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FORUM

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Multilocus minisatellite DNA fingerprinting and cooperative breeding

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McRae and Amos (1999) make the valuable point that, when incest has occurred in cooperatively breeding birds, multilocus minisatellite DNA fingerprinting is limited in its ability to resolve parentage. Their general point is a good one, and one that they rightly point out has not been fully appreciated in studies of cooperative breeding. We nonetheless feel that they have overemphasized the problems associated with the application of multilocus minisatellite fingerprinting, as well as the advantages of using single-locus microsatellites.

The main difficulty McRae and Amos (1999) identify is that, when an offspring (*I*) results from an incestuous mating between a mother (*M*) and her son (*S*), it will not be possible to exclude the paternal grandfather (*F*) from paternity of *I*, by conventional band-matching analyses. If the son's father *F* is the dominant male in the group, he may be "assigned" paternity despite the inability to exclude the son *S*. This will lead to underestimates of the actual rates of incest. This is a valid point worthy of attention. However, it is premature to abandon minisatellite DNA fingerprinting in these applications in favor of microsatellites because (a) except in populations with unusually low genetic polymorphism, it will usually be possible to determine when incest has *not* occurred (i.e., it will be possible to exclude *S* when he is *not* the father); (b) when mother-son incest has occurred, it will often be possible to exclude *F* by other (bandsharing) analyses, as McRae and Amos (1999) mention (again, the exceptions will be in populations with low genetic polymorphism), and (c) the conditions of low genetic polymorphism under which (a) and (b) represent difficulties can also affect the variability of microsatellite loci. We will remark on each of these points in turn.

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Probability of detecting non-incest with fingerprinting

When son *S* is not the father of *I*, it is possible to estimate the confidence with which son *S* could be excluded, or the probability that he might be mistakenly assigned as the father by providing all of the exclusively paternally derived bands in *I*. Background bandsharing (*a*), the proportion of bands shared between dyads of unrelated adults, is related to the allele frequency *q* across the family of loci surveyed by a multilocus minisatellite probe, as $a=2q-q^2$ (Jeffreys et al. 1985). From there, a series of predictive equations has been developed by Jeffreys and others (Georges et al. 1988) for bandsharing coefficients for a variety of classes of relatives. For example, the expected proportion of bands shared (*s*) between a parent and its offspring is $(1+q-q^2)/(2-q)$ (Georges et al. 1988). In our experience, these equations predict quite accurately the bandsharing coefficients found for several categories of relatives (Piper and Parker Rabenold 1992).

For studies in which an average number of fingerprinting bands scored per individual (*x*) is about 25, as is typically the case either within or across independent probe-enzyme combinations, then the average number of maternally derived bands scored will be 25*s*. This leaves the number of exclusively paternally derived bands with which paternity can be determined, *p*, as $(25-25s)$, or $x(1-s)$ (Parker Rabenold et al. 1991). As the number of bands scored increases, the number of exclusively paternally derived bands, and thus the resolution with which parentage determinations are made, increase as well. For the son *S* to be mistakenly identified as the father when in fact he was not, he would have to provide all of the exclusively paternally derived bands, which means that

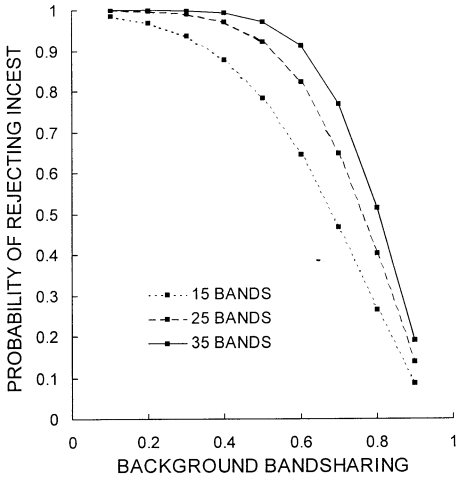

Fig. 1 The probability of rejecting son *S* as the father of a full sibling offspring of its mother *M* and father *F*, as a function of background bandsharing. This graph represents a typical range for the numbers of bands scored per individual in multilocus minisatellite DNA fingerprint lanes. Typically, with one probe-enzyme combination, 15–25 bands per lane can be scored; additional probe-enzyme combinations would usually be necessary to score as many as 35 bands per individual. The probability that a son could be assigned as parent depends on his having all of the paternally derived bands, and therefore sharing them with the actual father (his own father). The likelihood of this happening is s^p, where *s*=the likelihood that father and son would share any particular band (or expected father-son bandsharing) and *p*=the number of exclusively paternally derived bands (see text). Here we plot ($1–s^p$), the probability of rejecting incest when the son fails to match paternal bands in the offspring, its sibling

