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Can incest within cooperative breeding groups be detected using DNA fingerprinting?

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Although generally considered a rare phenomenon, incest has been observed in a number of populations of birds and mammals in which dispersal is limited (Greenwood et al. 1978; Bulger and Hamilton 1988; Gibbs and Grant 1989; Packer and Pusey 1993; Keller and Arcese 1998). In cooperatively breeding animals where offspring remain in their natal territory as adults, the opportunity for incest may be substantial (Ligon and Ligon 1990). Inbreeding avoidance mechanisms ensure that cases of incest in the cooperatively breeding acorn woodpecker (*Melanerpes formicivorus*) are exceedingly rare, and groups whose membership consists of only closely related members of the opposite sex may forego breeding for up to 3 years if membership remains unaltered (Koenig et al. 1998). Incest is avoided in the superb fairy wren *Malurus cyaneus* through a different mechanism: although offspring remain on their natal territory, females seek extra-group matings, particularly when many sons remain as helpers in the group (Brooker et al. 1990; Mulder et al. 1994). Few other studies of cooperatively breeding populations have verified genetically whether or not incest occurs.

To date, most studies that have looked at genetic relationships within groups of cooperatively breeding animals have used multilocus DNA fingerprinting (Rabenold et al. 1990; Jones et al. 1991; Packer et al. 1991; Bruce et al. 1996; Whittingham et al. 1997; Lundy et al. 1998). In several cases, the authors appear not to have appreciated that this technique does not allow the detection of parent-offspring incest. While some have acknowledged the limitations of the data and were careful not to make inferences beyond the scope of the technique (Haig et al. 1994; Dickinson et al. 1995; McRae 1996; Koenig et al. 1998), others have simply ignored the possibility of incest. Since genetic data of this nature are often compiled to draw general conclusions about levels of reproductive skew (Reeve and Keller 1995), and inbreeding (Heinsohn et al. 1990; Pusey and Wolf 1996), it is important that this possibility is not overlooked. We outline here why conventional DNA fingerprinting is limited, and in some cases unsuitable, for studying parentage in social animals, and discuss the power of typing with microsatellite markers.

The technique of multilocus DNA fingerprinting suffers from the constraint that individual bands cannot be assigned to known loci making it impossible to determine whether a given band is of paternal or maternal origin (Jones et al. 1991; McRae 1996; Danforth and Freeman-Gallant 1996). Careful analyses are required to determine whether or not bands are segregating independently. For example, bands may be consistently scored together (linkage), or they may appear mutually exclusive (allelism). Although segregation analyses can be used to calculate the probability of error due to non-independence, this is not always helpful for parentage analysis when parental candidates are the first-order relatives of other parental candidates.

Paternity analysis with multilocus DNA fingerprints relies on the existence of ‘diagnostic’ bands to exclude individuals from parentage. The problem with analyzing fingerprints of cooperative groups arises uniquely when there is an adult helper in the group that is the full offspring of the dominant pair. This is a very common group structure among avian cooperative breeders referred to as ‘simple’ family groups (Emlen 1995). Take the example of a dominant pair whose adult son remains in the group as a helper. When a chick is the offspring of the dominant male and female (i.e., the full sibling of the helper), assuming the identity of the mother is reliable, there will often be (paternal) bands found in the fingerprints of the chick and the dominant male that are not found in that of the helper son. Thus it is usually possible to exclude the son from paternity. However, if a chick is the product of mother-son incest, it is impossible to exclude the dominant male (i.e., the actual grandfather

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of the chick) from paternity in this manner since there will be no bands in that chick's fingerprint that are not found in the fingerprint of either the dominant female or the dominant male (McRae 1996). The alternative hypothesis, that the son could be the father, should be tested directly. In studies that assume no incest a priori, its probability of being overlooked is high since no attempt is made to reject the hypothesis of incest through statistical analysis of the genetic data.

