DNA fingerprinting reveals relation between tail ornaments and cuckoldry in barn swallows, *Hirundo rustica*

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In an experimental study in Denmark, it was previously found that male barn swallows (*Hirundo rustica*) with elongated tail streamers obtained an apparent fitness advantage through earlier pairing, an increased frequency of second clutches, and higher total reproductive success per season. In a parallel study of five barn swallow colonies in Ontario, Canada, we also found that elongated males paired earlier and thus were apparently preferred by females. Now, using DNA fingerprinting on families from two of those Ontario colonies, we show that five elongated males fathered only 59% of the offspring in their nests, whereas six shortened males fathered 96% of their nestlings. Thus, although elongated males were clearly preferred by females at the time of pair formation, tail elongation may have hampered the ability of a male to guard his mate, resulting in an increase in extrapair fertilizations (EPFs). A significant negative correlation between the number of EPFs and natural tail length in this experimental study also suggests that tail streamer length may reflect male quality. [Behav Ecol 1990; 2:90–98]

Even though Darwin (1871) clearly saw that the long and elaborate tails characteristic of many male birds during the breeding season deserved explanation, it was not until Malte Andersson’s (1982a) classic experimental study of the long-tailed widowbird (*Euplectes progne*) that detailed study of such tail ornamentation began. Since then, a variety of both experimental intraspecific and comparative interspecific studies have appeared, confirming that elaborate tails are ornamental traits under the influence of sexual selection through female choice as Darwin had deduced. Indeed, experimental studies showing that male birds with longer tails obtain a mating advantage provide some of the clearest evidence in support of Darwin’s theory.

Such experimental studies have found evidence that females prefer males with longer tails in both long-tailed (Andersson, 1982a) and Jackson’s widowbirds (*E. jacksoni*) (Andersson, 1990), in shaft-tailed whydahs (*Vidua regia*) (Barnard, 1990), and in the barn swallow (*Hirundo rustica*) (Møller, 1988; Smith and Montgomery, 1991). Using the comparative method, it has also been shown that tail ornaments are more variable, within species, than traits (like tarsi) under the influence of viability selection (Alatalo et al., 1988); that paternal care (incubation) occurs less often in swallow species (genus *Hirundo*) with longer-tailed males (Smith and Montgomery, 1991); and that there is a positive relation between tail length and degree of polygyny among species (Winquist T, personal communication).

All of these relations are expected if tails are under the influence of sexual selection and if sexual selection is more intense in polygynous species (Arnold, 1983).

Experimental studies so far have all used a similar protocol. Before females choose their mates, males are captured, and their tail length is manipulated either by adding or removing a section of the longest feathers, resulting in up to a 50% shortening or elongation of the tail. As a result of these manipulations, males with elongated tails have obtained mates earlier in the season (Møller, 1988; Smith and Montgomery, 1991) or more mates (Andersson, 1982a), or females have behaved in ways indicating a preference for elongated males (Andersson, 1990; Barnard, 1990). In each case it is assumed that elongated males obtained a fitness advantage from the female choice even if that advantage accrued only through earlier breeding (Kirkpatrick et al., 1990). Even in barn swallows, where monogamy is the rule, males with artificially elongated tail streamers (i.e., the longest outer tail feather on each side) paired earlier (Møller, 1988; Smith and Montgomery, 1991). In Denmark (Møller, 1988), elongated males also had second clutches more often, raised more nestlings per season, and obtained more ex-
trapair copulations (EPCs) than males with shortened streamers. Thus, sexual selection appears to act through an omnibus fitness advantage to males with longer tails.

While the results of the barn swallow studies seem straightforward, Möller’s (1987) finding of relatively high EPC rates in this species suggested to us that a simple counting of fledglings would not provide a reliable index of reproductive success. Indeed, in Möller’s (1988) study, females mated to elongated males engaged in significantly fewer EPCs than those mated to shortened males, suggesting that elongated males may be cuckolded less frequently, thereby achieving even higher reproductive success than shortened males. To evaluate the reproductive success of males with elongated and shortened tails more accurately, we employed DNA fingerprinting (for review see Burke, 1989) to determine the actual paternity of nestlings in our own experimental study (Smith and Montgomerie, 1991). We report on the results of that analysis here.

