Sexual selection and reproductive strategies in songbirds

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Chapter 7

Opposing effects of male heterozygosity on female promiscuity in the winter wren

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ABSTRACT

Females in many socially monogamous avian species engage in extra-pair copulations (EPCs) which lead to extra-pair fertilizations (EPFs). A leading explanation put forward to explain this is that females engage in EPCs to increase the genetic quality of their offspring. One process through which this may occur is for females to seek more EPFs when paired with less genetically diverse (measured by standardized heterozygosity, internal relatedness or d^2) males. We examined the occurrence of EPFs in the winter wren (*Troglodytes troglodytes*), and tested the hypothesis that female promiscuity will be higher when paired to a less genetically diverse male. We genotyped wren families at five microsatellite loci over four years in The Netherlands. We detected extra-pair young (EPY) at eleven of 29 nests (37.9%), accounting for 25 of 134 typed offspring (18.7%). Our study provided two novel lines of evidence that male heterozygosity influenced the rate of extra-pair paternity in their own nests. Surprisingly, we found that more heterozygous males were more likely to have EPY in their brood than less heterozygous males. However, once cuckolded, more heterozygous males had fewer EPY than less heterozygous males. Moreover, males with larger d^2 (size difference between alleles) had higher body condition, although we found no associations between male morphology and paternity loss. We did not find a difference in the genetic diversity of within-pair and extra-pair offspring, but we had no information on the genetic diversity of extra-pair sires. We discuss the implications of these results for genetic hypotheses for female mate choice.

INTRODUCTION

In many bird species, females copulate with males other than their social mate. Recent advances in genetic techniques have revealed that such extra-pair copulations (EPCs) often result in extra-pair fertilizations (EPFs; e.g. Westneat *et al.* 1990; Birkhead & Møller 1992a; Griffith *et al.* 2002). In most species, females appear to play an active role in obtaining EPCs (e.g. Birkhead & Møller 1993; Kempenaers & Dhondt 1993). However, despite much intensive research, the selective forces behind the evolution of such female promiscuity remain largely unresolved.

Several processes have been proposed to explain the benefits of EPCs for females. In many ways these processes mirror those proposed to explain the evolution of sexually selected traits (Griffith *et al.* 2002). These explanations can be grouped broadly into two categories: (1) those which deal with direct benefits to females, and (2) those which propose that females gain indirect (genetic) benefits from EPCs (Birkhead & Møller 1992a; Griffith *et al.* 2002). Direct benefits consist of immediate benefits that can improve brood fitness, such as access to better foraging areas or more paternal care. However, to date there has been little empirical support for the direct benefits hypotheses and in most cases males do not provide additional care or other direct benefits to extra-pair females (but see Gray 1997; Hunter & Davis 1998).

The second group of hypotheses revolves around potential indirect or genetic benefits to females of mating with extra-pair males. These hypotheses suggest that females may enhance the genetic quality of their offspring through EPFs (reviewed in Griffiths *et al.* 2002). So far, two specific forms of genetic benefits have received most support from empirical studies. First, females may prefer to mate with high quality or more attractive males, thereby securing genes for quality or attractiveness for their offspring ('good genes' hypothesis; e.g. Petrie 1994; Hasselquist *et al.* 1996; Kempenaers *et al.* 1997). This hypothesis assumes that there is a uniformly 'good' genotype to be chosen. Second, females may prefer to mate with males who are less related to themselves, in order to increase offspring genetic diversity and reduce the effects of inbreeding depression ('genetic compatibility' hypothesis; Blomqvist *et al.* 2002; Tregenza & Wedell 2002; Foerster *et al.* 2003; Eimes *et al.* 2005; but see Schmoll *et al.* 2005). According to this hypothesis, the best male genetically will be relative to the female's own genotype.

A further genetic benefits hypothesis, the 'genetic diversity' hypothesis (Williams 1975; Westneat *et al.* 1990), also postulates that females seek EPFs to maximise the genetic diversity of their offspring. However, this hypothesis differs from the genetic compatibility hypothesis in not assuming that females can assess the extent of genetic similarity between themselves and males. Simply mating randomly with multiple males should increase the genetic diversity in broods (Williams 1975; Westneat *et al.* 1990; Griffith *et al.* 2002). However, Brown's (1997) heterozygosity theory of mate choice provides an extension of this idea, whereby females may prefer to mate with males with greater heterozygosity or genic diversity in order to produce more genetically diverse offspring. As such, this hypothesis combines principles of the two aforementioned hypotheses: the good genes that females seek when choosing mates may be individual genetic diversity (Brown

1997; Weatherhead *et al.* 1999). Heterozygosity and genetic diversity has been positively associated with fitness, survival and recruitment in several taxa, especially in inbred populations (Allendorf & Leary 1986; Keller & Waller 2002). Furthermore, in at least three avian species male genetic diversity is revealed by sexually selected traits including plumage coloration (Foerster *et al.* 2003) and song complexity (Marshall *et al.* 2003; Seddon *et al.* 2004).

