. From the simulations, we computed the *P*-value as the proportion of times that the measures for the Bahamas population were higher than those of any of the continental populations.

Population structure

We conducted a cluster analysis with the microsatellite data using STRUCTURE v.2.3.3 (Pritchard *et al.*, 2000). For *K* clusters from 1 to 8, 10 replicate runs were performed, each with a 100 000 generation burn-in followed by 1 000 000 generations. We then constrained the number of populations to two, generated a single *Q*-matrix for $K = 2$ and used CLUMPP v.1.2.2 (Jakobsson & Rosenberg, 2007) to summarize and align clusters. The same steps were repeated for $K = 3$. Cluster assignment and admixture were visualized with R scripts, one of which calculated delta-*K* to evaluate the fit of each *K*-value (Evanno *et al.*, 2005; M. G. Johnson, personal communication).

To assess divergence between the continental and Bahamas populations, we sequenced the mitochondrial ND2 region for 14 continental birds (two from each of the seven study populations) and 14 Bahamas birds. Because of its length, the gene was split into two pieces and amplified with two primer sets, L5216-H5766 and L5758-H6313 (Sorenson, 2003). Each primer pair amplified a ~500-bp fragment. For the initial PCR, reactions consisted of 2.0 μL of DNA, 8.9 μL of distilled water, 2.0 μL of 10× buffer, 3.2 μL of dNTPs, 1.0 μL each of 10 μM forward and reverse primer, 1.5 μL of bovine serum albumin (BSA) and 0.4 μL of Taq (Denville Scientific, South Plainfield, NJ, USA). PCR cycles were initiated at 95 °C for 5 min, followed by 12 touchdown annealing cycles from 58 to 52 °C. Poor results for the second primer pair (L5758-H6313) were repeated using touchdown cycles from 60 to 54 °C. Touchdown cycles were followed by 28 additional cycles at 52 °C (or 54 °C). Each cycle consisted of denaturation at 95 °C for 0:30, annealing for 30 s and extension at 72 °C for 60 s. The final extension was at 72 °C for 7 min.

Gels were run after each reaction to verify successful amplification. The DNA template was then purified with ExoSAP. To each template, we added 2.6 μL of distilled water, 0.2 μL of exonuclease I (ExoI) and 0.2 μL of shrimp alkaline phosphatase (SAP). The reaction was initiated at 37 °C for 30 min, followed by 80 °C at 15 min to deactivate ExoI. Plates were processed by Eton Bioscience and edited in Sequencher (Gene Codes, Ann Arbor, MI, USA).

Sequences from each primer pair were aligned in MEGA v.5.2 (Tamura *et al.*, 2011), trimmed with PhyDE v.0.9971 (Müller *et al.*, 2010) and merged using a custom Python script. There was no overlap between the two sequences, indicating a middle portion of the gene was unsequenced and the reading frame may have differed across sequences. Because the goal was to align

sequences and not to analyse coding regions, potential frame shifts were not an issue. The complete NEXUS file was imported to PAUP v.4.0a129 (Swofford, 2003) for the construction of a neighbour-joining tree. We also ran a 1000-replicate bootstrap analysis to calculate majority-rule consensus values and determine which clusters were statistically distinct. Finally, we calculated mean between-group distances for the continental vs. Bahamas individuals in MEGA.

Calculation and comparison of EPP rates

Extra-pair paternity in previously studied populations was measured using microsatellites or DNA fingerprinting (Westneat, 1993b; Weatherhead & Boag, 1995; Gray, 1996; Westneat & Mays, 2005; Yasukawa *et al.*, 2009), whereas EPP in the newly studied populations (Pennsylvania, Michigan, and the Bahamas) was measured using microsatellites. An exclusion analysis on GenAlEx for these populations showed that, with both parents' genotypes available, the probability of paternity exclusion reached 100% with four loci for Pennsylvania and Michigan and eight loci for the Bahamas. Therefore, we used six loci for the Pennsylvania and Michigan populations and eight loci for the Bahamas population. The use of two to four additional markers to measure genetic diversity reduced the bias towards finding heterozygosity when there is complete overlap in markers to detect both paternity and heterozygosity (Wetzel & Westneat, 2009).

Extra-pair young (EPY) were defined as chicks whose genotypes were incongruous with the social father's at a minimum of two loci. Single-locus incongruencies were ascribed to allelic dropout or single-locus mutations (Westneat & Mays, 2005). There were occasional mismatches with the maternal genotypes, but they did not affect diagnoses of EPY and were almost all due to allelic dropout in the nestling genotypes. We measured the frequency of EPP by calculating two proportions: the number of EPY out of the total number of chicks and the number of nests containing at least one EPY out of the total number of nests.

We used generalized linear models (GLMs) to assess how the likelihood of being an EPY or a nest with EPY varied across populations. Binary logistic regression models were appropriate because of the response variables (i.e. whether offspring were EPY or whether a nest contained EPY) and because they were more robust than simple tests of proportion to sample size heterogeneity (range = 56 chicks across 20 nests in Bahamas to 1479 chicks across 537 nests in Kentucky). The simplest GLM (Model 1) was a logistic regression with a single intercept μ, the log odds that a chick was an EPY or a nest contained an EPY [i.e. $\Pr(EP_i = 1) = e^{\mu}/1 + e^{\mu}$]. Obtaining a maximum-likelihood estimate of μ is the equivalent of performing an ANOVA for a binary response with only a grand mean.

Next, we added population as a predictor variable, allowing the intercepts to differ across populations (Model 2). This model was the equivalent of a one-way ANOVA with a binary response, producing eight values of μ [i.e. $\Pr(EP_i = 1|x_i) = e^{\mu_{x_i}}/1 + e^{\mu_{x_i}}$] instead of a global intercept. To test whether adding population as a factor improved our model, we performed an analysis of deviance in which we calculated the difference in the deviance of the two models using a chi-squared test (analogous to calculating an *F*-statistic for linear regression model fit).

We also tested whether the EPP rate of the Bahamas population was significantly different from the average EPP rate across all continental populations (Model 3). This regression was estimated by pooling the continental populations into a single group and testing them against the Bahamas population. We again ran a comparison against Model 1 to test whether adding this 'island vs. continental' term improved the model.