he would have to share them with the actual father. The probability of father *F* and son *S* sharing the *p* bands that occur in *I* is the probability that they would share any particular band, *s*, raised to the number of exclusively paternally derived bands, *p*, or s^p (Parker Rabenold et al. 1991). The probability of rejecting incest is ($1–s^p$). The probability that *S* would be excluded from paternity is quite high at low values of background bandsharing (Fig. 1), but declines rapidly when background bandsharing rises above 50–60%. At any background bandsharing level, the likelihood of falsely assigning the son *S* declines as more bands are scored. For values of background bandsharing <50%, as most are, the application of multilocus minisatellite fingerprinting is significantly more efficient at rejecting incest than a microsatellite locus, even one with many alleles (Fig. 2 in McRae and Amos 1999). For cooperatively breeding stripe-backed wrens (*Campylorhynchus nuchalis*) and bicolored wrens (*C. griseus*), the probability of rejecting the older siblings as fathers of current nestlings is >0.9998 for both species (Rabenold et al. 1990; Haydock et al. 1996). Fingerprinting is still more powerful than all eight of McRae and Amos' (1999) microsatellite loci combined (97.2%), when bandsharing is 40% or lower and 25 bands are scored, or when bandsharing is 50% or lower and 35 bands are scored (Fig. 1). Studies that do not meet these criteria are the exception. Even for Arabian babblers (*Turdoides squamiceps*), with their unusually high background bandsharing of 0.66, the probability of being able to exclude the older brother as father is 0.952 (Lundy et al. 1998), counting only two of the six probe-enzyme combinations applied to the difficult cases.

Possibility of exclusion of *F* in cases of *M-S* incest

The problem identified by McRae and Amos (1999) is that all fingerprinting bands in *I* (the offspring resulting from an incestuous mating between a mother *M* and her son *S*) will be fully attributable to the combinations *M-S*, the actual parents, and *M-F* (where *F* is the social mate of *M* and the father of *S*). This occurs because all of the bands in *I* that came from *S* came from either *M* or *F*.

However, the proportion of *I*'s bands shared with older birds in the group will differ predictably depending on whether incest has occurred. McRae and Amos (1999) suggest that, when incest has occurred, the proportion of bands shared between the offspring and its mother should be $0.75(1+a)$, where *a* is the mean background bandsharing among non-relatives. Likewise, they suggest that the bandsharing between *I* and *S* (its father) should be $0.50(1+a)$, and between *I* and *F* (its grandfather) should be $0.25(1+a)$. We find these expressions to be erroneous in two important ways.

First, each expression incorrectly accounts for the addition of *a* (the background bandsharing). In general, sharing of bands among non-relatives will increase the overall bandsharing values between members of any category of relatives. Obviously, bandsharing between relatives will not be *less* than bandsharing between non-relatives (as can occur in McRae and Amos' calculation of bandsharing between *I* and *F*), nor can bandsharing ever exceed 1.0 (as can occur in McRae and Amos' calculation of bandsharing between *I* and *M*). Each expression should indicate the extent to which bandsharing is elevated above background in each category of relative, and thus will be better estimated by the background among non-relatives plus the proportion of bands shared by common descent (*r*) among the variable bands. They have correctly identified this modifier as 0.25 for the grandfather *F* in the case of incest, and 0.75 for the mother *M* in the case of incest. However, they have incorrectly identified the modifier as 0.50 for the father *S* in the case of incest. Since this individual is both the father and half-brother of *I*, the appropriate *r* is 0.75 as in the mother. Thus, using this approach, the more accurate expressions predicting bandsharing between the different categories of relatives are: $[a+0.75(1–a)]$ for *I* with *S* and *I* with *M*; $[a+0.50(1–a)]$ for offspring with outbred parents, and $[a+0.25(1–a)]$ for *I* with *F*. However, since fingerprinting cannot clearly distinguish between the heterozygous and homozygous state for particular alleles, the use of *r* as the modifier is problematic in the case of inbreeding, which increases homozygosity in the inbred individual. This suppresses the value of bandsharing between *S* and *I* (by inability to detect double-hits) to something closer to $[a+0.67(1–a)]$.

