Multiple Sirehood in Free-Ranging Twin Rhesus Macaques (Macaca mulatta)

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Rhesus macaque females regularly copulate with a number of partners, and produce a single offspring per reproductive cycle in over 99% of cases. We used genotyping of 10 STR markers to determine paternity in the Cayo Santiago population of rhesus macaques. About 1,500 monkeys have been analyzed to date, with their marker genotypes entered into a computerized database. These data enable us to report the first documented case in any cercopithecine nonhuman primate species of the production of twin offspring sired by different males. Am. J. Primatol. 57:31–34, 2002. © 2002 Wiley-Liss, Inc.

Key words: rhesus macaques; twins; heteropaternity; STR marker typing

INTRODUCTION

In some human populations [Bulmer, 1970; Eriksson, 1973; Schmidt et al., 1983], as well as in captive chimpanzees (Pan troglodytes) [Geissmann, 1990b], twinning tends to occur within families, and a genetic locus for twinning has been identified in humans [Busjahn et al., 2000]. Dizygotic twins produced by different men have been well documented [e.g., Teraski et al., 1978; Verma et al., 1992], with an estimated frequency of about 2.5% of twin cases, based upon paternity suits [Wenk et al., 1992]. In some mammalian species that bear litters of more than one animal, genetic analysis has also revealed multiple sirehood [e.g., Hanken & Sherman, 1981; Xia & Millar, 1991; Stockley et al., 1993]. Rhesus macaques usually produce single offspring, but free-ranging animals sometimes

Contract grant sponsor: NIH; Contract grant sponsor: NSF; Contract grant sponsor: DFG; Contract grant sponsor: H.F. Guggenheim Foundation; Contract grant sponsor: UPR-MSC; Contract grant sponsor: DAAD.

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Received 30 July 2001; revision accepted 31 January 2002
DOI: 10.1002/ajp.1085
Published online in Wiley InterScience (www.interscience.wiley.com).
have twins [Koford et al., 1966; Rawlins & Kessler, 1983, 1986a; Rawlins et al., 1984; Geissmann, 1990a].

Rhesus macaques live in multimale–multifemale societies where females regularly attempt to mate with multiple partners [Manson, 1992]. On average, females copulate with three different males during each reproductive cycle [Bercovitch, 1997], but copulatory frequency is a poor predictor of actual sirehood [Berard et al., 1994]. In the course of our longitudinal studies using genetic markers to track paternity and construct pedigrees, we discovered one set of twins, out of five born between 1997 and 1999, that had different sires.

METHODS

The study population of rhesus monkeys was resident on the 15-ha island of Cayo Santiago (18°09′N; 65°44′W), located about 1 km off the southeast coast of Puerto Rico. The island has been inhabited by multiple troops of rhesus macaques since 1938, with matrilineal relationships known from demographic census data collected on a daily basis since 1956 [Rawlins & Kessler, 1986c]. Troops are designated by letters, and all animals are recognized on an individual basis using both physical features and tattoos affixed at the age of 1 year. Births are seasonal, with a peak occurring between December and January [Rawlins & Kessler, 1985].

Once per year, immature animals and targeted older subjects are caught for research and colony management purposes by entering a holding cage from a chute connected to one of the large feeding corrals. Blood samples are obtained and frozen at –20°C pending DNA extraction and paternity analysis. Paternity has been assessed in the study population since 1989 using both classical DNA fingerprinting and STR marker genotyping with up to 15 polymorphic microsatellites [Krawczak et al., 1993; Nürnberg et al., 1998]. The genetic database contains microsatellite profiles from about 1,500 monkeys. For 10 polymorphic loci, genotyping has been completed in this population, and these markers were used for paternity assessment in the twins. The markers had between four and 15 alleles, with an average heterozygosity of 0.74 (range: 0.67–0.85) that resulted in an exclusion rate of over 99.9% for all loci combined [Nürnberg et al., 1998].