Although the genetic diversity hypothesis has received recent theoretical support (Brown 1997), to date there has been only limited empirical support for a link between genetic diversity on extra-pair mating in birds. In a comparative analysis of 432 bird studies, Petrie *et al.* (1998) found a positive correlation between the genetic variability of populations and the proportion of extra-pair paternity. In a study on great tits (*Parus major*), Otter *et al.* (2001) reported a negative association between male cuckoldry (i.e. loss of paternity in their own nest) and a measure of genic diversity (mean d^2) at five microsatellite loci.

Here we examine the occurrence of extra-pair parentage in the winter wren (*Troglodytes troglodytes*). Furthermore, we test a major prediction of the genetic diversity hypothesis, extended according to Brown (1997), that rates of female promiscuity will be higher when paired to less genetically diverse males (Bartos Smith *et al.* 2005). The wren is a small polygynous oscine songbird, in which the sexes are morphologically similar, although males are slightly larger. Male wrens sing regularly throughout the breeding season and vigorously defend exclusive breeding territories to which they try to attract females (Armstrong 1955). Males commonly provide care to their within-pair nestlings (Armstrong 1955; Burn 1996; pers obs). Only one previous study has investigated extra-pair paternity in a population of wrens in Oxfordshire, United Kingdom (Burn 1996). Using multilocus (minisatellite) DNA fingerprinting, Burn (1996) found that 13 of 94 offspring (13.8%) were sired by extra-pair males, and six of 19 broods (31.6%) contained extra-pair young. This study was designed to address three questions. First, is the rate of cuckoldry reported by Burn (1996) similar in a Dutch population of wrens, using data collected over four years and more reliable microsatellite markers for parentage analysis? Second, is there evidence that, once cuckolded, male genetic diversity influences the rate of extra-pair fertilizations by their social mate? Third, is male quality (morphometrics, body condition and feather mite infection) related to the rate of extra-pair mating or individual genetic diversity?

METHODS

Field work

We collected data during the main breeding seasons (April to June) from families of free-living winter wrens (1999-2002). Data was collected from two nearby study sites, Friescheveen and Vosbergen forest reserves, Paterswolde, The Netherlands (*ca.* 50 ha, 53°08' N, 6°35' E). We caught adults in mist-nets, which were then banded with a numbered aluminium band and with a unique combination of three colour bands for individual identification. We collected several morphological variables. Wrens were weighed to

the nearest 0.1 g with a spring balance (Pesola), right tarsus length was measured to the nearest 0.1 mm with vernier calipers, and wing and tail length were measured to the nearest 1 mm with a stop rule. Feather mite prevalence (infected or not, $n = 23$ males) and abundance (number of mites on each infected individual, $n = 16$ males) were estimated by counting the number of feather mites visible with the naked eye on the primary and secondary feathers of both wings when held up to the light. This technique has been shown to provide a reliable estimate of total feather mite load in several other species (see Dowling *et al.* 2001 and references therein). We collected 50-100 μl of blood from all individuals, which was stored in 1-1.5 ml of 95% ethanol at room temperature. Blood was collected by venipuncture at the brachial vein of adults or medial metatarsal vein of nestlings, using a sterile needle. Nestlings were blood sampled as soon as possible after hatching to minimise the chance of depredation before sampling. Tissue samples from unhatched embryos and other dead individuals were collected and stored at –20 °C. Thus, in most cases all offspring in each nest were sampled. Care was taken to avoid contamination with maternal or other tissue.

We identified the parents of nests through a combination of territory mapping and observations at nests. We mapped territories by regularly plotting the location of catching and resighting sites, singing males, and territorial interactions on detailed maps of the field sites. We found nests by searching systematically through occupied territories at least weekly. The nests of wrens are highly distinctive. All potential nest sites (i.e. vegetation or structures such as tree stumps above ground) were examined carefully for the nests. We marked all nests with a small piece of green or yellow weather-proof plastic tape on a twig 5 m either side of the nest. This method allowed exact relocation of each nest even if it disappeared due to depredation or adverse weather. Nest location coordinates were also determined with a handheld satellite global positioning system (Garmin GPS 12®) to a precision of < 5 m. All eggs were marked upon laying and we subsequently monitored all known nests every two days throughout the breeding seasons for changes in their contents.