From the models testing the likelihood of being an EPY, we found that the log odds differed significantly across populations and that the Bahamas EPP rate was not significantly different from the continental average rate (see Results). However, the latter conclusion was based on a model (Model 3, which pooled continental populations) that would be rejected relative to the model supporting the former conclusion (Model 2, which reported significant variation across populations). Therefore, we conducted an additional Bayesian analysis with a random-effects model, allowing for both different intercepts for all the populations and a central measure of the EPP rate for the continental population, given by the random-effects mean (Data S1). We then compared results from treating the continental populations as fixed vs. random effects.

Relationship between genetic diversity and EPP rate

From the bootstrap simulations, we obtained sample-size-adjusted estimates of mean (± SD) allelic diversity for each population. Using the estimates from the simulations with 11 individuals – the largest sample size used that included Ontario among the populations – we tested for a correlation between population allelic diversity and EPP rate, defined as proportion of EPY (Table 4).

Results

Calculation and comparison of genetic diversity

Measurements of genetic diversity are summarized in Table 2. Within individual loci, the Bahamas population had the fewest alleles at every locus except one (Ap107, data not shown), with a mean raw allelic diversity of 10.6 alleles (Fig. 1a; see Table 2 for values). This maximum was lower than for any other population,

Table 2 Mean sample size (N), raw (N_a) and effective (N_e) number of alleles, Shannon diversity index (I), observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficient (F_{IS}) for 10 loci in eight populations of red-winged blackbirds. Numbers in parentheses are standard error.

Site	N	N_a	N_e	I	H_o	H_e	F_{IS}
Bahamas	66	10.60 (2.78)	5.80 (1.45)	1.71 (0.24)	0.72 (0.05)	0.73 (0.05)	0.005 (0.014)
KY	32	16.10 (2.31)	9.17 (1.55)	2.31 (0.18)	0.83 (0.03)	0.85 (0.03)	0.012 (0.018)
MI	51	17.80 (2.71)	9.65 (1.66)	2.36 (0.19)	0.81 (0.03)	0.85 (0.03)	0.050 (0.019)
NY	31	15.30 (1.88)	8.90 (1.45)	2.30 (0.16)	0.82 (0.03)	0.85 (0.02)	0.043 (0.026)
Ontario	13	10.80 (1.09)	7.06 (0.97)	2.07 (0.14)	0.84 (0.04)	0.83 (0.03)	−0.015 (0.032)
PA	60	19.10 (2.85)	9.84 (1.65)	2.41 (0.17)	0.86 (0.02)	0.87 (0.02)	0.009 (0.015)
WA	31	13.30 (2.01)	8.21 (1.46)	2.17 (0.18)	0.81 (0.03)	0.83 (0.03)	0.030 (0.016)
WI	22	14.20 (1.93)	9.47 (1.52)	2.30 (0.17)	0.86 (0.04)	0.86 (0.03)	−0.005 (0.028)

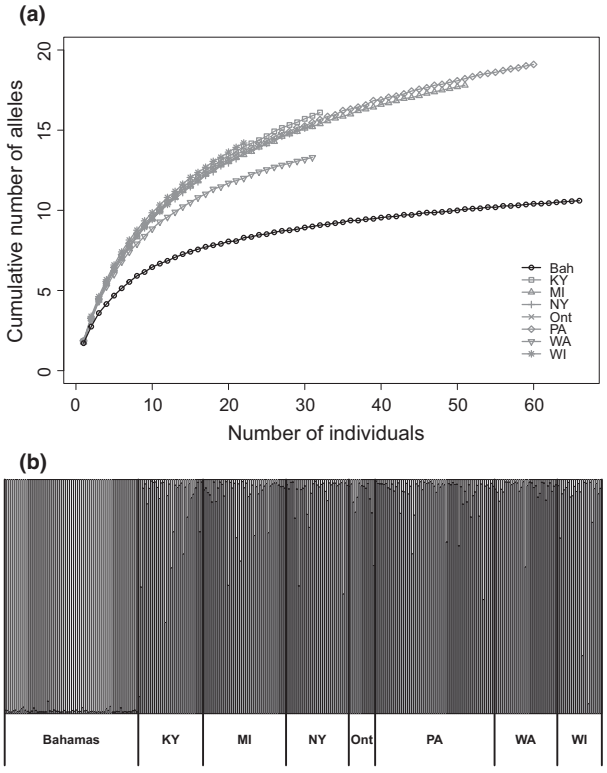

Fig. 1 (a) Rarefaction curve for mean raw allelic diversity, showing the Bahamas population (black) approaches a lower maximum than the continental populations (grey). (b) STRUCTURE barplot showing the presence of population structure as measured from ten microsatellites for $K = 2$ clusters. Each bar represents an individual. Shading represents proportion of membership in either cluster.

including Ontario. At 13 samples, the Ontario population did not approach a plateau, but it followed the same trajectory and likely had comparable allelic diversity to the other six continental populations (mean = 16.0 ± 0.9 alleles). The bootstrap found that the Bahamas population had significantly lower allelic diversity, Shannon diversity index and expected heterozygosity (Table 3). However, the Bahamas population was not significantly more inbred than the continental populations (Table 2; ANOVA on all populations, $F_{1,7} = 1.44$, $P = 0.19$).

Table 3 Outcomes of bootstrap simulation testing whether the Bahamas has lower genetic diversity (measured by three different variables) than the continental populations. 'Proportion' refers to the proportion out of 1000 trials that the above statement was true. Simulation-derived P-values are 1 minus the bootstrap proportions. Simulations with resample sizes of 15, 16 and 17 omit the Ontario population. The Bahamas population was found to have significantly lower genetic diversity across all metrics than the continental populations.

	Allelic diversity		Expected heterozygosity		Shannon diversity index	
N	Proportion	P	Proportion	P	Proportion	P
9	0.953	0.047	0.994	0.006	0.988	0.012
10	0.959	0.041	0.993	0.007	0.992	0.008
11	0.960	0.040	0.995	0.005	0.990	0.010
15	0.999	0.001	1	< 0.001	1	< 0.001
16	1	< 0.001	1	< 0.001	1	< 0.001
17	1	< 0.001	1	< 0.001	1	< 0.001

Population structure

For microsatellite analysis, the delta-K script determined the optimal K-value to be 2 (Fig. S1). Cluster assignment at $K = 2$ showed the continental populations were essentially a single population that differentiated strongly from the Bahamas population (Fig. 1b). At $K = 3$, the Washington population emerged as a separate cluster from the other continental populations, but the Bahamas population remained distinct (Fig. S2).