**METHODS**

**Field study**

We studied barn swallow colonies in the vicinity of Chaffey’s Locks, Ontario, Canada (44°53’ N, 76°19’ W), during their May–August breeding season in 1989 (for details see Smith and Montgomerie, 1991). For the parentage investigation reported here, we analyzed data only from first broods in two colonies (of 7 and 11 pairs) in which we were able to alter the streamer length of all males experimentally. We focus on first broods because few pairs were successful in raising second broods and the success of second broods was not significantly related to the tail manipulation (Smith and Montgomerie, 1991).

We captured each male and all but two females in these two colonies as soon as possible after they arrived in spring. All birds captured were given one or two plastic color bands and one numbered metal band so that they could be individually recognized. In addition, we marked their wings (with acrylic paint) and/or their chest (with an aniline dye) to facilitate later identification from a distance. We determined the identity of pair members by observing nests during incubation and nesting periods. Each color-marked male and female was unequivocally assigned to a nest in each colony.

Captured birds were sexed using tail length and plumage criteria adapted from Pyle et al. (1987; see also Smith and Montgomerie, 1991). We determined body mass to the nearest 0.1 g using a Pesola balance and flattened wing length to the nearest millimeter using a ruler with a perpendicular stop at 0 (see Pyle et al., 1987). We used dividers and a ruler to measure the total length (to the nearest 0.5 mm) of each tail streamer from its base to its distal end. Using dial calipers, we also measured the tip of each streamer as the distance (to the nearest 0.1 mm) from its distal end to the distal end of the feather beside it. We measured the streamer on each side of a male’s tail and used the mean of these two measurements in all analyses of original streamer length reported here.

All males captured had both streamers shortened or elongated by 20 mm. For details about the manipulation technique, see Möller (1988) and Smith and Montgomerie (1991). In each colony, we alternated consecutively between shortening and elongating streamers to provide (1) a random treatment effect, (2) sections of streamer for tail elongation, and (3) approximately equal numbers of shortened and elongated males in each colony. We did not perform any of the sham and unmanipulated controls that were performed by Möller (1988) (see Discussion).

We collected 100–150 μl of blood from most birds by jugular venipuncture. This method provided more than enough blood for DNA fingerprinting analysis and had no noticeable effect on the birds. Birds captured by day were released immediately after processing (usually less than 60 min after capture); birds captured by night were held in cloth bags for release at daybreak. Immediately after collection, blood was transferred to vacutainers coated with EDTA (pH 8.0). These vacutainers were then kept on ice for up to 4 h before being transferred to storage at −20°C for up to 8 months before further processing.

Nests were inspected every 1–3 days during the egg-laying period in each colony so that the dates of clutch initiation and completion could be established. We checked each nest daily around the estimated hatch date so that nestling ages could be determined. When nestlings were about 8 days old, we banded them and collected blood, as described above.

For this paternity analysis, we included only those families in which the attending male was manipulated more than 5 days before the female laid her first egg and for which we had usable blood samples from the male, the female, and all of their nestlings that lived to be 8 days old. We used this criterion because the 5 days before egg laying begins are believed to be the period of maximum female fertility (Möller, 1988). Thus, we included only those males whose manipulated tail lengths could have had an effect on the paternity of their nestlings. In these 11 families, males were manipulated 8–34 days (x = 17.2 days) before their mates began egg laying.
DNA fingerprinting

To prepare blood for DNA fingerprinting, we added 3.5 ml of a lysis buffer (4 M urea, 0.2 M NaCl 0.1 M Tris-HCl at pH 8.0, 0.5% n-lauroylsarcosine, and 10 mM EDTA) and 0.5 ml proteinase-K (approximately 80 units) to 50 µl and 75 µl of thawed adult and nestling blood samples, respectively. These samples were then incubated overnight at 37°C.

DNA was extracted from these samples and purified by two phenol: chloroform (70:30 ratio) extractions and one chloroform extraction using a Nucleic Acid Extractor (Applied Biosystems Inc., Model 340A). DNA was precipitated with the addition of 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 95% ethanol and recovered by filtration through Teflon-coated filters. This DNA was then dissolved in an appropriate volume of a Tris-EDTA buffer (0.1 M Tris-HCl at pH 8, 10 mM NaCl, and 2 mM EDTA), and concentrations were determined by absorbance reading at 260 nm using a Beckman DU-8 spectrophotometer. Typically, 50–75 µl of blood yielded 200–400 µg of DNA.