When the helper son is a full sibling of the chicks in the brood, he will share on average half of his maternal and half of his paternal bands with each chick. However, if the son breeds with his mother, it is not possible to determine whether 'maternal' bands in a chick's multilocus DNA fingerprint were inherited directly from the mother or via the son. Determination of whether each of the chicks is really the offspring of the dominant pair or the product of mother-son incest must be based on band-sharing coefficients which must then be compared with expected values under different parentage scenarios. In the above example with one adult son helper, the offspring of a mother-son mating would be expected to share $0.75(1+a)$ of its bands with its mother, where a=the level of background band-sharing for non-relatives, $0.50(1+a)$ with its father (the helper), and $0.25(1+a)$ with the dominant male (its grandfather). An offspring of the dominant pair would be the full sibling of the helper, and share $0.50(1+a)$ of its bands with each adult. If there are sexually mature helper sons in cooperative groups, and multilocus DNA fingerprinting is used to determine parentage, the distributions of band-sharing coefficients between chicks and adult group members should at the very least be compared with the expected distributions and reported. Depending on the level of variability, the result may be that the distributions are not statistically different from either of the expected distributions. In this case, there is no genetic basis on which to exclude the adult son helper from paternity of one or more of the chicks.

In a typical DNA fingerprint, not all fragments segregate independently, and it is rare that an equivalent number of maternal and paternal fragments are scored. This could arise because some alleles of a given locus lie outside the range of fragment sizes being scored. Ultimately, we do not really know how many independent loci we have. Band-sharing coefficients on their own are not reliable for diagnostic purposes because the levels of homozygosity within the population are unknown (Lynch 1990). Futhermore, few studies attempt to assess the degree of inbreeding in their population(s) by looking at background levels of band-sharing (Danforth and Freeman-Gallant 1996).

Single-locus genetic markers have the potential to be more useful in this regard because, for any given locus, bands can be assigned as either paternal or maternal when one or both parents are known. Thus, an offspring that is the product of an incestuous mating, as between mother and son, can be identified when for a given autosomal locus, the offspring matches two alleles from its mother (the dominant female) and none from the dominant male; whereas paternity can be assigned to the dominant male when the offspring's paternal allele is matched only with the dominant male's genotype and not that of the helper son (Fig. 1). To ensure paternity exclusion of one or other male requires variable loci. For a given locus, the probability of detecting incest increases logistically with the number of alleles (Fig. 2). Interpolating and substituting the number of alleles for seven autosomal microsatellite loci isolated for a study of

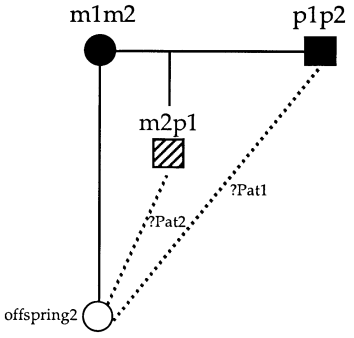

Fig. 1 Excluding incestuous matings at a single locus. In the diagram, a female genotype *m1m2* has mated a male genotype *p1p2* to yield an offspring with (in this example) genotype *m2p1*. All alleles are distinct. A subsequent offspring, *offspring2*, could have been fathered either by the original male (line *?Pat1*), or incestuously by the first offspring (line *?Pat2*). If offspring2 inherits allele p1, neither male can be excluded. However, inheritance of p2 or m2 would allow exclusion of Pat2 and Pat1, respectively. Exclusion is less likely when the male and female share common alleles

