

Sexual selection and individual genetic diversity in a songbird

Rupert C. Marshall^{1*}, Katherine L. Buchanan² and Clive K. Catchpole¹

¹*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK*

²*School of Biosciences, Cardiff University, Park Place, Cardiff CF10 3TL, UK*

* Author for correspondence (r.marshall@rhul.ac.uk).

Recd 20.06.03; Accptd 09.07.03; Online 20.08.03

Here, we report for the first time, to our knowledge, a strong correlation between a measure of individual genetic diversity and song complexity, a sexually selected male trait in sedge warblers, *Acrocephalus schoenobaenus*. We also find that females prefer to mate with males who will maximize this diversity in individual progeny. The genetic diversity of each offspring is further increased by means of non-random fertilization, as we also show that the fertilizing sperm contains a haplotype more genetically distant to that of the egg than expected by chance. These findings suggest that species' mating preferences may be subject to fine tuning aimed at increasing offspring viability through increased genetic diversity. This includes external and internal mechanisms of selection, even within the ejaculate of a single male.

Keywords: genetic diversity; sexual selection; mean d^2 ; song; repertoire size

1. INTRODUCTION

Inbreeding occurs when close relatives reproduce together, often resulting in low genetic diversity among their offspring. This can have a deleterious effect upon phenotypic traits, leading to decreased fertility, fecundity, offspring viability and growth rates (Charlesworth & Charlesworth 1987). Non-incestuous inbreeding, involving mating between genetically similar but not closely related individuals, has also been found to negatively influence an individual's ability to survive and reproduce, affecting sperm quality (Wildt *et al.* 1987), offspring survival (Hansson *et al.* 2001) and reproductive success (Amos *et al.* 2001). Sexually selected ornaments favoured by female choice may also be affected by the level of individual genetic diversity. However, no studies, to our knowledge, have investigated whether known sexually selected traits could have evolved as an indicator of genetic diversity (Brown 1997).

Song complexity, as measured by syllable repertoire size, is an established sexually selected trait exhibited by male sedge warblers, *Acrocephalus schoenobaenus*. In the field and under controlled conditions in the laboratory, females have been shown to prefer males with larger repertoires (Catchpole 2000). Repertoire size in this species is indicative of parasite load, level of paternal offspring pro-

visioning and the size of the main song control nucleus (the higher vocal centre) in the brain (Catchpole 2000). Here, we investigate the relation between repertoire size and individual genetic diversity in a wild population of sedge warblers. We then proceed to investigate the effects of mate choice and internal mechanisms of selection on this level of diversity.

2. METHODS

We studied a population of individually colour-ringed sedge warblers at Wraysbury, England, from 1993–1999. Males were recorded and blood samples taken under UK Home Office licence within a few days of their arrival.

All sampled males within a radius of 200 m from the focal male's territory (the auditory limit) were included when assessing female mate choice. Recordings were analysed by using AVISOFT-SASLAB PRO (see electronic Appendix A, available on The Royal Society's Publications Web site). Song complexity was measured in terms of repertoire size using standard techniques to estimate the total number of different syllable types sung by each male (Catchpole 2000).

(a) DNA analysis

Standard phenol/chloroform DNA extraction and polymerase chain reaction (PCR) techniques were used to amplify microsatellite DNA (see electronic Appendix A). Four of our loci (Fhu2 (Ellegren 1992); Ppi2 (Martinez *et al.* 1999); Pca3 (Dawson *et al.* 2000); Ase18 (Richardson *et al.* 2000)) displayed 17 alleles. Pocc1 & Pocc8 (Bensch *et al.* 1997) displayed 15 alleles, Ase37 & 48 (Richardson *et al.* 2000) displayed 13 alleles and Pca5 (Dawson *et al.* 2000) 11 alleles. No parent was included in any part of the analysis more than once. Broods containing extra-pair young were excluded to limit interference from male–male sperm competition.

(b) Genetic distance

We measured individual genetic diversity using mean d^2 (Coulson *et al.* 1998), the squared distance between microsatellite alleles, averaged across loci. Mean d^2 takes account of allele length variation as well as heterozygosity *per se* and is particularly powerful with highly polymorphic loci where homozygosity is rare (Coulson *et al.* 1998; Tsitroni *et al.* 2001). Mean d^2 incorporates heterozygosity (Coulson *et al.* 1998) and, although models of d^2 at a single locus have suggested that it may not be as powerful as heterozygosity (Tsitroni *et al.* 2001), averaging across loci (mean d^2) reduces the inherent level of variation in single locus studies, increasing its validity as a measure of diversity.