Next, we digested 15 µg of this DNA with Alu I (using conditions recommended by the manufacturer, Bethesda Research Labs) plus 4 mM spermidine at 37°C for 3 h. The DNA fragments were ethanol precipitated and dissolved in the Tris-EDTA buffer. Three micrograms of DNA were then mixed with 5 µl of loading buffer (0.5 Orange G, 15% ficoll, 50 mM EDTA) at pH 8.0, 5 ng of a lambda DNA marker (BstE II + Hind III/EcoRI cocktail), and distilled water to a total volume of 30 µl.

Samples were loaded onto and electrophoretically size-fractionated at 1.3 volts/cm through a 20 cm long 0.8% agarose gel for 48 h until all fragments <0.9 kilobases (kb) long had migrated off the gel. The male, female, and all of the nestlings from each nest were always loaded onto the same gel to facilitate comparisons. Subsequently, the gel was depurinated by treatment with 0.25 M HCl for 10 min, denatured with 0.4 M NaOH and 0.6 M NaCl, neutralized with 0.5 M Tris-HCl (pH 7.0) and 1.5 M NaCl, and Southern blotted onto GeneScreenPlus transfer membrane. The Southern blots were then prehybridized for 3–8 h using the method of Westneat et al. (1988).

We used three different 32P-labeled probe DNAs: Jeffreys 33.15 (Jeffreys et al., 1985), the M2.5 repetitive sequence from the Per gene in mice (Georges et al., 1988), and bacteriophage lambda DNA (Galbraith et al., 1991). These probes were prepared by primer extension and were separately added to the Southern blots to produce three different autoradiograms, as described below. Hybridization was carried out at 65°C overnight. We washed the blots at 65°C in 2 × SSC, 0.1% SDS for Jeffreys 33.15 and lambda DNA probing and at 65°C in 0.5 × SSC, 0.1% SDS for Per probing. Each blot was autoradiographed for 1–14 days at −70°C using Cronex 4 or Kodak XAR 5 films and a single Cronex intensifying screen. Blots were stripped with 0.4 M NaOH at room temperature for 20–30 min and neutralized for 20–30 min before re-probing.

Based on the number of bands detected (Table 1) and their segregation in sibships, both Per and Jeffreys 33.15 probes appear to produce DNA fingerprints for barn swallows similar to those described for other bird species (Burke and Bruford, 1987; Burke et al., 1989; Gibbs et al., 1990; Gyllensten et al., 1990; Westneat, 1990; Watton et al., 1987). Thus, all bands present in a nestling should also be present in one or both true parents.

Analysis of fingerprints

All fingerprint autoradiograms were scored independently by H.G.S. and R.M. so that the reliability of our methods could be assessed quantitatively. In general, we followed the methods described by Galbraith et al. (1991) for scoring fingerprints by using acetate overlays on which we marked the positions of bands and their uniqueness with respect to those of other family members. On each autoradiogram, only family units and the sexes of adults were identified so that all scoring could be accomplished without reference to the actual identity or experimental status of individuals.

For the analysis presented here we scored only those bands in the 3.5–21.2 kb range (Figure 1) where bands were most distinct, but our results were not changed by scoring from 0.95 to 21.2 kb (unpublished data). Bands were considered to be the same if their centers were ≤1 mm apart and if they were not exceptionally different in density (Figure 1). We used this technique to provide an objective and repeatable method for scoring fingerprints because, as yet, there appears to be no consensus on how this should be done (see, for example, Burke and Bruford, 1987; Galbraith et al., 1991; Gyllensten et al., 1990; Jeffreys et al., 1985; Watton et al., 1987).