DNA analyses

DNA was extracted from either whole blood samples or tissue samples (embryos or dead nestlings) stored in ethanol. We used a salt extraction method followed by precipitation in ethanol, as described in Richardson *et al.* (2001). We assessed parentage using five polymorphic microsatellite loci (see Table 2.4). There was no evidence of non-amplifying (null) alleles for any of these loci from statistical analysis of allele frequencies (chapter 2) or when examining known mother-offspring pairs ($n = 38$ pairs with 8 mothers). PCR conditions and the visualization of PCR products were as described for wrens in chapter 2. Due to PCR failure, some individuals used in the parentage analysis were analysed at only three (2.5%) or four (14.6%) loci (mean loci typed per individual 4.8 ± 0.5 SD).

Microsatellite banding patterns were analysed with GENOTYPER software (Applied Biosystems). Basic genetic statistics (observed (H_O) and expected heterozygosity (H_E), polymorphic information content (PIC), Hardy-Weinberg probability and estimation of null allele frequency) were calculated using CERVUS 2.0 software (Marshall *et al.* 1998).

We used the likelihood-based approach implemented by the CERVUS software (Marshall *et al.* 1998) to assess the parentage in wren nests. CERVUS compares the likelihood of the most likely candidate parent against the likelihood of the next most likely candidate parent, using the statistic Delta. The confidence level reported, determined by simulation, indicates the probability that an unrelated individual would by chance obtain a Delta score of this value or larger. CERVUS provides a Delta score and confidence level, set as 80% (relaxed) and 90% (strict), that indicates how the candidate parent who matches best compares to all the other possible candidate parents, and hence whether it can be identified with confidence as the true parent. A further advantage of CERVUS is that it provides a feature to take into account genotyping errors. We used CERVUS to assess parentage in wrens (i.e. true father-offspring relationships). We tested two candidate males, including the putative father and an unsampled candidate father, using the following CERVUS simulation parameters: 10,000 cycles, 2 candidate parents present and 50% candidate parents sampled, genotypes available for 94% of loci and 0.01% of loci were assumed to be mistyped. Candidate fathers were only accepted as the genetic parent with at least 80% confidence level and ≤ 1 mismatching allele. All comparisons used at least 3 loci.

To test the relationship between individual genetic diversity of male wrens and cuckoldry, we calculated three measures of individual heterozygosity or genetic diversity for each male from their microsatellite genotypes. These measures are all designed to indicate inbreeding depression in the absence of individual inbreeding coefficients. First, we calculated standardized individual heterozygosity (hereafter H_S), such that H_S = proportion of heterozygous typed loci/mean heterozygosity of typed loci (Coltman *et al.* 1999). Higher H_S values indicate more heterozygous individuals at the loci considered. H_S is an improvement over unstandardized individual heterozygosity (i.e. the proportion of heterozygous loci) when not all individuals are typed at all loci, because the scores at each locus are weighted by the heterozygosity at that locus and consequently heterozygosity for each individual is measured on the same scale (Coltman *et al.* 1999; Amos *et al.* 2001). Most males in our study were typed at all five loci, and we found a very strong correlation between unstandardized heterozygosity and H_S (Spearman correlation, $r_s = 0.976$, $n = 23$, $P < 0.001$). Second, we calculated internal relatedness (IR), such that IR = ((2 x number homozygous loci)-$\sum f_i$)/((2 x number typed loci)-$\sum f_i$), where f_i = the frequency of the ith allele contained in the genotype (Amos *et al.* 2001). Unlike H_S, IR is approximately normally distributed and centred approximately on zero (for individuals born to unrelated parents), with more negative values indicating relatively outbred individuals and more positive values indicating relatively inbred individuals (Amos *et al.* 2001). Third, we calculated mean standardized d^2 (hereafter d^2_S) for each individual, such that for each locus $d^2_S = d^2$ for the individual/maximum d^2 for the given locus (Coulson *et al.* 1998). For each typed locus, d^2 was calculated as (size of first allele-size of second allele)2. d^2_S has a more even weighting over all alleles than unstandardized d^2 due to the differences in allele size ranges between loci (Amos *et al.* 2001), although these two measures were significantly correlated ($r_s = 0.447$, $n = 23$, $P < 0.032$). Like IR, d^2_S displayed a normal distribution in our dataset. While H_S and IR give a direct measure of individual heterozygosity (marker polymorphism), d^2 provides an alternative measure of individual genetic