Mean d^2 was calculated for all males for whom recordings were available and who were successfully profiled at the six most powerful loci available (in terms of number of alleles exhibited). Where individuals lacked data at a locus (e.g. PCR failure, null allele) the next most powerful locus was used instead. Comparisons between individuals always used the same loci.

To calculate the suitability of each pairing, in terms of maximizing offspring genetic diversity, we calculated an expected mean d^2 score for a hypothetical offspring from each possible pairing. First, we calculated d^2 for each possible combination of maternal and paternal alleles at each locus. We then calculated the mean for all possible combinations of these d^2 scores across all loci. We defined the expected score as the median of all possible mean d^2 scores (MeAPS) (one mean d^2 score for each possible combination of maternal and paternal alleles at each locus; six loci equal 4096 combinations). Assuming fertilization to be random, actual offspring mean d^2 scores should be equally distributed above or below this value. If females choose males who maximize the genetic diversity of their offspring, they should choose the male who provides the greatest MeAPS.

To determine whether offspring mean d^2 scores were larger than expected by chance, we calculated an expected value for each chick. The maternal haplotype present in an egg was identified by profiling parents and offspring from each brood at the same six loci (the six most powerful loci available for all of them), allowing 64 possible mean d^2 scores per offspring, representing all possible combinations of parental alleles given the maternal alleles present in the egg. The expected value was the MeAPS. We assumed equal numbers of sperm containing each possible combination of alleles. If fertilization is random, the probability of a chick having a mean d^2 score higher or lower than the MeAPS is 0.5.

3. RESULTS

We found a highly significant positive correlation between repertoire size and mean d^2 ($r_s = 0.437$, $p = 0.005_{2 \text{ tail}}$, $n = 40$; figure 1).

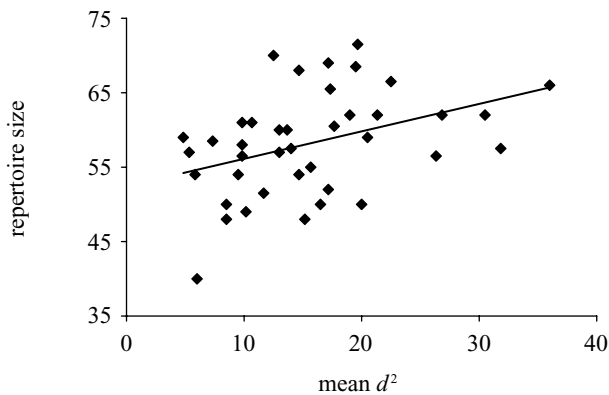


Figure 1. Correlation of mean d^2 with song repertoire size.

As male repertoire size is related to individual genetic diversity, females selecting males with large repertoires may increase the genetic diversity of individual offspring. The extent to which such diversity is increased also depends on the genetic distance between the male and female genotypes. We investigated this in two ways. First, we asked whether females chose males who would provide the most genetically diverse individual offspring compared with other males that were available (i.e. unpaired) on the day of pairing. We found that, where there was a choice of at least two males, the female paired significantly more often with the male who was most genetically distant to her (i.e. the male who provided the highest MeAPS score in relation to her genotype; 10 out of 12; $\chi^2 = 4.08$, $p < 0.05$).

Second, we asked whether offspring genetic diversity could be further increased, beyond that obtained through initial mate choice, by considering the role of gametes. The haplotype contained within each gamete is unique, so each sperm varies in its genetic distance to the haplotype contained within the egg. In birds, once an ejaculate is inside the female tract, each sperm may be regarded as an individual (Birkhead & Moller 1998), sharing a common ancestor with other sperm from the same male progenitor. Fertilization by a sperm whose haplotype is distant to that of the egg may bestow an advantage on the offspring through increased genetic diversity. We investigated whether the mean d^2 scores of individual offspring were higher than expected from random fertilization.