For mtDNA analysis, trimmed and concatenated ND2 sequences were 1119 bp long. Seven of 18 polymorphic

sites were parsimony-informative. Out of those seven sites, three were fixed differences between Bahamas and continental individuals, giving a between-group distance of 0.004. This result does not give temporal context to the island-continental divergence, as it could potentially arise from founder effects following the arrival of any individual harbouring three singletons. However, it is consistent with the signal from the nuclear DNA that gene flow has been absent between the two groups, especially considering the slower evolutionary rate of mtDNA sequences relative to microsatellites (e.g. Brohede *et al.*, 2004).

The neighbour-joining tree showed that the 14 Bahamas individuals clustered to form a polytomy with short branches, indicating few overall mutations across individuals (Fig. 2). The 14 individuals across the seven continental populations also formed their own polytomy, but varying branch lengths in the continental group indicated greater nucleotide diversity within the continental population than within the Bahamas. Additionally, inconsistent sorting of the individuals from Michigan, Washington and New York reflects insufficient population-level resolution and supports the view that continental individuals belong to the same genetic population (Ball *et al.*, 1988).

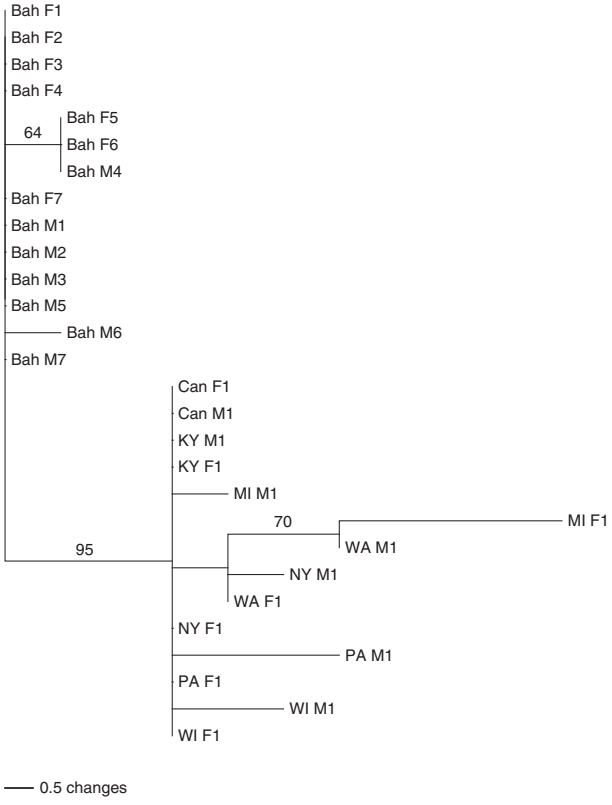

Fig. 2 Neighbour-joining tree showing relationships between the eight populations using the ND2 gene in mtDNA. 'F' = female, 'M' = male. Majority-rule consensus values over 60% are included.

Calculation and comparison of EPP rates

Table 4 shows the measures of EPP in each population, defined first as the proportion of nestlings that were genotyped as EPY and then as the proportion of nests containing at least one EPY.

In testing the likelihood that a nestling was EPY, Model 1 estimated μ to be -0.62 ± 0.04 ($Z = -15.5$, $P < 0.001$), indicating that the overall probability of being an EPY was $e^{-0.62}/1 + e^{-0.62}$, or 0.35, and that this probability was significantly different from 0.5. Model 2, adding population as a predictor variable, found no difference between the odds of being an EPY in the Bahamas population vs. any of the continental populations (Table 5a) when the tests were conducted one at a time. However, this approach did not control for multiple comparisons, and thus, we also compared Model 2 to Model 1 using analysis of deviance. In this test, we rejected the null hypothesis that the odds of being an EPY were the same across all populations ($\chi^2_7 = 48.54$, $P < 0.001$).

This analysis of deviance indicated that significant variation in EPY existed across populations. To test whether the probability of being EPY in the Bahamas differed from the overall probability of being EPY in the continental populations, we estimated Model 3, which showed no difference between the odds of being an EPY in the Bahamas population vs. the pooled continental populations (Table 5b). In an analysis of deviance between Model 1 and Model 3, we failed to reject the null hypothesis that the odds of being EPY were the same in the continental and Bahamas populations ($\chi^2_1 = 1.04$, $P = 0.31$).

The Bayesian random-effects model confirmed the conclusions of Model 3 while also revealing differences between the fixed- and random-effects models. The

Table 4 Rates of extra-pair paternity in each population, measured by number of extra-pair young (EPY); number of within-pair young (WPY); and number of nests with and without EPY. Asterisks indicate the data were reprinted from published measures of EPP (see Table 1 for references).

Population	EPY	WPY	Prop. EPY	Nests with EPY	Nests with no EPY	Prop. nests with EPY
Bahamas	16	40	0.29	10	10	0.50
KY*	593	886	0.40	295	242	0.55
MI	32	93	0.26	20	20	0.50
NY*	55	177	0.24	28	40	0.41
Ontario	64	179	0.26	30	48	0.38
PA	23	64	0.26	13	14	0.48
WA*	136	267	0.34	72	62	0.54
WI	31	66	0.32	20	12	0.62

Table 5 (a) Summary of output for Model 2, the GLM incorporating population as a variable, testing for variation in the log odds that a chick is EPY. This model was found to be a significantly better fit than Model 1 with only the intercept. For the Bahamas population, 'estimate' indicates the maximum-likelihood estimation (MLE) of μ, the log odds. For all other populations, this value is the MLE of the difference in log odds relative to the reference Bahamas population. SE is standard error, while the z-score is the number of standard deviations away from the mean. The *P*-value for the Bahamas population indicates the estimate is significantly different from 0, whereas the *P*-values for the other populations indicate the estimated differences from the Bahamas intercept are not significant. (b) Summary of output for Model 3, the GLM comparing the log odds of being an EPY, between the Bahamas and the pooled continental populations. The Bahamas does not have a significantly different probability from the continental populations.