diversity. d^2 is thought to indicate long-term mutational differences, so should reflect the genetic distance between the two parental gametes (Coltman & Slate 2003). However, although d^2 has been associated with fitness in several studies (Tsitrone *et al.* 2001; Slate & Pemberton 2002), actual heterozygosity is generally considered a more robust measure of inbreeding (Amos *et al.* 2001; Tsitrone *et al.* 2001; Goudet & Keller 2002; Slate & Pemberton 2002; Coltman & Slate 2003). For clarity, throughout this paper we use the term 'genetic diversity' to refer to genetic variation as measured by any of the above measures, while restricting the term 'heterozygosity' to measures of the number of alleles present at individual loci (i.e. H_S and IR).

Statistical analyses

Statistical analyses were performed in SPSS 12.0.1 for Windows (SPSS Inc., USA) and followed Quinn & Keough (2002). When data were not normally distributed or displayed unequal variances between groups, we used non-parametric tests. The proportion of extra-pair young (EPY) in broods was arcsin-squareroot transformed prior to analyses to improve normality. A body condition index was calculated for each male as the residuals from a linear regression of body mass on tarsus length ($r = 0.446$, df = 22, $P = 0.033$, B = 0.658 ± 0.288 SE; Packard & Boardman 1987). Male wrens were caught from March 26 to May 15, and from 07.00 h to 16.30 h. Due to this variation, we tested for associations between date and time and the flexible morphological variables, body mass and feather mite abundance. We found no significant correlations between capture time and body mass (Pearson correlation, $r = 0.015$, $n = 23$, $P = 0.947$) or feather mite abundance ($r = 0.224$, $n = 16$, $P = 0.404$), nor between capture date and body mass ($r = -0.064$, $n = 23$, P = 0.771). However, we found a tendency for feather mite abundance to decline with capture date ($r^2 = -0.226$, $n = 16$, $P = 0.063$, B = -0.311 ± 0.154). Therefore, we controlled feather mite abundance for capture date in all analyses, although all conclusions were robust to excluding capture date. We weighted all regressions involving the proportion of EPY by the brood size to account for the greater confidence in this value with larger broods. All *P*-values are two-tailed and the null hypothesis was rejected when $P < 0.05$.

RESULTS

Frequency of extra-pair parentage

We were able to assign paternity in nests of 29 wren pairs. Of these, eleven contained at least one extra-pair young (EPY; 37.9%). Overall, 25 of 134 offspring typed were fathered by extra-pair males (18.7%), with the mean proportion of EPY in broods a similar 20%. The proportions of EPY tended to be evenly distributed across broods, except for a large excess of nests with no EPY (Fig. 7.1). We found no evidence of conspecific brood parasitism: all putative mothers that were sampled ($n = 8$) were assigned to all offspring in their broods (>95% confidence). Furthermore, we never observed two eggs to appear in any nest in one day, nor were marked eggs observed to disappear from nests in the absence of nest depredation (Yom-Tov 1980).

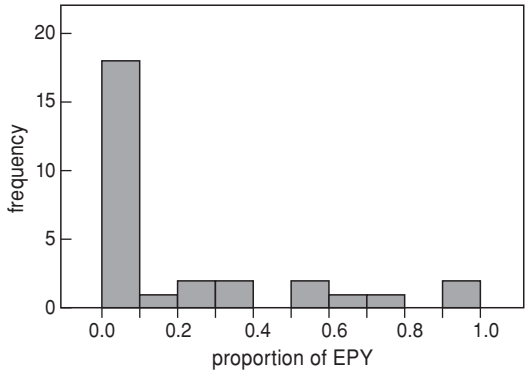

Figure 7.1. Observed distributions of extra-pair young across twenty-nine broods of winter wrens.

Between sites, the proportion of nests containing at least one EPY was very similar (Friescheveen: 38.9%, $n = 18$; Vosbergen: 36.4%, $n = 11$). There was a larger but non-significant difference in the proportions of EPY between the sites (Friescheveen: 24.0%, Vosbergen: 11.9%; $\chi^2_1 = 2.610$, $P = 0.106$). Similarly, we found no significant difference in the proportions of EPY across the four study years ($\chi^2_3 = 4.308$, $P = 0.230$).