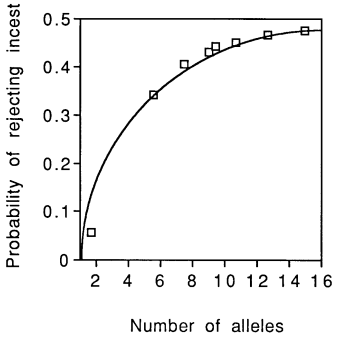

Fig. 2 Probability of rejecting incest. The graph depicts the probability of being able to exclude an incestuous mating by an older sibling using a single microsatellite locus in relation to the number of microsatellite alleles at that locus. The curve is fitted by eye and is expected to increase asymptotically from zero at a monomorphic locus towards its maximum value of 0.5 when all alleles are unique. For maximal realism, the data were generated by stochastic simulations, using a one-step, symmetric stepwise mutation model of the microsatellite in a finite population of 100. Allele number was varied by varying the mutation rate. Each simulation involved allowing evolution to occur for $4n$ (=400) generations to generate an allele frequency distribution. Then, 1000 mother-father pairs were created by random sampling of alleles, allowed to produce two offspring and then assayed for whether the first offspring could be eliminated as the father of the second. For each mutation rate, 100 replicate simulations were run and the mean number of alleles and probability of exclusion recorded

white-browed sparrow weavers *Plocepasser mahali* (McRae and Amos 1999), we calculated that we are able to exclude 95.6% of incestuous matings.

The situation described above is peculiar to cooperative breeding groups in which full offspring are retained beyond sexual maturity and overlap with subsequent broods. A different problem involving cooperative breeding groups is where one needs to distinguish paternity by, for example, two males that are first-order relatives but not related to the breeding female. This type of breeding group may arise when, for example, males disperse as coalitions of brothers (lions *Panthera leo*: Packer et al. 1991; acorn woodpeckers: Koenig et al. 1998). The difference here is that, with an unrelated female, there is not the potential problem of incest. In this case, although typing a large number of single loci is again the best method, multilocus DNA fingerprinting can be sufficient (or better if only a few single-locus markers are available). Nevertheless, in some cooperative breeding systems, groups can be either simple or complex (e.g., Whittingham et al. 1997). In these cases, again it is only possible to determine parentage in the simple family groups using single-locus methods.

Single-locus microsatellite markers constitute the most appropriate currently available molecular technique to use in parentage analysis of most conventional cooperatively breeding species. The limitations of multilocus DNA fingerprinting outlined above make it inappropriate for parentage analysis in some such cooperative breeding systems. To date, many studies have either ignored the problem of potentially misassigning parentage within cooperatively breeding groups completely, or simply stated that incest does not occur because it has never been observed directly (Lundy et al. 1998). Yet, genetic techniques have previously revealed unexpected levels of reproductive success among individuals, and have provided evidence of breeding by individuals assumed to be non-breeders (Burke et al. 1989; Gibbs et al. 1990; Dixon et al. 1994; Allen et al. 1995). Incest has been observed directly in a number of avian populations (see above), and been confirmed genetically in one of these populations by multilocus DNA fingerprinting analysis where eggs were marked as they were laid and the mother's identity could be deduced from distinctive egg shell patterns (McRae 1996). We cannot hope to understand the actual extent of incest in wild populations, where and why it occurs, nor whether it has deleterious effects when many researchers dismiss it without adequately testing for it.

It is particularly important to estimate accurately levels of inbreeding in cooperatively breeding populations in light of increasing interest in the idea that inbreeding avoidance is a major force driving the evolution of reproductive skew within social groups (Clutton-Brock 1998; Koenig et al. 1998). If incest is found to be rare in cooperatively breeding populations, at least we can be confident that we have tested for it adequately.

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References

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Mol Ecol 4:653–662

Brooker MG, Rowley I, Adams M, Baverstock PR (1990) Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? Behav Ecol Sociobiol 26:191–199

Bruce JP, Quinn JS, Sloane SA, White BN (1996) DNA fingerprinting reveals monogamy in the bushtit, a cooperatively breeding species. Auk 113:511–516

Bulger J, Hamilton WJ III (1988) Inbreeding and reproductive success in a natural chacma baboon *Papio cynocephalus ursinus* population. Anim Behav 36:574–578

Burke T, Davies NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251

Clutton-Brock TH (1998) Reproductive skew, concessions and limited control. Trends Ecol Evol 13:288–292