	Estimate	SE	z	*P*
(a)				
Bahamas	−0.92	0.30	−3.10	0.0020
KY	0.51	0.30	1.71	0.087
MI	−0.15	0.36	−0.42	0.68
NY	−0.25	0.33	−0.76	0.45
Ontario	−0.11	0.33	−0.34	0.73
PA	−0.11	0.38	−0.28	0.78
WA	0.24	0.31	0.77	0.44
WI	0.16	0.37	0.44	0.66
(b)				
Continental	−0.62	0.04	−15.21	0.0000
Bahamas	−0.30	0.30	−1.00	0.32

fixed-effects models (Models 2 and 3) and the random-effects model yielded similar estimates for population-specific intercepts (Fig. 3, panels a–h) but different estimates for the mean continental intercept (Fig. 3, panel i). In the latter case, the random-effects model controlled for heterogeneity in sample size to estimate the continental random-effects mean. Conversely, Model 3's single parameter controlling EPY for all continental populations was heavily influenced by Kentucky's large sample size and relatively higher proportion of EPY (40%). The difference between the random-effects mean in the Bayesian hierarchical model and the continental parameter in Model 3 suggests that treating the continental populations' EPY rates as distinct but dependent yields qualitatively different conclusions about the overall continental EPY rate than when considering them as a single population. Nonetheless, the Bayesian model supported the conclusion that the odds of being EPY in the Bahamas did not differ from the odds of being EPY in the continental populations (posterior probability that $\mu_{\text{Continental}} > \mu_{\text{Bahamas}} = 0.54$). This result is supported visually by the similarity between the estimated Bahamas intercept (from either the random- or fixed-effects model) and the estimated continental random-effects mean (Fig. 3).

In testing the likelihood that a nest contained EPY, Model 1 estimated μ to be 0.08 ± 0.06, indicating that the overall probability of a nest containing EPY is $e^{0.08}/1 + e^{0.08}$, or 0.52. This value was not significantly different from 0.5 ($Z = 1.31$, $P = 0.19$). Model 2 was not a significantly better fit than Model 1 ($\chi^2_7 = 12.66$, $P = 0.08$), suggesting that, unlike the likelihood of being an EPY, the likelihood of being a nest with EPY does not vary significantly across populations. Similar to the results above, a model separating the Bahamas from the pooled continental populations was not a better fit than the model with only the intercept (Model 3 vs. Model 1, $\chi^2_1 = 0.037$, $P = 0.85$). Model outputs are shown in Table S2. Overall, the probability that a nest contained EPY appeared relatively uniform for all populations, possibly due to the lower resolution of comparing whole broods instead of individual chicks.

Relationship between genetic diversity and EPP rate

Plots of the proportion of EPY against sample-size-adjusted estimates of mean allelic diversity for each population showed that, despite its lower genetic diversity, the Bahamas population had similar proportions of EPY as the continental populations (Fig. 4). A Pearson's correlation test found no relationship between genetic diversity and the EPP rate ($r = 0.05$, $P = 0.90$).

Discussion

We found that population genetic diversity does not predict EPP rate in red-winged blackbirds. We took advantage of the species' North American distribution, including its occurrence on an island, to predict that continental populations with high genetic diversity would have higher levels of EPP than the island population with low genetic diversity. However, the EPP rate of the island population was not significantly different from those of the continental populations, nor did genetic diversity account for the significant variation in EPP rate (defined as the number of EPY) across the continental populations (Fig. 4). These results raise questions of why genetically distinct populations had similar levels of EPP, and why the continental populations showed significant variation in EPP rates despite being genetically undifferentiated.

No difference between EPP in island and continental populations

Our results contrast with studies reporting lower rates of EPP in island vs. continental populations (Griffith *et al.*, 1999; Griffith, 2000) and studies reporting positive correlations between genetic diversity and EPP rate (Petrie *et al.*, 1998; Gohli *et al.*, 2013). Conversely, they are concordant with studies where island and continental populations had similar rates of EPP (Fridolfsson

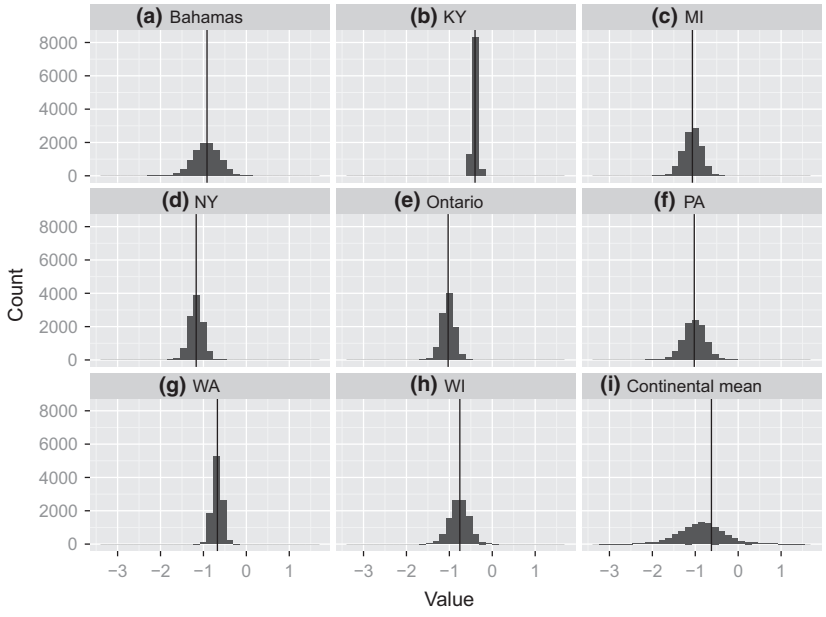

Fig. 3 Distribution of posterior samples for the eight population-specific intercepts (panels a–h) and the continental random-effects mean (panel i). The histograms for each population are overlaid with vertical lines indicating the maximum-likelihood estimates for μ from the fixed-effects model for EPY (Model 2; see Table 5a), whereas the histogram for the continental random-effects mean is overlaid with the estimate for the single pooled continental parameter μ from Model 3.

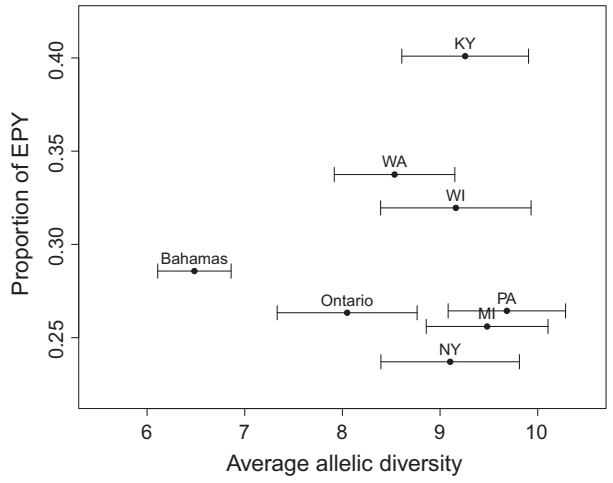

Fig. 4 Relationship between average genetic diversity (measured as number of alleles) and proportion of EPY. The Bahamas population has less genetic diversity than the continental populations yet has an intermediate proportion of EPY.

et al., 1997; Krokene & Lifjeld, 2000; Conrad *et al.*, 2001; Charmantier & Blondel, 2003), including one in which the island population – a closely related species to the mainland population – was known to have lower genetic diversity (García del Rey *et al.*, 2012).