Male quality and extra-pair paternity

The mean male H_S was 1.00 ± 0.06 SEM (range 0.32-1.35, n = 23), IR was –0.007 ± 0.070 SEM (-0.534-0.708), while d^2_S was 0.074 ± 0.011 SEM (0.004-0.196). Higher H_S values and lower IR values indicate more outbred individuals. H_S and IR were strongly correlated ($r_s = -0.952$, $n = 23$, $P = <0.001$), while d^2_S was less strongly but still significantly correlated with both H_S ($r_s = 0.504$, $n = 23$, $P = 0.014$) and IR ($r_s = -0.477$, n = 23, $P = 0.021$). Males that were cuckolded and for which we had genetic and morphometric data ($n = 9$) had significantly higher H_S (Mann-Whitney test, $Z = 2.698$, $n = 23$, $P = 0.007$) and significantly lower IR (*t*-test assuming equal variance, $t_{21} = 3.058$, $P = 0.006$, 95% CI of difference 0.119-0.625; Fig. 7.2) than males that were not cuckolded ($n = 14$). This effect size corresponds to approximately one heterozygous loci out of five. In contrast, there was no significant difference in d^2_S between cuckolded and non-cuckolded males, and the effect size approximated to zero ($t_{21} = 0.752$, $P = 0.460$, 95% CI of difference –0.063-0.030; Fig. 7.2A). When considering only broods with one or more extra-pair offspring, there was a significant positive effect of IR on the proportion of EPY (linear regression, $r = 0.854$, $n = 9$, $P = 0.003$; Fig. 7.3A). All nine cuckolded males had negative IR values (mean IR of cuckolded males –0.234 ± 0.195 SD, range -0.534 to –0.027); Fig. 7.3A), while only six out of 14 non-cuckolded males had negative IR values (43%, mean IR of non-cuckolded males 0.138 ± 0.328 SD, range –0.453 to 0.708)). There was also a significant negative effect of H_S the proportion of EPY ($r = -0.770$, $n = 9$, $P = 0.015$), but this effect was not present when considering d^2_S ($r = -0.464$, $n = 9$, $P = 0.208$; Fig. 7.3B). To investigate whether females might pursue EPFs to increase the genetic diversity of offspring, we compared the genetic diversity of within-pair and extra-pair young in mixed

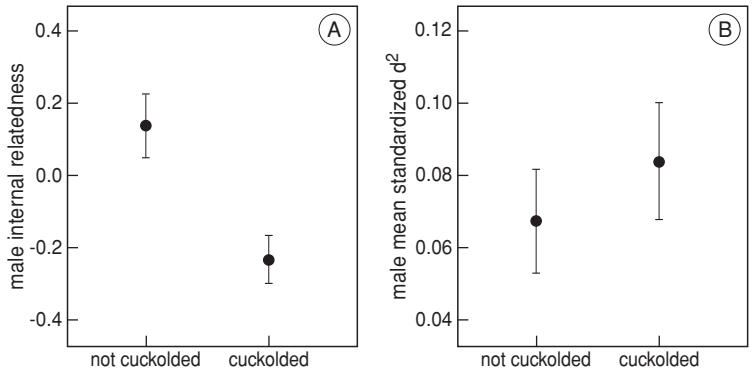

Figure 7.2. The differences in genetic diversity (mean ± SEM) between non-cuckolded (n = 14) and cuckolded (n = 9) male wrens expressed as (A) internal relatedness and (B) mean standardized d^2. For internal relatedness, more positive values indicate less heterozygosity (more inbred), while for mean standardized d^2 higher values indicate more genetic diversity (less inbred).

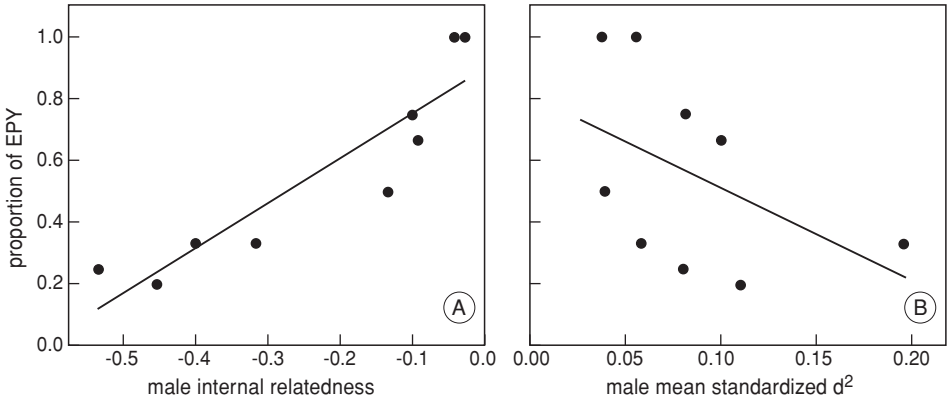

Figure 7.3. The relationships between genetic diversity and the proportion of extra-pair young (EPY) in the nests of cuckolded male wrens (n = 9). Genetic diversity is expressed as (A) internal relatedness and (B) mean standardized d^2. For internal relatedness, more positive values indicate less heterozygosity (more inbred), while for mean standardized d^2 higher values indicate more genetic diversity (less inbred). Least-squares regression lines are weighted by brood size to account for the greater confidence in the proportion of extra-pair young in larger broods.