We analysed all offspring from all broods as a single group: a significant number of offspring demonstrated a higher level of individual genetic diversity than expected by chance (52 out of 82; $\chi^2 = 5.4$, $p < 0.05$). However, parental genotypes affect the size and range of scores obtained and differ between broods. In addition, chicks within a brood cannot be regarded as independent. We controlled for these factors by using a matched-pair within-brood analysis, comparing the proportions of each brood that did and did not demonstrate a higher than expected level of individual genetic diversity. We found that a significant proportion of offspring in each brood exhibited greater individual genetic diversity than expected from random fertilization (Wilcoxon $Z = -1.969$, $n = 19$, $p < 0.05$; figure 2). (There was no correlation between brood size and the proportion of the brood exhibiting greater individual genetic diversity ($r_s = 0.3$, $n = 19$, $p > 0.1$).

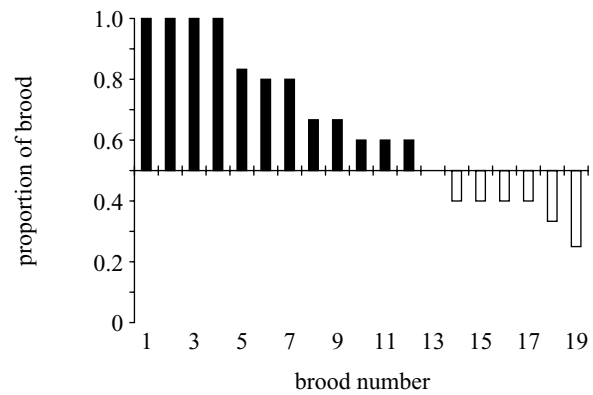


Figure 2. Proportion of brood with higher than expected levels of individual genetic diversity.

4. DISCUSSION

This is the first investigation, to our knowledge, to report a link between individual genetic diversity and a sexually selected trait favoured by female choice in a wild population. Our results lend weight to studies suggesting that females engage in active avoidance of inbreeding to improve offspring quality both pre- and post-copulation (e.g. Tregenza & Wedell 2002).

Male sedge warblers cease all song upon pairing (Catchpole 2000) so female offspring do not have an opportunity to gain familiarity with their father's song during their development and to compare its acoustic characteristics with those of prospective mates. Only peacocks have previously been shown to discriminate between kin and non-kin when deprived of both social learning and environmental cues (Petrie *et al.* 1999).

Previous work has shown that female sedge warblers use repertoire size as a cue in mate choice, suggesting directional selection for song complexity (Catchpole 2000). Female choice for song complexity and genetic distance are not mutually exclusive behaviours and may act to reinforce the occurrence of some form of sperm selection (Colegrave *et al.* 2002). If the cost of mating with an incompatible male is sufficiently high, females should favour males with compatible genotypes over high-quality males with 'good genes' (Colegrave *et al.* 2002). This may explain why the patterns of female choice when establishing social pair bonds are not always mirrored in the patterns of mate choice for extra-pair copulations in many species. Microsatellite loci are not thought to be under direct selection and therefore individuals that exhibit similarity at microsatellite loci are likely to be more similar in general if this similarity is due to inbreeding (Coulson *et al.* 1998).

Our new finding, that individual genetic diversity in offspring is greater than expected from random fertilization of eggs by sperm from a single male suggests that attention should also be focused on within-male sperm variability as a method for inbreeding avoidance (Haig & Bergstrom 1995). Similar results were obtained in an experimental study of full-sibling flat oysters, which found a higher than expected frequency of heterozygotes at microsatellite loci among offspring (Bierne *et al.* 1998). Although variation in sperm fertilization success due to female genotype occurs in a wide range of species, it has been in relation to either a specific protein or allele (e.g. major histocom-

patibility complex), or where repeated exposure to sperm of a particular genotype results in discrimination against that genotype in future encounters, or where sperm from more than one male are present (Birkhead & Moller 1998). An effect of individual genetic diversity upon offspring recruitment has already been reported in the closely related great reed warbler, *A. arundinaceus*, by using comparable methods (Hansson *et al.* 2001).

At present we are unable to detect the specific mechanism by which any differential fertilization or sperm survival might occur. Although many sperm come from one male, each individual sperm contains a slightly different haplotype as a result of recombination at meiosis. In the absence of intense inter-male sperm competition, altruism towards other sperm from the same male requires fractionally more than one sperm to fertilize an egg to be stable (Hamilton 1964*a,b*). If sperm characters relate to the haplotype they contain, then a form of gametic polyandry may occur within the female tract with a multitude of sperm, each containing a different haplotype, competing to fertilize a single egg. Such a mechanism of post-copulatory competition within the ejaculate of a single male would increase offspring viability, thereby also increasing parental reproductive success, as has been shown to occur when sperm from more than one male are in competition (Tregenza & Wedell 2002).