Methodological differences may explain some of the discrepancies between studies. For example, Gohli *et al.* (2013) and Petrie *et al.* (1998)'s use of interspecific data covered a broad taxonomic scale but did not account for the often high intraspecific variation in both variables (Garamszegi & Møller, 2010; Spurgin, 2013). Additionally, Griffith's (2000) meta-analysis included early studies that inferred paternity using low numbers of loci, including single-locus minisatellite and microsatellite markers. Where too few loci are used in low-diversity populations, the limited combinations of multilocus genotypes will bias against the correct identification of EPY (Wetzel & Westneat, 2009). Finally, our use of microsatellite loci could have failed to capture measures of genetic diversity that directly relate to the indirect benefits hypotheses of EPP. We recognize that neutral markers are unlikely to be the underlying molecular targets of selection and that variation in these markers may not reflect the variation in male fitness traits thought to drive female participation in EPP (reviewed in Reid *et al.*, 2011). Nevertheless, as neutral markers, microsatellites offer an unbiased estimate of genetic diversity by reflecting the effects of drift alone (but see Li *et al.*, 2004), unlike coding regions whose evolutionary patterns require distinguishing between effects of drift and selection. Microsatellite estimates of neutral diversity additionally are concordant with estimates from other methods such as intron nucleotide diversity (Väli *et al.*, 2008). Moreover, microsatellites are robust for detecting population structure (Haasl & Payseur, 2011), increasing our confidence of differentiation between the continental and island populations. If this differentiation has led to a loss in microsatellite diversity in the Bahamas, then it is possible that diversity in other, potentially functional, loci could have decreased as well. These predictions can be tested with measurements of genetic diversity at both neutral and adaptive loci to assess the genomic or adaptive genetic variance implicated in hypotheses for EPP (Gohli *et al.*, 2013; Hartmann *et al.*, 2014).

Beyond methodology, why else might EPP rates be similar between the Bahamas and continental populations? One possibility is that the genetic diversity in the Bahamas population, although relatively low, may still have been sufficient to benefit females participating in EPP. Similar to the thresholds observed for female preferences, sexual ornaments or regulatory mechanisms (Roff, 1996; Jennions & Petrie, 1997; Emlen & Nijhout, 2000), there may be a level of genetic diversity above which EPP is adaptive and below which the costs of EPP outweigh its benefits. EPP could lose its selective advantage once genetic diversity has dropped below a certain threshold, leading to an eventual decrease in EPP not captured in this data set.

An alternative explanation is that any decline in EPP resulting from decreases in population genetic diversity may be counteracted by an increase in EPP driven by inbreeding avoidance (Tregenza & Wedell, 2000). This hypothesis is supported by our finding that although the Bahamas population had lower expected heterozygosity than the continental populations, its inbreeding coefficient (F_{IS}) was as low as that of the continental populations. Because heterozygosity erodes in the face of random mating, this pattern suggests that the Bahamas population may have engaged in disassortative mating to sustain its standing heterozygosity. In this case, the value of a rare, distinct male would increase as genetic diversity decreases, leading to a negative frequency-dependent dynamic (Knoppien, 1985; Partridge, 1988) opposite our prediction that females in a low-diversity population would not seek EPP. One caveat is that the rare-male effect may only be viable in species with mating systems featuring low costs of finding additional mates (e.g. adders, *Vipera berus*, Madsen *et al.*, 1992; flour beetles, *Tribolium castaneum*, Michalczyk *et al.*, 2011), which may not be the case for island populations exhibiting low breeding densities and potentially high search costs for females.

Our goal with the mtDNA sequence analysis was to determine whether population structure could explain potential differences in EPP rates, given that island residents may prefer assortative mating to outbreeding (Ockendon *et al.*, 2009; Bichet *et al.*, 2014). In finding population structure but no difference in EPP between the Bahamas and continental populations, we aimed to determine the time as divergence to infer the evolutionary lability of this behaviour. Although nuclear and mitochondrial DNA both gave signals of divergence, the three fixed SNPs in the mtDNA did not provide sufficient molecular resolution to reveal the time of separation. Ideally, a longer, more polymorphic region would clarify whether divergence is recent (in which case, the Bahamas' observed EPP rate could be a carryover of reproductive behaviour in the continental populations, with the potential for future differentiation) or whether it occurred long ago (in which case, EPP in the Bahamas may have remained unchanged from that of continental populations, even after the loss of gene flow).

Continental populations vary significantly in EPP rate

Because genetic diversity was not correlated with EPP, differences in EPP rate within the continental populations suggest the variation in payoffs is unrelated to population genetic profiles. In red-winged blackbirds, benefits associated with extra-pair mating appear to vary geographically, possibly as a response to local opportunities or benefits of EPP. Females in a Washington population solicit EPCs from males (Gray, 1996) and gain both indirect and direct benefits through improved fledging success and increased access to food and nest defence, respectively (Gray, 1997a,b). In a New York population, fledging success also increases slightly with the number of sires in a brood, although EPC is resisted or at least never initiated by females (Westneat, 1992). By contrast, fledging success decreases in an Ontario population, possibly through reduced nest defence by territorial males (Weatherhead *et al.*, 1994). Although behavioural variables were not standardized across studies, the diversity in reported behaviour indicates that cost–benefit calculations across populations are dynamic and cannot easily be conveyed by linear variables such as genetic diversity.

Variation in immediate payoffs could be shaped by ecological factors such as breeding density, breeding synchrony and latitude. When tested alone, hypotheses for density and synchrony have generally received weak empirical support in songbirds (Petrie & Kempenaers, 1998; Griffith *et al.*, 2002). Studies of latitude or migration as predictors of EPP in other species both implicate (Stutchbury & Morton, 2001; Spottiswoode & Møller, 2004; Bonier *et al.*, 2014; Taylor *et al.*, 2014) and discount (Albrecht *et al.*, 2013; Eikenaar *et al.*, 2013) their effects on the relative strength of sperm competition. Considering the present study, in which a sedentary low-latitude population showed no difference in EPP rate compared to several migratory temperate populations, these factors by themselves do not seem to predict EPP rate.