paternity broods (when more than one within-pair young or EPY were present we took the average). However, we found no evidence that extra-pair young had different heterozygosity (H_S, Wilcoxon signed ranks: Z = -0.140, n = 8, P = 0.889; IR, paired t-test: t_7 = -1.213, P = 0.264, 95% CI of difference –0.364 to 0.117; Fig. 7.4A) or d^2_S (Wilcoxon signed ranks: Z = -0.911, n = 8, P = 0.362; Fig. 7.4B) than their within-pair siblings. Similarly, overall within-pair young did not have different genetic diversity than EPY by any measure of diversity ($p \geq$ 0.2).

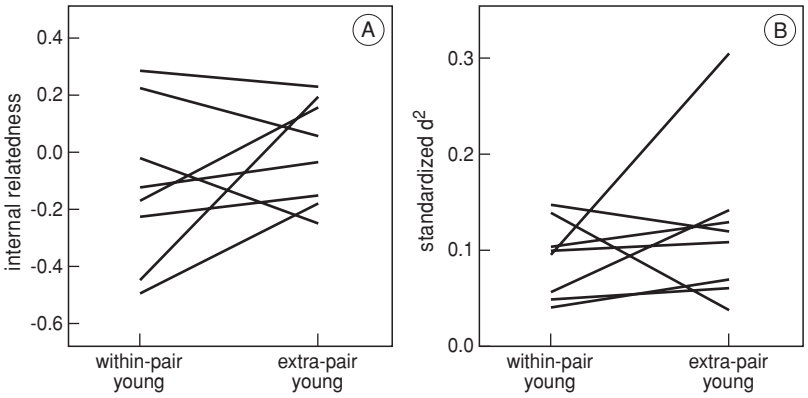

Figure 7.4. The differences in mean genetic diversity of within-pair young and extra-pair young for mixed paternity broods (n = 8). Genetic diversity is expressed as (A) internal relatedness and (B) mean standardized d^2. For internal relatedness, more positive values indicate less heterozygosity (more inbred), while for mean standardized d^2 higher values indicate more genetic diversity (less inbred).

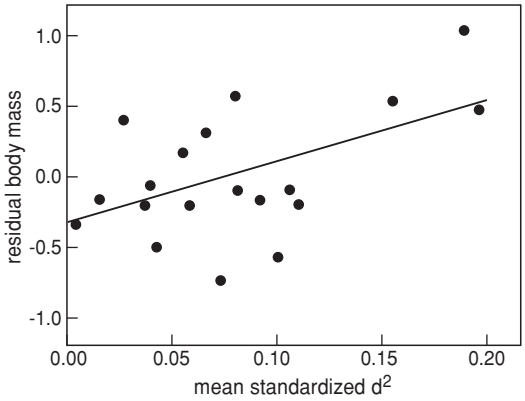

Figure 7.5. The relationship between mean standardized d^2 and a body condition index (residuals from a linear regression of body mass on tarsus length) for male wrens (n = 23). Higher mean standardized d^2 values indicate greater genetic diversity (less inbred).

We found significant correlations between d^2_S and both body mass (r = 0.463, n = 23, P = 0.026, B = 4.151 ± 1.736) and body condition index (r = 0.534, n = 23, P = 0.009; Fig. 7.5), although the former comparison can not be considered significant after sequential Bonferroni correction for multiple comparisons involving d^2_S (adjusted _ = 0.0125). We did not find any significant correlations between the other male morphometric variables (tarsus length, wing length, tail length) and d^2_S (all comparisons, r < 0.130, n = 23, P > 0.558). Similarly, there were no significant correlations between any of the male morphometric variables and either H_S (all comparisons, r_s < 0.301, n = 23, P > 0.164) or IR

Table 7.1. Comparison of male morphometrics and feather mite abundance between cuckolded (n = 9) and non-cuckolded (n = 14) male wrens. Condition index is the studentized residual of body mass regressed over tarsus length. Values show mean ± SEM.