We used two methods in combination to detect illegitimate offspring. First, bands occurring in a nestling that were not present in either parent were considered to be diagnostic (Burke, 1989). Thus, we considered these unique bands to indicate that one (or both) of the putative parents in the family was not the true genetic parent of that nestling. Following the method of Watton et al. (1987), we calculated the probability of erroneously as-
Table 1
Summary of one fingerprinting analysis for each nest studied using blots probed with Per.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Tail</th>
<th>Bands/bird</th>
<th>No.</th>
<th>$D_m$</th>
<th>$D_l$</th>
<th>Unique bands/bird</th>
<th>No.</th>
<th>$D_m$</th>
<th>$D_l$</th>
</tr>
</thead>
<tbody>
<tr>
<td>QB-101</td>
<td>S</td>
<td>13-17</td>
<td>3</td>
<td>0.48-0.50</td>
<td>0.47-0.58</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QB-103</td>
<td>E</td>
<td>8-12</td>
<td>6</td>
<td>0.50-0.80</td>
<td>0.50-0.73</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QB-106</td>
<td>S</td>
<td>9-11</td>
<td>5</td>
<td>0.50-0.67</td>
<td>0.30-0.57</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QB-109</td>
<td>E</td>
<td>10-15</td>
<td>2</td>
<td>0.64</td>
<td>0.56</td>
<td>3-6</td>
<td>0.15-0.26</td>
<td>0.40-0.50</td>
<td></td>
</tr>
<tr>
<td>CS-202</td>
<td>S</td>
<td>6-11</td>
<td>2</td>
<td>0.56-0.67</td>
<td>0.31</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS-206</td>
<td>E</td>
<td>8-12</td>
<td>4</td>
<td>0.64-0.83</td>
<td>0.50-0.67</td>
<td>1</td>
<td>3</td>
<td>0.30</td>
<td>0.63</td>
</tr>
<tr>
<td>CS-209</td>
<td>S</td>
<td>13-19</td>
<td>5</td>
<td>0.56-0.71</td>
<td>0.47-0.60</td>
<td>0</td>
<td>1</td>
<td>0.27</td>
<td>0.64</td>
</tr>
<tr>
<td>CS-214</td>
<td>S</td>
<td>9-12</td>
<td>4</td>
<td>0.48-0.67</td>
<td>0.57-0.64</td>
<td>1</td>
<td>3</td>
<td>0.27</td>
<td>0.64</td>
</tr>
<tr>
<td>CS-227</td>
<td>S</td>
<td>9-12</td>
<td>3</td>
<td>0.46-0.63</td>
<td>0.50-0.78</td>
<td>1</td>
<td>6</td>
<td>0.07</td>
<td>0.58</td>
</tr>
<tr>
<td>CS-245</td>
<td>E</td>
<td>11-20</td>
<td>2</td>
<td>0.52-0.57</td>
<td>0.39-0.52</td>
<td>4</td>
<td>0-3</td>
<td>0.10-0.20</td>
<td>0.53-0.78</td>
</tr>
</tbody>
</table>

* See text for details.
* Identity of nests in colonies QB or CS (for details see Smith and Montgomery, 1991).
* Tail length manipulation: S = shortened, E = elongated.

Band similarity coefficients ($D$) (see Methods) were calculated for each offspring relative to the male ($D_m$) and female ($D_l$) that attended their nest (i.e., the putative parents).

signing an unrelated male as the true father (assuming correct maternity) by this method as $< 4 \times 10^{-3}$. Second, we also calculated band-sharing coefficients ($D$) as described in Wetton et al. (1987). These $D$ values represent the proportion of bands shared in common between two individuals. In other studies of birds, unrelated individuals have been generally found to have $D \leq 0.30$, whereas parents and their offspring have $D \geq 0.50$ (Gibbs et al., 1990; Reeves et al., 1990; Westneat, 1990).

Unique diagnostic bands and relatively low band-sharing coefficients ($D \leq 0.30$) were used to identify illegitimate nestlings. We used this combination of methods because mutations sometimes result in unique bands on fingerprints (e.g., Burke and Bruford, 1987; Burke et al., 1989), and $D$-values between unrelated individuals can occasionally be high (e.g., Burke and Bruford, 1987; Burke et al., 1989; Gibbs et al., 1990; Reeves et al., 1990; Westneat, 1990).

In this paper we also report only on the results obtained from blots probed with Per because these produced the clearest autoradiograms for all individuals studied. Although Per and Jeffreys 33.15 probes often produced a different number of unique bands per individual and different band-sharing coefficients ($D$) between individuals, both resulted in the same assessment of illegitimate offspring from all blots. A similar difference was found between the results obtained by the two scorers, but both scorers and both probes produced qualitatively identical results with respect to the assessment of parenthood (unpublished data). For example, the band-sharing coefficients obtained by both scorers were significantly correlated (e.g., for $D$ values comparing nestlings and each putative parent on Per blots; $r = .79$, $p = .0001$, $n = 90$ birds), but, for a variety of reasons, $D$ values calculated for the same individual bird were rarely identical. Thus, both scorers obtained identical $D$ values for only 1 of the 90 nesting versus putative parent comparisons on blots probed with Per, even though 68% of the values were within 0.1, and none were more different than 0.25. Because the final assessment of parentage of each nestling was entirely concordant between scorers (unpublished data), we present only the results obtained by H.G.S. here.