An even more accurate approach is to extend the logic of Jeffreys et al. (1985) and Georges et al. (1988) mentioned above to include the cases of incest. We have developed equations expressing the predicted bandsharing for *I* with *S* and *I* with *F* based on population allele frequency *q*. Any particular band found in *I* could be in either the homozygous or heterozygous state, with the sum probability of being in either state given by q^2+2pq, or $(2q–q^2)$. If *I* has a particular band, the probability that it is shared with *S* is expressed in two parts, representing the two possible conditions under which the allele occurs in *I*:

$$\frac{q^2}{2q-q^2}+\frac{2q(1-q)}{2q-q^2}[0.5+0.5(0.5q+0.5(0.5+0.5q))]$$

Using Georges et al.'s (1988) logic for the expected bandsharing between an offspring and parent, the probability of *I* being a homozygote, $q^2/(2q–q^2)$, is multiplied by one because in that case *I* necessarily shares the band with *S*. The probability of *I* being a heterozygote, $2q(1–q)/(2q–q^2)$, is multiplied by the probability that *I* got the band from *S* (0.5) or that *I* got the band from *M* (0.5). If *I* is a heterozygote and got the band from *M*, *I* could still share the band with *S* if *S* has the band, but did not give it to *I*. This latter probability $[0.5q+0.5(0.5+0.5q)]$ includes the probability that *S* got the same band from *F* $(0.5q)$ or that *S* got it from *M* $[0.5(0.5+0.5q)]$, which accounts for the probability that the band is the same band that *M* gave to *I* (0.5), or that *M* had that band anyway in the position not passed to *I* $(0.5q)$. This expression simplifies to $(5+2q–3q^2)/(8–4q)$.

The bandsharing between *I* and *F* can also be expressed as a function of the allele frequency *q* as follows:

$$\frac{q^2}{2q-q^2}[0.5+0.5q]+\frac{2q(1-q)}{2q-q^2}[0.25+0.75q]$$

Here, the probability of *I* being homozygous, $q^2/(2q–q^2)$, is multiplied by the probability that its paternal copy of the band was given to *S* by *F* (0.5), or that it was not, and *F* had the band anyway $(0.5q)$. The probability of *I* being heterozygous, $2q(1–q)/(2q–q^2)$, is multiplied by the probability that it got the band from *F* through *S* (0.25) or that it did not, and *F* had the band anyway $(0.75q)$. This equation simplifies to $(1+3q–2q^2)/(4–2q)$.

These expectations are plotted in Figure 2 as a function of population background bandsharing *a*, which is related to q as $(a=2q–q^2)$. At low background bandsharing values, the distinction between the two possible fathers (*F* and *S*) in terms of their bandsharing values with *I*, is quite large (see dotted and solid lines in Fig. 2), and much larger than predicted by the erroneous expressions of McRae and Amos (1999). However, as background bandsharing values increase, all categories of relatives become somewhat more difficult to distinguish. At the highest background bandsharing values, the problem identified by McRae and Amos (1999) comes into play. However, the low polymorphism indicated by this high background bandsharing will hinder the application of any marker in this situation, even microsatellites.

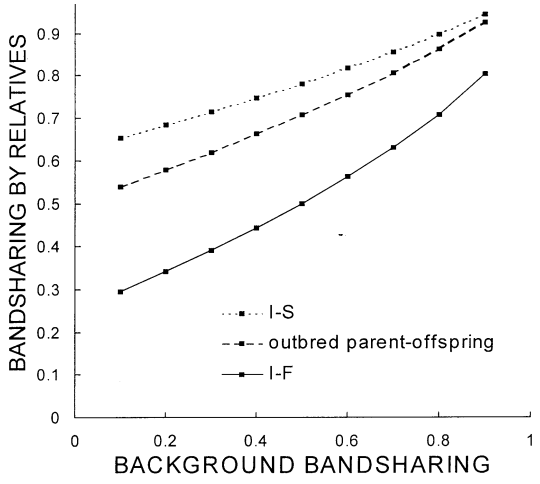

Fig. 2 The expected proportions of bands shared between offspring and their parents under outbreeding and close inbreeding. Expectations are based on the logic presented by Jeffreys et al. (1985) and Georges et al. (1988). The background bandsharing value (*a*) is related to the population allele frequency *q* as $a=2q–q^2$ (Jeffreys et al. 1985). The *dashed line* depicts the bandsharing expected between outbred young and their parents $(1+q–q^2)/(2–q)$ (Georges et al. 1988). When incest occurs between a mother *M* and son S, while the son's father *F* is still present, the top and bottom lines represent expected proportions of bands shared between the incestuous offspring *I* and its father *S* [*dotted line*; bandsharing $I\text{-}S=(5+2q–3q^2)/(8–4q)$], and between the incestuous offspring and its grandfather *F* [*solid line*; bandsharing $I\text{-}F=(1+3q–2q^2)/(4–2q)$] (see text for derivations)

Difficulties presented by microsatellites

The great advantage afforded by microsatellite markers is their ability to establish allelic identity – to allow a researcher to establish each individual's heterozygosity or homozygosity for particular alleles on a locus-by-locus basis. In this application, this advantage frequently allows the identification of paternal alleles in *I*, and therefore allows us to exclude *F* (if *S* passes on a maternally derived allele not shared with *F*) or *S* (if *F* passes on an allele different from the one he transmitted to *S*). Once again, the power of this ability to resolve parentage in the case of incest requires having a sufficient number of sufficiently polymorphic loci at which the mother and father share no alleles, as depicted by McRae and Amos (1999) in their Fig. 1. When parents share alleles at some or all loci, some ambiguity remains.