Male trait	Non-cuckolded males	Cuckolded males	t	P	95% CI of difference
Body mass (g)	10.50 ± 0.13	10.60 ± 0.21	0.243	0.810	-0.372-0.471
Tarsus length (mm)	17.20 ± 0.07	17.17 ± 0.19	0.324	0.749	-0.241-0.330
Wing length (mm)	49.7 ± 0.21	50.0 ± 0.58	0.276	0.948	-0.780-0.732
Tail length (mm)	32.3 ± 0.61	32.7 ± 1.20	0.223	0.984	-1.657-1.625
Condition index	-0.398 ± 0.276	-0.107 ± 0.270	0.346	0.871	-0.856-1.003
Mite abundance	17.2 ± 6.4	34.3 ± 9.6	2.100*	0.171	-42.5-8.4

* ANCOVA controlling for capture date (F statistic)

(all comparisons, $r < 0.243$, $n = 23$, $P > 0.265$). There were no correlations between feather mite abundance (controlled for capture date) and any of our measures of genetic diversity (all comparisons, partial correlation, $r < 0.212$, $n = 13$, $P > 0.450$).

Cuckolded males did not differ morphometrically in comparison to non-cuckolded males, and there was no significant difference in the feather mite abundance between cuckolded and non-cuckolded males (Table 7.1). We found no significant effects of any male morphometric variables on the proportion of EPY in broods. This was true both when considering non-cuckolded and cuckolded males together (all comparisons, $r < 0.306$, $n = 23$, $P > 0.156$), and when considering only cuckolded males (all comparisons, $r < 0.579$, $n = 9$, $P > 0.102$).

DISCUSSION

Extra-pair parentage in the wren

Extra-pair paternity has been found to occur frequently in 86% of passerines studied so far (Griffith *et al.* 2002). The wrens in our study population were no exception, and we found that 37.9% of broods contained at least one extra-pair young (EPY) and that extra-pair paternity accounted for 18.7% of offspring. There was no evidence for conspecific brood parasitism (egg dumping). To our knowledge, only one other study has attempted to assess extra-pair parentage in winter wrens. Burn (1996), studying a population in Oxfordshire, England, reported that 13 of 94 of offspring (13.8%) were attributable to extra-pair paternity, and that six of 19 broods (31.6%) contained at least one EPY. Again, Burn (1996) found no evidence of conspecific brood parasitism, and detected no cases of multiple paternity. In both our study and Burn's (1996), two nests were found to contain only EPY (6.9% and 10.5%, respectively). In many species, considerable variation in the rates of extra-paternity has been reported between study years and populations, which is generally considered to be a result of variation in the opportunities or the benefits to

females of promiscuity (Petrie & Kempenaers 1998). In this respect, the variation we see in the rates of extra-pair paternity between these two populations of wrens is not remarkable. A further finding of Burn (1996) was that larger males were less likely to be cuckolded than smaller males, and that smaller males were cuckolded by larger males. This suggests that female wrens may seek EPCs with larger males. In contrast, we found no evidence that male size or condition was related to cuckoldry.

Male quality, genetic diversity and female promiscuity

Our study provided two novel lines of evidence that male heterozygosity influenced the rate of extra-pair paternity in their own nests. First, more heterozygous males were more likely to be cuckolded. Second, once cuckolded, more heterozygous males had a lower proportion of EPY in their brood. These patterns were not reflected in male d^2_S. Taken together, these results indicate that male heterozygosity could be an important factor in the evolution of female promiscuity. However, they suggest opposing directions of the effect of male heterozygosity on the rate of extra-pair paternity. We currently have no clear explanation for why less heterozygous males were less likely to be cuckolded. If mating with more heterozygous males does provide fitness benefits to females (Allendorf & Leary 1986; Brown 1997; Keller & Waller 2002), this may explain why more heterozygous males, when cuckolded, suffered from a lower proportion of EPY in their broods than less heterozygous males that were cuckolded. Females paired socially to males with low heterozygosity might be more likely to engage in EPCs in order to increase the heterozygosity and hence the viability of their offspring, by choosing extra-pair mates that are either more genetically diverse or that possess different alleles to the female (genetic compatibility hypothesis). We found no evidence that females increase the genetic diversity of their offspring by pursuing EPCs, because within-pair young did not have significantly different genetic diversity than their extra-pair nest-mates. This is in line with Masters *et al.* (2003), who found no difference in heterozygosity between within-pair and extra-pair males. In our sample, we were unable to identify enough extra-pair sires to compare these directly with the extra-pair females that they fertilized or the males that they cuckolded.

