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Problems with pair-wise comparisons: does certainty of paternity covary with paternal care?

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In a recent interspecific comparative study of birds, Møller & Birkhead (1993) used two comparative techniques, the comparison of independent linear contrasts (Harvey & Pagel 1991) and pair-wise comparisons of sister taxa (Møller & Birkhead 1992), to show that male parental care (feeding of chicks) is significantly negatively related to extra-pair paternity. The pair-wise comparisons were included to show that the main result was independent of statistical technique (Møller & Birkhead 1993), and to control many confounding variables (Møller & Birkhead 1992). These comparisons appeared strongly to support their argument because 10 of 11 pairs varied in the predicted direction (sign test: $P=0{\cdot}012$).

There are at least four major problems with Møller & Birkhead's (1993) pair-wise comparisons. In going through these below, I shall show that further testing of Møller & Birkhead's (1993) hypothesized relationship between extra-pair paternity and paternal care is necessary and that pair-wise comparisons can often be an inadequate test for behavioural questions. I have re-analysed the corrected data, and the revised pair-wise comparisons no longer support the hypothesis that male parental care covaries with extra-pair paternity (sign test: $P=0{\cdot}55$).

(1) In a comparison of two parids, Møller & Birkhead (1993) used extra-pair paternity estimates of 17·9% for blue tits, *Parus caeruleus*, and 15·9% for great tits, *P. major*. These values are incorrect for the following reasons.

(a) The general reference the authors used for estimates of extra-pair paternity is Birkhead & Møller (1992). For species where more than one estimate of extra-pair paternity was available, Møller & Birkhead (1993) used the mean value. Thus, for great tits, they used 15·9%, the mean of the three cited studies in Birkhead & Møller (1992; 23·9, 8·6 and 15·2%). For the blue tit, however, they used the higher of two cited studies (17·9%) when to be consistent they should have used 15·1% (the mean of 12·3 and 17·9%).

(b) Updated estimates of extra-pair paternity for these two species also yield different results. Using DNA fingerprinting, Gullberg et al. (1992) estimated extra-pair paternity to be 14·9% in great tits (seven out of 47 offspring) and 5·9% in blue tits (three out of 51 offspring). Furthermore, the paternity estimate for Kempenaers' study population of blue tits (17·9%: five out of 28 offspring, cited in Birkhead & Møller 1992), can now be revised downwards to 10·5% (33 out of 314 offspring; Kempenaers et al. 1992). By taking the mean values of all available studies one now gets extra-pair paternity estimates of 15·7% for great tits (mean of 23·9, 8·6, 14·9 and 15·2%) and 9·6% for blue tits (mean of 12·3, 5·9 and 10·5%).

Correcting for either of the above two points causes the parid comparison to vary in the reverse direction from that stated by Møller & Birkhead (1993).

(2) Based on a multi-locus DNA fingerprinting study by Morton et al. (1990), Birkhead & Møller (1992) used the estimate of 34·6% (18 of 52 offspring) extra-pair offspring for purple martins, *Progne subis*. This value is surely an overestimate for two reasons. First, it confuses extra-pair offspring and some offspring resulting from intraspecific brood parasitism together as extra-pair offspring and second, it overestimates the number of excluded young because of inappropriate data analysis (see below). Since the dependent variable in Møller & Birkhead's (1993) comparative study is the ratio of paternal care to total parental care by a pair, the relevant independent variable is extra-pair paternity among the mate's offspring. Thus the frequency of extra-pair offspring should be calculated as in Birkhead et al. (1990): the percentage of offspring that are unrelated to the

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putative fathers out of all offspring that are related to the putative mothers.

When comparing DNA fingerprints between offspring and putative parents, individuals arising from extra-pair fertilizations will usually have three or more novel bands, a low band-sharing coefficient with the father and a high band-sharing coefficient with the mother (Westneat 1990). In their analysis on purple martins, Morton et al. (1990) considered band-sharing coefficients independently of novel bands and they used the criteria of a low band-sharing coefficient (<0·4) alone to identify offspring unrelated to their parent. Thus, there are 18 nestlings with band-sharing coefficients with their putative fathers that are less than 0·4 (Table 1 in Morton et al. 1990). However, not all of these 18 can be considered as extra-pair offspring. One chick had only one novel band and since its band-sharing coefficient with its putative father was marginally below 0·4 (0·367), it was almost certainly not extra-pair. The remaining 17 offspring all had at least three novel bands, but six of them had band-sharing coefficients with their putative mothers that were also less than 0·4, suggesting that they were unrelated to both their parents (i.e. they were likely to be a result of intraspecific brood parasitism). Since these offspring should be excluded from the calculation of extra-pair paternity, a more appropriate estimate of the frequency of extra-pair offspring in this population is 23·9% (11 out of 46).

Revising the extra-pair estimate of purple martins from 34·6 to 23·9% reverses the direction of variation in the pair comparison of purple martins with tree swallows, *Tachycineta bicolor*, from that stated by Møller & Birkhead (1993).

(3) Møller & Birkhead (1993) compared barn swallows, *Hirundo rustica*, with purple martins and tree swallows with purple martins. They did not include a comparison of tree swallows and barn swallows. Based on the phylogeny they used (Fig. 1), there is no reason not to include this comparison. If tree swallows were more closely related to purple martins than to barn swallows, then it might be justified to leave this comparison out. However, based on the given phylogeny, tree swallows are equally related to barn swallows and purple martins, and so by using the tree swallow/purple martin comparison alone their results are biased because the tree swallow/barn swallow comparison is in the opposite direction to Møller & Birkhead's (1993) prediction.