Taken together, our findings also suggest a mechanism that may contribute to the rapid recovery of inbred populations after the introduction of new individuals. Non-random selection of genetically distant sperm haplotypes could significantly affect the occurrence of certain genotypes within the population. Such a demonstration would transform our view of fertilization as a random event based largely on sperm numbers (Birkhead & Moller 1998) and have fundamental implications for gene flow within populations. Identification of haplotype-determined gamete characteristics affecting fertilization ability may also offer applications for sperm choice in *in vitro* fertilization.

Acknowledgements

We thank T. Burke and the NERC Sheffield Molecular Genetics Facility, and NERC (UK) for financial support. We are grateful to T. Tregenza, H. E. Nice and two anonymous referees for comments on an earlier version of the manuscript.

Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001 The influence of parental relatedness on reproductive success. *Proc. R. Soc. Lond. B* **268**, 2021–2027. (DOI 10.1098/rspb.2001.1751.)

Bensch, S., Price, T. & Kohn, J. 1997 Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Mol. Ecol.* **6**, 91–92.

Bierne, N., Launey, S., Naciri-Graven, Y. & Bonhomme, F. 1998 Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* **148**, 1893–1906.

Birkhead, T. & Moller, A. 1998 *Sperm competition and sexual selection*. London: Academic.

Brown, J. L. 1997 A theory of mate choice based on heterozygosity. *Behav. Ecol.* **8**, 60–65.

Catchpole, C. K. 2000 Sexual selection and the evolution of song and brain structure in *Acrocephalus* warblers. *Adv. Study Behav.* **29**, 45–97.

Charlesworth, D. & Charlesworth, B. 1987 Inbreeding depression and its evolutionary consequences. *A. Rev. Ecol. Syst.* **18**, 237–268.

Colegrave, N., Kotiaho, J. S. & Tomkins, J. L. 2002 Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol. Ecol. Res.* **4**, 911–917.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. 1998 Microsatellites reveal heterosis in red deer. *Proc. R. Soc. Lond. B* **265**, 489–495. (DOI 10.1098/rspb.1998.0321.)

Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K. & Burke, T. 2000 Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. *Mol. Ecol.* **9**, 1941–1944.

Ellegren, H. 1992 Polymerase chain reaction (PCR) analysis of microsatellites: a new approach to studies of genetic relationships in birds. *Auk* **109**, 886–895.

Haig, D. & Bergstrom, C. T. 1995 Multiple mating, sperm competition and meiotic drive. *J. Evol. Biol.* **8**, 265–282.

Hamilton, W. D. 1964*a* The genetical evolution of social behaviour I. *J. Theor. Biol.* **7**, 1–16.

Hamilton, W. D. 1964*b* The genetical evolution of social behaviour II. *J. Theor. Biol.* **7**, 17–52.

Hansson, B., Bensch, S., Hasselquist, D. & Akesson, M. 2001 Microsatellite diversity predicts recruitment of sibling great reed warblers. *Proc. R. Soc. Lond. B* **268**, 1287–1291. (DOI 10.1098/rspb.2001.1640.)

Martinez, J. G., Soler, J. J., Soler, M., Moller, A. P. & Burke, T. 1999 Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution* **53**, 269–278.

Petrie, M., Krupa, A. & Burke, T. 1999 Peacocks lek with relatives even in the absence of social and environmental cues. *Nature* **401**, 155–157.

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2226–2231.

Tregenza, T. & Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature* **415**, 71–73.

Tsitronis, A., Rousset, F. & David, P. 2001 Heterosis, marker mutational processes and population inbreeding history. *Genetics* **159**, 1845–1849.

Wildt, D. E., Bush, M., Goodrowe, K. L., Packer, C., Pusey, A. E., Brown, J. L., Joslin, P. & Obrien, S. J. 1987 Reproductive and genetic consequences of founding isolated lion populations. *Nature* **329**, 328–331.

Visit <http://www.pubs.royalsoc.ac.uk> to see an electronic appendix to this paper.