In a population characterized by low genetic polymorphism, it is unlikely that many highly polymorphic microsatellite loci will be easily found. It is important to recognize that the situations limiting the application of multilocus minisatellite fingerprinting will create difficulties for the already difficult development of polymorphic markers for microsatellites as well. These two variable number of tandem repeat (VNTR) approaches provide the greatest possible resolution of the genetic polymorphism available in natural populations. Minisatellite and microsatellite loci represent two points along a continuum based on size of repeat unit. Because minisatel-

lite repeat units are large compared to microsatellites, they are not easily accessible on a locus-by-locus-basis through the polymerase chain reaction. They are instead assayed through gel-blot hybridization, using the repeat sequence itself as a probe. This allows the screening of a great many loci simultaneously. Estimates of mutation rates for minisatellites exceed those for microsatellite loci, meaning that minisatellite markers should typically be more variable than microsatellites (Queller et al. 1993; Fleischer 1996). When minisatellite fingerprinting comes up dry, showing little variability, that tells us something important about the history or structure of the population, which in turn is likely to mean that VNTR polymorphism at less mutable loci like microsatellites will be very, very difficult to find. Refer to McRae and Amos (1999) Fig. 2; when genetic polymorphism is so low that fingerprinting is problematic, it is unlikely that many microsatellite loci will be found with many alleles. Those that are polymorphic might be expected to have a small number of alleles, making the probability of rejecting incest low for microsatellites.

Most studies of cooperatively breeding birds of which we are aware have background bandsharing values within the range for which fingerprinting is quite useful. Among our studies, background bandsharing for stripe-backed wrens (*C. nuchalis*) was 0.27 for one fingerprinting probe and 0.26 for the other (Rabenold et al. 1990); for bicolored wrens (*C. griseus*), 0.37 for one probe and 0.24 for the other (Haydock et al. 1996); for acorn woodpeckers (*Melanerpes formicivorus*), 0.23 for one probe (Dickinson et al. 1995), and for western bluebirds (*Sialia mexicana*), 0.23 for one probe (Dickinson and Akre 1998). However, we have been involved in two studies for which bandsharing is in the range that causes problems, one of which McRae and Amos (1999) highlighted. In Arabian babblers (*Turdoides squamiceps*), background bandsharing exceeded 60% (Lundy et al. 1998). The problem of low polymorphism was even worse for our study of Galapagos hawks (*Buteo galapagoensis*) (Faaborg et al. 1995), where background bandsharing was 0.63 for one probe and 0.74 for another. (And this was on the "good" island of Santiago, which had some genetic polymorphism; on the island of Santa Fe, background bandsharing was >90% and determination of parentage was not attempted.) However, since the cooperating males in groups of Galapagos hawks are not retained young of a dominant pair, but unrelated males instead, we expect cooccurrences of fathers and sons in these groups to be exceedingly rare. Nonetheless, we will return to the hawk and babbler data sets to reexamine our findings as a result of this discussion.

We appreciate that McRae and Amos (1999) have brought this problem into sharp focus, instigating this most constructive exercise, so that we can begin to clarify the situations under which alternative techniques should be sought. Our conclusion is that multilocus minisatellite DNA fingerprinting is the best starting point for studies of cooperatively breeding birds, and will likely be entirely sufficient for populations with standard low background bandsharing values (<50–60%), a point whose implications we had not fully appreciated before. As McRae and Amos (1999) suggest, explicit comparisons of bandsharing values against those predicted by incest should become a standard part of such an analysis. Background bandsharing values greater than 50–60% are unusual, but when they occur, additional techniques might be considered. However, we expect that they, too, will be problematic.

Our laboratories have full minisatellite and microsatellite capability, and we use microsatellites for other studies. Our choosing fingerprinting for studies of cooperative breeding is not just a choice of convenience, although it is true that fingerprinting is significantly more efficient in terms of time and money. One great advantage of microsatellites in any study of parentage is their ability to help identify the father of chicks whose social father has been excluded by fingerprinting. This application would benefit studies of cooperative breeders with extragroup paternity, as well as studies of socially monogamous or polygynous systems, particularly when neighborhoods are large.

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