More likely, interactions between ecological and genetic factors may drive variation in EPP levels (Arnold & Owens, 2002; Westneat & Stewart, 2003; Arct *et al.*, 2013). An integrative model could evaluate whether the effects of genetic diversity are enhanced or diminished by ecological variables influencing resource distribution and mate availability (*sensu* Emlen & Oring, 1977) to shape the relative payoffs of EPP. In addition, an empirical comparison between an island and nearby continental population (e.g. Prather & Cruz, 2006) could decouple the ecological and genetic factors experienced by lower-latitude populations. The inclusion of a continental population with predicted high genetic

diversity, but similar latitude and ecology to the island population, would help show whether EPP is impervious to genetic, ecological or both types of factors.

Indirect benefits hypotheses for EPP revisited

Our results do not support the idea that females in populations with higher levels of genetic diversity engage in more frequent extra-pair copulations. Instead, the present study joins a growing number of studies showing no clear results in tests of hypotheses that assume EPP to be an exclusively female-driven behaviour (Kleven *et al.*, 2006; Taylor *et al.*, 2014). Alternative explanations contrasting the indirect benefits of EPP with direct costs of sexual conflict, or framing EPP as a product of indirect selection on males or genetic constraint on females, have been advanced to account for mating system variation (Westneat & Stewart, 2003; Arnqvist & Kirkpatrick, 2005; Forstmeier *et al.*, 2011, 2014). These hypotheses propose that the relative payoffs of, and thereby the strength of selection on, EPP must be determined with respect to interactions between the female, the within-pair male and the extra-pair male(s) involved. Although assessing these interactions would require more extensive field observations, the observed lack of a direct relationship between genetic diversity and EPP rate gives reason to suspect that population-wide patterns of extra-pair mating may depend on decisions beyond those of the female.

In this broader context, EPP should not be expected to exert the same costs and benefits on individuals of different species or populations. For instance, EPY of song sparrows (*Melospiza melodia*) and house sparrows have lower lifetime reproductive success than within-pair young (Sardell *et al.*, 2012; Hsu *et al.*, 2014), representing the first reports that being an EPY could have not only neutral but adverse effects on lifetime fitness. Such heterogeneity in fitness consequences highlights the need for work on individual species to verify the exact payoffs to males or females, and it may also explain why certain meta-analyses detect greater costs of multiple mating than benefits (Arnqvist & Kirkpatrick, 2005) while others estimate positive genetic benefits (Griffith, 2007; Slatyer *et al.*, 2012).

Finally, extra-pair mating may be a behaviour under phylogenetic control, with intraspecific variation due to variation in stochastic factors (Kokko & Mappes, 2013). While the idea of EPP as a species-wide trait does not account for the variation observed across the continental populations, it refers to the argument that EPP is a fundamental reproductive strategy expressed broadly across passerines. Different evolutionary explanations are valid at different scales of comparison, with variation within species likely to be determined by differences in the local ecological and genetic factors discussed above (Arnold & Owens, 2002). Future comparisons of EPP in closely related species will clarify how species are united or distinguished by their genetic mating systems.

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References

Albrecht, T., Kleven, O., Kreisinger, J., Laskemoen, T., Omotoriogun, T.C., Ottosson, U. *et al.* 2013. Sperm competition in tropical versus temperate zone birds. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **280**: 20122434.

Arct, A., Drobniak, S., Podmokła, E., Gustafson, L. & Cichoń, M. 2013. Benefits of extra-pair mating may depend on environmental conditions – an experimental study in the blue tit (*Cyanistes caeruleus*). *Behav. Ecol. Sociobiol.* **67**: 1809–1815.

Arnold, K.E. & Owens, I.P.F. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **269**: 1263–1269.

Arnqvist, G. & Kirkpatrick, M. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. *Am. Nat.* **165**: S26–S37.

Ball, R.M., Freeman, S., James, F.C., Bermingham, E. & Avise, J.C. 1988. Phylogeographic population structure of red-winged blackbirds assessed by mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* **85**: 1558–1562.

Bichet, C., Penn, D., Moodley, Y., Dunoyer, L., Cellier-Holzem, E., Belvalette, M. *et al.* 2014. Females tend to prefer genetically similar mates in an island population of house sparrows. *BMC Evol. Biol.* **14**: 47.

Bollmer, J.L., Dunn, P.O., Freeman-Gallant, C.R. & Whittingham, L.A. 2012. Social and extra-pair mating in relation to major histocompatibility complex variation in common yellowthroats. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **279**: 4778–4785.

Bonier, F., Eikenaar, C., Martin, P.R. & Moore, I.T. 2014. Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *Am. Nat.* **183**: 54–61.

Bray, O.E., Kennelly, J.J. & Guarino, J.L. 1975. Fertility of eggs produced on territories of vasectomized red-winged blackbirds. *Wilson Bull.* **87**: 187–195.

Brohede, J., Møller, A.P. & Ellegren, H. 2004. Individual variation in microsatellite mutation rate in barn swallows. *Mutat. Res.* **545**: 73–80.

Charmantier, A. & Blondel, J. 2003. A contrast in extra-pair paternity levels on mainland and island populations of Mediterranean blue tits. *Ethology* **109**: 351–363.

Conrad, K.F., Johnston, P.V., Crossman, C., Kempenaers, B., Robertson, R.J., Wheelwright, N.T. *et al.* 2001. High levels of extra-pair paternity in an isolated, low-density, island population of tree swallows (*Tachycineta bicolor*). *Mol. Ecol.* **10**: 1301–1308.

Eikenaar, C., Bonier, F., Martin, P.R. & Moore, I.T. 2013. High rates of extra-pair paternity in two equatorial populations of rufous-collared sparrow, *Zonotrichia capensis*. *J. Avian Biol.* **44**: 600–602.

Emlen, D.J. & Nijhout, H.F. 2000. The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.* **45**: 661–708.

Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science* **197**: 215–223.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H. & Kempenaers, B. 2011. Female extrapair mating behavior can evolve via indirect selection on males. *Proc. Natl. Acad. Sci. USA* **108**: 10608–10613.