Statistical methods

Before performing statistical tests, we checked residuals for normality using the Lilliefers test (Lilliefers, 1967). Data were transformed to help normalize when the departures from normality were significant ($p < .05$).

RESULTS

Illegitimate nestlings

We identified a total of 10 illegitimate nestlings occurring in 5 of the 11 families studied (Table 1). Thus, in five families there were from one to four nestlings that were not the true genetic offspring of one or both of the adults attending the nest. These offspring had, on average, 3.2 ($\pm 0.71$ SE) unique bands (Table 1). For two of these nestlings (nest CS-253, see Table 1), there were no unique bands on the Per autoradiograms scored by H.G.S.,

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but for both of these nestlings $D$ values were very low (0.10 and 0.11), and the Jeffreys 33.15 probe revealed two and four unique bands, respectively. Similarly, one nestling (in nest CS-227) had a single unique band when probed with the Jeffreys 33.15 but none when probed with Per. Because $D$ values for both parents with both probes were high (for Per, $D = 0.78$ and 0.46; for Jeffreys 33.15, $D = 0.52$ and 0.71, for putative mother and father, respectively), we considered this to be a legitimate offspring in this nest.

Based on band-sharing coefficients, all of these offspring were the progeny of the female attending the nest and not of the putative father (Table 1). The average $D$ ($\pm SE$) for 35 legitimate nestlings and their true parents was 0.59 ($\pm 0.09$) for fathers and 0.54 ($\pm 0.12$) for mothers. For 10 nestlings identified as illegitimate, average $D$ was 0.18 ($\pm 0.08$) with their putative father and 0.57 ($\pm 0.12$) with their putative mother. Furthermore, we detected no irregularities in the timing of egg laying that might indicate intraspecific nest parasitism (see Möller, 1987) in any of these nests.

**Streamer length and paternity**

Since we manipulated males at random, no significant differences between morphological traits were expected. With small sample sizes, however, mean values are unlikely to be identical. Thus, the mean original tail length of shortened males was longer (93.3 mm $\pm 2.3$ SE, $n = 6$) than that of elongated males (88.5 mm $\pm 1.6$, $n = 5$) but not significantly so ($t = 1.64$, $p = .13$). Small differences in wing length ($t = 1.12$, $p = .29$) and mass at capture before breeding ($t = 0.29$, $p = .78$) between males with elongated and shortened streamers were also not significant.

Interestingly, males did not receive the expected fitness advantage (Möller, 1988; Smith and Montgomery, 1991) from elongated streamers (Figure 2A). Although the mean clutch size of elongated males ($4.4 \pm 0.51$, $n = 5$) was slightly higher than that of shortened males ($3.8 \pm 0.54$, $n = 6$), the difference is far from significant ($t = 0.75$, $p = .47$). Moreover, DNA fingerprinting revealed that elongated males had significantly more illegitimate offspring in their nest ($\bar{x} = 1.8 \pm 0.73$, $n = 5$) than did shortened males ($\bar{x} = 0.17 \pm 0.17$, $n = 6$; $t = 2.37$, $p = .04$).

**Figure 2**

Percent of nestlings that were illegitimate (i.e., due to extrapair fertilizations) in the nests of male barn swallows with shortened ($n = 6$) or elongated ($n = 5$) tail streamers, as a function of (A) experimental manipulation (dot size reflects the number of coincident points) and (B) original (premanipulation) tail streamer length.

Since the clutch sizes of elongated and shortened males were not significantly different, we focus (in the remaining analyses) mainly on the proportion of nestlings fathered as an indication of the degree of cuckoldry incurred by different males. In all analyses, proportions were arcsine-transformed to help normalize (using Equation 14.5 in Zar, 1984).