The target population for paternity testing among the rhesus macaques on Cayo Santiago comprised the birth cohorts from 1988 to 1998 (652 animals). Any male 1,250 days older than a given infant born into these cohorts, and residing on the island 200 days prior to the birth of the infant, was considered a potential sire. We assigned paternity to a male when the log_{10}-likelihood ratio for paternity vs. nonpaternity was larger than 2 (corresponding to a paternity probability in excess of 99%) and at the same time exceeded that of any other male in the population by at least 1 unit. Log_{10}-likelihood ratios were calculated as described in detail elsewhere [Krawczak, 1999].

RESULTS

In the Cayo Santiago population, the maternal twinning rate from 1956 to 1963 was 0.21% [Koford et al., 1966]. No twins were born between 1963 and 1976, and the twinning rate was 0.09% between 1976 and 1983 [Rawlins & Kessler, 1986b], with production of twins occurring in different matrilines.

On 27 January 1997, an 8-year-old female in group F, R68, gave birth to twin daughters, 17G and 91F. In the previous year, she had given birth to a daughter, who was the third offspring produced. One year after bearing twins, she produced a son. Genetic analysis using 10 microsatellite markers not only
confirmed that R68 was the mother of the twins, but paternity testing in the population revealed that 17G was sired by male X94, while 91F was sired by male O19 (Table I). In both cases, the respective male was at least 500 times more likely to have been the true sire than the next most likely male (Table II).

The two sires are descended from a common matriline and are distant relatives (first cousins once removed), but are not related to the mother of the twins. Both had notably different life histories: male X94 was born in group F on 31 December 1991, sired 17G while still resident in his natal troop, and did not disperse from his natal troop until migrating into group R in August 2000. Male O19 was born on 10 January 1988, also in group F, but his natal dispersal occurred on 3 September 1993, when he left and became a roving solitary male until joining group V in May 1994. He then alternated between life as an extra-group male and residency in group R. When female 91F was conceived, male O19 was an extra-group male. In summary, at the time the twins were conceived, one sire was a 4.5-year-old natal male, while the other was an 8.5-year-old extra-group male.

DISCUSSION

We have shown for the first time that twin rhesus macaques can be produced by different males. Multiple sirehood in twin rhesus macaques is uncommon, but, as with humans, when periovulatory females mate with multiple partners, they can conceive twins that have different fathers. Although our study

<table>
<thead>
<tr>
<th>Marker</th>
<th>R68</th>
<th>17G</th>
<th>X94</th>
<th>91F</th>
<th>O19</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S367</td>
<td>139–139</td>
<td>115–139</td>
<td>115–141</td>
<td>115–139</td>
<td>115–139</td>
</tr>
<tr>
<td>D6S474</td>
<td>141–145</td>
<td>141–141</td>
<td>141–141</td>
<td>141–145</td>
<td>141–145</td>
</tr>
<tr>
<td>SCA1REP</td>
<td>166–166</td>
<td>166–166</td>
<td>166–166</td>
<td>166–178</td>
<td>163–178</td>
</tr>
</tbody>
</table>

Log₁₀-likelihood ratios are only listed for the five most likely sires.

TABLE II. Log₁₀-Likelihood Ratios for Paternity vs. Non-paternity

<table>
<thead>
<tr>
<th>Infant</th>
<th>17G</th>
<th>91F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sire</td>
<td>Likelihood ratio</td>
<td>Sire</td>
</tr>
<tr>
<td>X94</td>
<td>4.205</td>
<td>O19</td>
</tr>
<tr>
<td>X16</td>
<td>1.205</td>
<td>O34</td>
</tr>
<tr>
<td>33A</td>
<td>–0.591</td>
<td>V45</td>
</tr>
<tr>
<td>Z88</td>
<td>–0.892</td>
<td>Z88</td>
</tr>
<tr>
<td>D71</td>
<td>–2.989</td>
<td>H89</td>
</tr>
</tbody>
</table>
is the first to demonstrate heteropaternity in cercopithecine nonhuman primates, we suggest that fine-tuned molecular genetic analyses of other nonhuman primate species will reveal additional cases.

ACKNOWLEDGMENTS

We thank Ulrike Sauermann and Rich Rawlins for comments on the text. The study was approved by the Institutional Animal Care and Use Committee of UPR-MSC, and conducted in accordance with NIH guidelines and USDA regulations.

REFERENCES