Danforth BN, Freeman-Gallant CR (1996) DNA fingerprinting data and the problem of non-independence among pairwise comparisons. Mol Ecol 5:221–227

Dickinson J, Haydock J, Koenig W, Stanback M, Pitelka F (1995) Genetic monogamy in single-male groups of acorn woodpeckers, *Melanerpes formicivorus*. Mol Ecol 4:765–769

Dixon A, Ross D, O'Malley SLC, Burke T (1994) Paternal investment inversely correlated to degree of extra-pair paternity in the reed bunting. Nature 371:698–700

Emlen ST (1995) An evolutionary theory of the family. Proc Natl Acad Sci 92:8092–8099

Gibbs HL, Grant PR (1989) Inbreeding in Darwin's medium ground finches (*Geospiza fortis*). Evolution 43:1273–1284

Gibbs HL, Weatherhead PJ, Boag PT, White BW, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. Science 250:1394–1397

Greenwood PJ, Harvey PH, Perrins CM (1978) Inbreeding and dispersal in the great tit. Nature 271:52–54

Haig SM, Walters JR, Plissner JH (1994) Genetic evidence for monogamy in the cooperatively breeding red-cockaded woodpecker. Behav Ecol Sociobiol 34:295–303

Heinsohn RG, Cockburn A, Mulder RA (1990) Avian cooperative breeding: old hypotheses and new directions. Trends Ecol Evol 5:403–407

Jones CS, Lessells CM, Krebs JR (1991) Helpers-at-the-nest in European bee-eaters (*Merops apiaster*): a genetic analysis. In: Burke T, Dolf G, Jeffreys AJ, Wolff R (eds) DNA fingerprinting: approaches and applications. Birkhäuser, Basel, pp 170–192

Keller L, Arcese P (1998) No evidence for inbreeding avoidance in a natural population of song sparrows (*Melospiza melodia*). Am Nat 152:380–392

Koenig WD, Haydock J, Stanback MT (1998) Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition. Am Nat 151:243–255

Ligon JD, Ligon SH (1990) Green woodhoopoes: life history traits and sociality. In: Stacey PB, Koenig WD (eds) Cooperative breeding in birds. Cambridge University Press, Cambridge, UK, pp 33–65

Lundy KJ, Parker PG, Zahavi A (1998) Reproduction by subordinates in cooperatively breeding Arabian babblers is uncommon but predictable. Behav Ecol Sociobiol 43:173–180

Lynch M (1990) The similarity index and DNA fingerprinting. Mol Biol Evol 7:478–484
McRae SB (1996) Family values: costs and benefits of communal breeding in the moorhen. Anim Behav 52:225–245
McRae SB, Amos W (1999) Characterization of hypervariable microsatellites in the cooperatively breeding white-browed sparrow weaver *Plocepasser mahali*. Mol Ecol 8:903–904
Mulder RA, Dunn PO, Cockburn A, Lazenby-Cohen KA, Howell MJ (1994) Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. Proc R Soc Lond B 255: 223–229
Packer C, Pusey AE (1993) Dispersal, kinship and inbreeding in African lions. In: Thornhill NW (ed) The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. University of Chicago Press, Chicago, pp 375–391
Packer C, Gilbert DA, Pusey AE, O'Brien SJ (1991) A molecular genetic analysis of kinship and cooperation in African lions. Nature 351:562–564
Pusey A, Wolf M (1996) Inbreeding avoidance in animals. Trends Ecol Evol 11:201–206
Rabenold PP, Rabenold KN, Piper WH, Haydock J, Zack SW (1990) Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. Nature 348:538–540
Reeve HK, Keller L (1995) Partitioning of reproduction in mother-daughter versus sibling associations: a test of optimal skew theory. Am Nat 145:119–132
Whittingham LA, Dunn PO, Magrath RD (1997) Relatedness, polyandry and extra-group paternity in the cooperatively breeding white-browed scrubwren (*Sericornis frontalis*). Behav Ecol Sociobiol 40:261–270

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