It is clear that elongated males suffered more cuckoldry than males with shortened streamers. For example, five of six nests of elongated males had illegitimate nestlings compared to only one of five nests of shortened males (Fisher’s Exact Test, $p = .08$). As a result, the average proportion of illegitimate offspring in the nests of males with elongated streamers (46%) was significantly higher than in nests of shortened males (3%; $t = 2.40$, $p = .04$, $n = 5$, 6). In all, 41% of all nestlings (9/22) raised by elongated males were illegitimate, whereas only 4% (1/23) were illegitimate in nests of shortened males.

We found no relations between mass at capture or wing length and proportion of offspring fathered (for length, $r = -0.38$ and $-0.02$, respectively, $p > .2$, $n = 11$), and males with and without
illegitimate offspring did not differ significantly in these respects ($t = 1.50$ and 0.42, respectively, $p > .1, n = 11$). There were, however, significant negative correlations between original tail length and both the number ($r = .70, p = .02$) and proportion ($r = .68, p = .02$) of illegitimate nestlings in a nest. Moreover, an analysis of covariance showed that the proportion of young fathered was significantly influenced by experimental category ($F = 13.4, p = .008, df = 1, 7$), original tail length ($F = 13.3, p = .004, df = 1, 7$), and the interaction between these two variables ($F = 12.6, p = .009, df = 1, 7$). Thus, the degree of cuckoldry was significantly influenced by both the original and the manipulated tail length of males.

DISCUSSION

Tail manipulation

Because we began this study (see Smith and Montgomerie, 1991) to evaluate the conclusions of a recent study of tail manipulation in barn swallows (Møller, 1988), we took particular care to replicate the methods used previously. For example, we manipulated tail streamers by the same amount (20 mm) using a technique similar to that used by Møller (1988). We also analyzed data only from males in relatively small colonies whose streamers were manipulated more than 5 days before their females laid their first egg (Møller, 1988), and we pooled data from different nesting colonies all studied during the same breeding season.

Two aspects of our study, however, differed from Møller's (1988). First, our study population was in North America rather than Europe. This is important because North American male barn swallows have shorter tail streamers and spend some time helping their partner with incubation duties, whereas in Europe only females incubate (Turner and Rose, 1989). These differences suggest that sexual selection may be less intense in North American populations of this species and may account for the fact that we found a less striking difference between the pairing dates of elongated and shortened males than did Møller (1988) and no difference in the numbers of offspring that they fledged in a season (Smith and Montgomerie, 1991).

Second, we did not conduct any of the sham or unmanipulated "controls" that were performed by Møller (1988); we compared only elongated and shortened males. Because our study was designed to test the hypothesis that females preferred elongated over shortened males, such controls were not necessary (see also Smith and Montgomerie, 1991). Because Møller (1988) found that both elongated and shortened males differed from controls and that there were no effects (on behavior or reproductive performance) irrespective of the changes in tail streamer length, we assumed that the results obtained from our two treatments would also have differed from unmanipulated birds. Further work will be needed on our study populations to test this assumption.

DNA fingerprinting

Although the methods of producing and analyzing DNA fingerprints have been reasonably well documented (for review see Burke, 1989), we have taken a relatively cautious approach to our analysis to minimize the chances of error in the paternity exclusions. First, we used two probes (Per and Jeffreys 33.15) so that we could produce two different fingerprints for each individual. This method gave us greater confidence in the paternity exclusions in those cases where the examination of band-sharing coefficients and unique bands appeared to give different results. We report the results of Per probing here because this probe usually produced clearer bands on the autoradiograms, and the exclusions based on unique bands were largely consistent with low band-sharing coefficients.

Second, we used in-track lambda DNA markers in each lane on every gel so that the sizes of bands could be estimated more accurately for comparisons among individuals. Because electrophoresis sometimes results in the DNA in some lanes running at a slightly different speed from others, this method allowed us to be more certain that bands that appeared to be the same molecular weight really were.

Third, two different observers analyzed each fingerprint independently. When scoring fingerprints we found (unpublished data) that the quantitative results were not always as repeatable as other published studies have implied (e.g., see Burke, 1989). Despite this fact, the paternity exclusions of both scorers were completely concordant, so we are confident that our paternity exclusions are reliable.

Cuckoldry

The most striking finding from the fingerprinting analysis is that males with elongated tail streamers were cuckolded more frequently than shortened males (Figure 2). This is particularly surprising because previous analyses of pairing dates strongly suggested that elongated males were preferred as mates in both Denmark (Møller, 1988) and Ontario.
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