If genetic diversity is associated with the genetic health of males (e.g. Hawley *et al.* 2005; MacDougall-Shackleton *et al.* 2005), this might be expressed in condition-dependent cues that females could use for assessment purposes (Otter *et al.* 2001). We found some evidence for this with a positive association between d^2_S and male body condition. However, we found no relationships between male d^2_S and female promiscuity, and no relationships between the other measures of heterozygosity that were related to female promiscuity, and either male size or condition. Further work will be required to uncover links between male genetic diversity and cues of potential use to females, which may include plumage features (Foerster *et al.* 2003), song (Marshall *et al.* 2003; Seddon *et al.* 2004; Reid *et al.* 2005) or territorial performance (Seddon *et al.* 2004).

As a whole, these surprising patterns raise questions about the proximate and ultimate factors underlying the role of male heterozygosity in male quality, female choice and male extra-pair mating success. To understand these phenomena further, there is a pressing need to determine whether females should prefer to mate with more heterozygous

males at all, and if so under which circumstances. This is complicated by several factors. Heterozygosity itself is not a heritable trait and is a property of both the male's and the female's genotypes. Nevertheless, females that mate with more heterozygous males can, on average, expect to produce more heterozygous offspring because heterozygosity of offspring is correlated with the heterozygosity of their parents (Mitton *et al.* 1993; Brown 1997; but see Tregenza & Wedell 2000). One way that this will occur is because heterozygous individuals carry more rare alleles (Mitton *et al.* 1993; Brown 1997). However, despite the well documented effects of inbreeding depression (Keller & Waller 2002) such alleles are more likely to be deleterious recessives and may not increase the fitness of the bearer (Tregenza & Wedell 2000, but see Masters *et al.* 2003). From this perspective, females may not benefit by mating with more heterozygous males. Males that possess a high proportion of heterozygous loci without carrying many rare alleles could still provide a benefit to females in terms of enhanced offspring viability through heterozygosity. Since the most rare alleles often occur towards the extremes of the size range (e.g. Primmer *et al.* 1995; Xu *et al.* 2000), such males might be characterized by high heterozygosity values (high H_S or strongly negative IR) but moderate or low d^2_S values.

On the other hand, females would benefit by mating with males that are heterozygous at loci showing a fitness advantage of rare alleles, such as major histocompatibility complex (MHC) loci (Edwards & Hedrick 1998; Tregenza & Wedell 2000). Masters *et al.* (2003) found that male house wrens (*Troglodytes aedon*) that carried rare microsatellite alleles were more successful at siring EPY. Variation at neutral genetic markers such as microsatellites may not necessarily reflect variation at unlinked fitness-related loci such as MHC loci (Jarne & Lagoda 1996; Reed & Frankham 2001; Westerdahl *et al.* 2004). This means that female wrens may achieve benefits related to genetic diversity that are not revealed consistently by the microsatellite markers used in this study.

To date, intra-specific empirical support for an association between paternity and male heterozygosity or d^2 comes from only one study. In the great tit (*Parus major*), Otter *et al.* (1998) found that males that were more variable at five microsatellite loci, measured as mean d^2, were less likely to have EPY in their nest than males with lower d^2 values. This pattern was not present in females, and there was no difference in d^2 between the putative parents of broods containing EPY. Otter *et al.* (1998) did not report on the relationship between male d^2 and the proportion of EPY in broods, and they did not present measures of genetic diversity other than d^2. The results of this study are in contrast to our study in two important ways. First, we found no relationships between a d^2 measure of genetic diversity and extra-pair paternity. Second, our study indicated that more heterozygous males were more likely to have at least one EPY in their nest.

Several other studies have not found an association between male genetic diversity (heterozygosity or d^2) and (extra-pair) mating success, although some of these studies have found that females chose extra-pair males more genetically dissimilar to themselves (Weatherhead *et al.* 1999; Aparicio *et al.* 2001; Foerster *et al.* 2003; Masters *et al.* 2003; Bartos Smith *et al.* 2005; Kleven & Lifjeld 2005). One explanation for the apparent discrepancy between studies may be that populations can differ markedly in their inbreeding history and levels of heterozygosity at microsatellite loci, even within species (e.g.

Dawson *et al.* 1997). Bartos Smith *et al.* (2005) also pointed out that the sample sizes and numbers of loci typed in most similar studies to date are far too small to reliably detect all but a pronounced effect. Accordingly, we must also consider the possibility that our failure to find similar associations between d^2 and mating success may be due to type II errors, rather than other biological explanations. Amos *et al.* (2001) have shown that the effect sizes when relating genetic diversity to life history and morphometric traits are smaller when using d^2 than heterozygosity. For this reason, future studies should endeavour to use a large number of markers and interpret negative results cautiously.

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