Forstmeier, W., Nakagawa, S., Griffith, S.C. & Kempenaers, B. 2014. Female extra-pair mating: adaptation or genetic constraint?. *Trends Ecol. Evol.* **29**: 456–464.

Frankham, R. 1997. Do island populations have less genetic variation than mainland populations? *Heredity* **78**: 311–327.

Fridolfsson, A.-K., Gyllensten, U.B. & Jakobsson, S. 1997. Microsatellite markers for paternity testing in the willow warbler *Phylloscopus trochilus*: high frequency of extra-pair young in an island population. *Hereditas* **126**: 127–132.

Garamszegi, L.Z. & Møller, A.P. 2010. Effects of sample size and intraspecific variation in phylogenetic comparative studies: a meta-analytic review. *Biol. Rev. Camb. Philos. Soc.* **85**: 797–805.

García del Rey, E., Kleven, O. & Lifjeld, J.T. 2012. Extrapair paternity in insular African blue tits *Cyanistes teneriffae* is no less frequent than in continental Eurasian blue tits *Cyanistes caeruleus*. *Ibis* **154**: 862–867.

Gohli, J., Anmarkrud, J.A., Johnsen, A., Kleven, O., Borge, T. & Lifjeld, J.T. 2013. Female promiscuity is positively associated with neutral and selected genetic diversity in passerine birds. *Evolution* **67**: 1406–1419.

Gray, E.M. 1996. Female control of offspring paternity in a western population of red-winged blackbirds (*Agelaius phoeniceus*). *Behav. Ecol. Sociobiol.* **38**: 267–278.

Gray, E.M. 1997a. Do female red-winged blackbirds benefit genetically from seeking extra-pair copulations? *Anim. Behav.* **53**: 605–623.

Gray, E.M. 1997b. Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Anim. Behav.* **53**: 625–639.

Griffith, S.C. 2000. High fidelity on islands: a comparative study of extrapair paternity in passerine birds. *Behav. Ecol.* **11**: 265–273.

Griffith, S.C. 2007. The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. *Am. Nat.* **169**: 274–281.

Griffith, S.C., Stewart, I.R.K., Dawson, D.A., Owens, I.P.F. & Burke, T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? *Biol. J. Linn. Soc.* **68**: 303–316.

Griffith, S.C., Owens, I.P.F. & Thuman, K.A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* **11**: 2195–2212.

Haasl, R.J. & Payseur, B.A. 2011. Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. *Heredity* **106**: 158–171.

Hartmann, S.A., Schaefer, H.M. & Segelbacher, G. 2014. Genetic depletion at adaptive but not neutral loci in an endangered bird species. *Mol. Ecol.* **23**: 5712–5725.

Hsu, Y.H., Schroeder, J., Winney, I., Burke, T. & Nakagawa, S. 2014. Costly infidelity: low lifetime fitness of extra-pair offspring in a passerine bird. *Evolution* **68**: 2873–2884.

Jakobsson, M. & Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801–1806.

Jaramillo, A. & Burke, P. 1999. *New World Blackbirds: The Icterids*. Princeton University Press, Princeton, NJ.

Jennions, M.D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev. Camb. Philos. Soc.* **72**: 283–327.

Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Camb. Philos. Soc.* **75**: 21–64.

Johnsen, A., Andersen, V., Sunding, C. & Lifjeld, J.T. 2000. Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**: 296–299.

Kempenaers, B., Verheyren, G.R. & Dhondt, A.A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behav. Ecol.* **8**: 481–492.

Kleven, O., Jacobsen, F., Izadnegahdar, R., Robertson, R.J. & Lifjeld, J.T. 2006. No evidence of paternal genetic contribution to nestling cell-mediated immunity in the North American barn swallow. *Anim. Behav.* **71**: 839–845.

Knoppien, P. 1985. Rare male mating advantage: a review. *Biol. Rev. Camb. Philos. Soc.* **60**: 81–117.

Kokko, H. & Mappes, J. 2013. Multiple mating by females is a natural outcome of a null model of mate encounters. *Entomol. Exp. Appl.* **146**: 26–37.

Krokene, C. & Lifjeld, J.T. 2000. Variation in the frequency of extra-pair paternity in birds: a comparison of an island and a mainland population of blue tits. *Behaviour* **137**: 1317–1330.

Li, Y.-C., Korol, A.B., Fahima, T. & Nevo, E. 2004. Microsatellites within genes: structure, function, and evolution. *Mol. Biol. Evol.* **21**: 991–1007.

Løvlie, H., Gillingham, M.A.F., Worley, K., Pizzari, T. & Richardson, D.S. 2013. Cryptic female choice favours sperm from major histocompatibility complex-dissimilar males. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **280**: 20131296.

Madsen, T., Shine, R., Loman, J. & Hakansson, T. 1992. Why do female adders copulate so frequently? *Nature* **355**: 440–441.

Michalczyk, L., Millard, A.L., Martin, O.Y., Lumley, A.J., Emerson, B.C., Chapman, T. *et al.* 2011. Inbreeding promotes female promiscuity. *Science* **333**: 1739–1742.

Müller, J., Müller, K., Neinhuis, C. & Quandt, D. 2010. *PhyDE: Phylogenetic Data Editor*. Dresden, Germany.

Neff, B.D. & Pitcher, T.E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* **14**: 19–38.

Ockendon, N., Griffith, S.C. & Burke, T. 2009. Extrapair paternity in an insular population of house sparrows after the experimental introduction of individuals from the mainland. *Behav. Ecol.* **20**: 305–312.

Orians, G.H. 1969. On the evolution of mating systems in birds and mammals. *Am. Nat.* **103**: 589–603.

Paquette, S.R. 2012. PopGenKit: useful functions for (batch) file conversion and data resampling in microsatellite databases.

Partridge, L. 1988. The rare-male effect: what is its evolutionary significance?. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* **319**: 525–539.

Peakall, R. & Smouse, P.E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295.

Peakall, R. & Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2537–2539.

Petrie, M. & Kempenaers, B. 1998. Extra-pair paternity in birds: explaining variation between species and populations. *Trends Ecol. Evol.* **13**: 52–58.

Petrie, M. & Lipsitch, M. 1994. Avian polygyny is most likely in populations with high variability in heritable male fitness. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **256**: 275–280.

Petrie, M., Doums, C. & Møller, A.P. 1998. The degree of extra-pair paternity increases with genetic variability. *Proc. Natl. Acad. Sci. USA* **95**: 9390–9395.