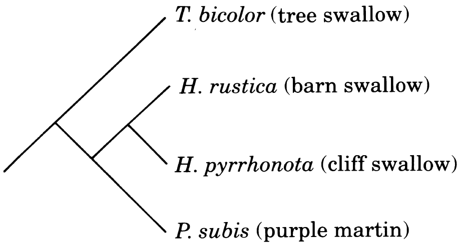

Figure 1. The relevant portion of the phylogeny used by Møller & Birkhead (1993) for their swallow comparisons. The axes of the above phylogeny have been rotated to illustrate my point more clearly. *Hirundo pyrrhonota* was not included in any of the pair-wise comparisons. Møller & Birkhead (1993) included a pair-wise comparison of purple martins and tree swallows, but not a barn swallow/tree swallow comparison, thus biasing their result.

(4) One of Møller & Birkhead's (1993) pairs was comprised of monogamous dunnocks, *Prunella modularis*, and polyandrous dunnocks from the same study population. Monogamous male dunnocks had no extra-pair offspring and provided 49·8% of the nestling feedings. Alpha male dunnocks in polyandrous 'trios' had 36·1% 'extra-pair' chicks in their nests (sired by the beta male; Burke et al. 1989) and provided 35·0% of the feedings. Two comments about the use of this pair are relevant.

(a) The drop in feedings by alpha males in polyandrous matings does not necessarily indicate a decrease in alpha male care relative to female care (the relevant variable) because there are three birds giving parental care. Indeed, in polyandrous trios, females and alpha males fed nestlings at similar rates (Hatchwell & Davies 1990), despite their differences in confidence of relatedness to the chicks.

(b) The validity of comparing data from the same species and population under two different mating systems is questionable. An assumption of pair-wise comparisons is that the variables of interest have a genetic basis (Møller & Birkhead 1992). More specifically, the assumption is that variation in the variables of interest has a genetic basis. In the dunnock comparison, variation in paternal care between monogamous males and polyandrously mated males is probably not genetically based, but a reflection of context-specific responses elicited by a common behavioural strategy all males share. Comparing context-specific parental care strategies, although insightful, will not answer the question at hand: how do changes in extra-pair paternity affect changes in paternal care on an evolutionary time scale?

The consequences of the above comments for Møller & Birkhead's (1993) pair-wise analysis are as follows. If one substitutes the updated estimates of paternity for great tits, blue tits and purple martins into the analysis, the result of the sign test changes from $P=0{\cdot}012$ (10 of 11 pairs in the predicted direction) to $P=0{\cdot}23$ (eight of 11 pairs). If one also excludes the dunnock comparison and includes the tree swallow/barn swallow comparison then the significance of the sign test drops to $P=0{\cdot}55$ (seven of 11 pairs). Furthermore, analysis of the revised data with Wilcoxon's matched-pairs test, a more powerful test than the sign test, also yields non-significant results ($z=1{\cdot}29$, $P=0{\cdot}20$; $z=1{\cdot}03$, $P=0{\cdot}31$, respectively).

The fact that the revised pair-wise comparisons yield different results from the analysis of independent contrasts (Møller & Birkhead 1993) suggests that the observed covariance between extra-pair paternity and paternal care is not independent of the comparative technique used. Whether this discrepancy between the two techniques is from the hypothesized relationship being spurious or from inadequacies of the pair-wise comparative method is unknown. Although some of the above criticisms of the pair-wise comparisons also apply to the analysis of independent contrasts (e.g. the revised paternity values), they should not affect the latter analysis as markedly since it is based on a much larger data set. Since the contrasts (the major analysis in Møller & Birkhead's (1993) study) show a strong relationship in the predicted direction, this suggests that the pair-wise analysis may be at fault. If so, then this paper clearly illustrates some of the problems associated with using this technique: (1) low sample sizes coupled with the inclusion of pair-wise comparisons with widely overlapping standard errors, (2) lack of rigorous criteria for determining which pair-wise comparisons are appropriate to include, and (3) uncritical use of published data.

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REFERENCES

Birkhead, T. R., Burke, T., Zann, R., Hunter, F. M. & Krupa, A. P. 1990. Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.*, **27,** 315–324.

Birkhead, T. R. & Møller, A. P. 1992. *Sperm Competition in Birds: Evolutinary Causes and Consequences*. London: Academic Press.

Burke, T., Davies, N. B., Bruford, M. W. & Hatchwell, B. J. 1989. Parental care and mating behavior of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature, Lond.*, **338,** 249–251.

Gullberg, A., Tegelström, H. & Gelter, H. P. 1992. DNA fingerprinting reveals multiple paternity in families of great and blue tits (*Parus major* and *P. caeruleus*). *Hereditas*, **117,** 103–108.

Harvey, P. H. & Pagel, M. 1991. *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.

Hatchwell, B. J. & Davies, N. B. 1990. Provisioning of nestlings by dunnocks, *Prunella modularis*, in pairs and trios: compensation reactions by males and females. *Behav. Ecol. Sociobiol.*, **27,** 199–209.

Kempenaers, B., Verheyen, G. R., Van den Broeck, M. V., Burke, T., Van Broeckhoven, C. V. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature, Lond.*, **357,** 494–496.

Møller, A. P. & Birkhead, T. R. 1992. A pairwise comparative method as illustrated by copulation frequency in birds. *Am. Nat.*, **139,** 644–656.

Møller, A. P. & Birkhead, T. R. 1993. Certainty of paternity covaries with paternal care in birds. *Behav. Ecol. Sociobiol.*, **33,** 261–268.

Morton, E. S., Forman, L. & Braun, M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. *Auk*, **107,** 275–283.

Westneat, D. F. 1990. Genetic parentage in the indigo bunting; a study using DNA fingerprinting. *Behav. Ecol. Sociobiol.*, **27,** 67–76.