Prather, J.W. & Cruz, A. 2006. Breeding biology of red-winged blackbirds in south Florida. *Southeast. Nat.* **5**: 547–554.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Pryke, S.R., Rollins, L.A. & Griffith, S.C. 2010. Females use multiple mating and genetically loaded sperm competition to target compatible genes. *Science* **329**: 964–967.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reid, J.M., Arcese, P., Sardell, R.J. & Keller, L.F. 2011. Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. *Am. Nat.* **177**: 177–187.

Roff, D.A. 1996. The evolution of threshold traits in animals. *Q. Rev. Biol.* **71**: 3–35.

Sardell, R.J., Arcese, P., Keller, L.F. & Reid, J.M. 2012. Are there indirect fitness benefits of female extra-pair reproduction? Lifetime reproductive success of within-pair and extra-pair offspring. *Am. Nat.* **179**: 779–793.

Searcy, W.A. & Yasukawa, K. 1995. *Polygyny and Sexual Selection in Red-Winged Blackbirds*. Princeton University Press, Princeton, NJ.

Sheldon, B.C., Merila, J., Qvarnstrom, A., Gustafsson, L. & Ellegren, H. 1997. Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **264**: 297–302.

Slatyer, R.A., Mautz, B.S., Backwell, P.R.Y. & Jennions, M.D. 2012. Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. *Biol. Rev. Camb. Philos. Soc.* **87**: 1–33.

Sorenson, M.D. 2003. Avian mtDNA primers. http://people.bu.edu/msoren/Bird.mt.Primers.pdf.

Spottiswoode, C. & Møller, A.P. 2004. Extrapair paternity, migration, and breeding synchrony in birds. *Behav. Ecol.* **15**: 41–57.

Spurgin, L.G. 2013. Comment on Gohli et al. (2013): "Does promiscuity explain differences in levels of genetic diversity across passerine birds?". *Evolution* **67**: 3071–3072.

Stutchbury, B.J. & Morton, E.S. 2001. *Behavioral Ecology of Tropical Birds*. Academic Press, San Diego, CA.

Swofford, D.L. 2003. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, MA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739.

Taylor, M.L., Price, T.A.R. & Wedell, N. 2014. Polyandry in nature: a global analysis. *Trends Ecol. Evol.* **29**: 376–383.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* **9**: 1013–1027.

Valera, F., Hoi, H. & Kristin, A. 2003. Male shrikes punish unfaithful females. *Behav. Ecol.* **14**: 403–408.

Väli, Ü., Einarsson, A., Waits, L. & Ellegren, H. 2008. To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? *Mol. Ecol.* **17**: 3808–3817.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**: 535–538.

Weatherhead, P.J. & Boag, P.T. 1995. Pair and extra-pair mating success relative to male quality in red-winged blackbirds. *Behav. Ecol. Sociobiol.* **37**: 81–91.

Weatherhead, P.J., Montgomerie, R., Gibbs, H.L. & Boag, P.T. 1994. The cost of extra-pair fertilizations to female red-winged blackbirds. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **258**: 315–320.

Wenzel, M.A., Webster, L.M.I., Blanco, G., Burgess, M.D., Kerbiriou, C., Segelbacher, G. *et al.* 2012. Pronounced genetic structure and low genetic diversity in European red-billed chough (*Pyrrhocorax pyrrhocorax*) populations. *Conserv. Genet.* **13**: 1213–1230.

Westneat, D.F. 1992. Do female red-winged blackbirds engage in a mixed mating strategy? *Ethology* **92**: 7–28.

Westneat, D.F. 1993a. Temporal patterns of within-pair copulations, male mate-guarding, and extra-pair events in eastern red-winged blackbirds (*Agelaius phoeniceus*). *Behaviour* **124**: 267–290.

Westneat, D.F. 1993b. Polygyny and extrapair fertilizations in eastern red-winged blackbirds (*Agelaius phoeniceus*). *Behav. Ecol.* **4**: 49–60.

Westneat, D.F. 1994. To guard mates or go forage – conflicting demands affect the paternity of male red-winged blackbirds. *Am. Nat.* **144**: 343–354.

Westneat, D.F. & Mays, H.L. 2005. Tests of spatial and temporal factors influencing extra-pair paternity in red-winged blackbirds. *Mol. Ecol.* **14**: 2155–2167.

Westneat, D.F. & Rambo, T.B. 2000. Copulation exposes female red-winged blackbirds to bacteria in male semen. *J. Avian Biol.* **31**: 1–7.
Westneat, D.F. & Stewart, I.R.K. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. *Annu. Rev. Ecol. Evol. Syst.* **34**: 365–396.
Wetzel, D.P. & Westneat, D.F. 2009. Heterozygosity and extra-pair paternity: biased tests result from the use of shared markers. *Mol. Ecol.* **18**: 2010–2021.
Wilk, T., Cichon, M. & Wolff, K. 2008. Lack of evidence for improved immune response of extra-pair nestlings in collared flycatcher *Ficedula albicollis*. *J. Avian Biol.* **39**: 546–552.
Yasukawa, K. 2013. Effects of age and experience on the responses of territorial and floater male red-winged blackbirds to models of receptive females. *J. Field Ornithol.* **84**: 377–388.
Yasukawa, K. & Searcy, W.A. 1995. Red-winged blackbird (*Agelaius phoeniceus*). In: *The Birds of North America Online* (A. Poole, ed.), pp. 1–28. Cornell Lab of Ornithology, Ithaca, NY.
Yasukawa, K., Enstrom, D.A., Parker, P.G. & Jones, T.C. 2009. Epaulet color and sexual selection in the red-winged blackbird: a field experiment. *Condor* **111**: 740–751.

Supporting information

Additional Supporting Information may be found in the online version of this article:
Data S1 Methods.
Table S1 Primers used in the study.
Table S2 (a) Summary of output for Model 2, the GLM incorporating population as a variable, testing for variation in the log odds that a nest contains EPY. This model was not found to be a significantly better fit than Model 1 with only the intercept. SE is standard error, while the z-score is the number of standard deviations away from the mean. (b) Summary of output for Model 3, the GLM comparing the log odds that a nest contains EPY, between the Bahamas and the pooled continental populations. The Bahamas does not have a significantly different probability from the continental populations.
Figure S1 Likelihood plot from delta-K script showing that $K = 2$ is the optimum cluster number for this data set.
Figure S2 STRUCTURE barplot with $K = 3$ clusters.

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