Lack of Mother–Offspring Relationships in White-Tailed Deer Capture Groups

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ABSTRACT Behavioral studies of white-tailed deer (Odocoileus virginianus) often assign mother–offspring relationships based on common capture of juveniles with adult deer, assuming that fawns associate closely with mothers. We tested this assumption using genetic parentage to assess mother–offspring relationships within capture groups based on data from 10 polymorphic microsatellite loci. At the 80% confidence level, we assigned maternity to 43% and 51% of juveniles captured with an adult female in 2 respective study areas. Capture with their mother did not differ by sex of juveniles in either study area, and limiting our analysis to capture groups that most represent family groups (i.e., one adult female with 1–3 juveniles) did not increase maternity assignment (35%). Our results indicate that common capture may be a poor indicator of mother–offspring relationships in many field settings. We recommend genetic verification of family relationships. (JOURNAL OF WILDLIFE MANAGEMENT 73(3):357–361; 2009)

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Studies of white-tailed deer (Odocoileus virginianus) behavior often depend on knowledge of family relationships. Maternal–offspring relationships have been used to study survival (Holzenbein and Marchinton 1992), dispersal (Woodson et al. 1980, Holzenbein and Marchinton 1992, Etter et al. 1995, Shaw et al. 2006), breeding behavior (Ozoga and Verme 1985), and migration (Nelson and Mech 1984). Some studies define mother–offspring relationships based, in part, on common capture or capture of a juvenile with an adult female (i.e., >1 yr old) at the same time in the same net or trap (Nelson and Mech 1984, Holzenbein and Marchinton 1992, Nelson 1993, Etter et al. 1995, Guiliano et al. 1999). Use of common capture as a criterion to identify family relationships assumes deer associate with family members when captured. However, several factors could reduce reliability of common capture as a criterion for identifying mother–offspring relationships. First, deer-capture techniques using bait may attract multiple family groups to a capture site (Thomas et al. 1965). Second, fawn associations with unrelated deer increase as fawns mature through 6 months of age (Schwede et al. 1994). Deer capture activities often occur when fawns are 6–10 months old and may be somewhat independent of their mother. Finally, in populations where capture occurs after the rut or hunting seasons, social groups may not consist of family members because of social factors or harvest-related mortality (Thomas et al. 1965).

Genetic analyses provide an objective means of determining white-tailed deer mother–offspring relationships (Anderson et al. 2002, Shaw et al. 2006). As part of an ongoing study of dispersal and survival (Long et al. 2005, Diefenbach et al. 2008), we captured white-tailed deer in drop and rocket nets in 2 areas of Pennsylvania and collected tissue samples for genetic analysis. We evaluated the validity of common capture as a means of determining mother–offspring relationships.

STUDY AREA

We captured white-tailed deer in 2 study areas of Pennsylvania from January 2003 to April 2003. One study area was in Armstrong County, in the Allegheny Plateau region of western Pennsylvania, east of the Allegheny River. Forests covered 51% of the landscape but were extensively fragmented by agricultural fields, and much of the forested landscape existed as isolated woodlots.

The other study area was located in Centre County, in the Ridge and Valley region, approximately 150 km east of the study area in the Allegheny Plateau. This study area was less fragmented and was composed of more forested land (61% forest cover). Land use was also primarily agricultural; however, long, parallel ridges were forested, and agriculture was predominately restricted to valleys. Long et al. (2005) provide additional study area details. In general, study areas were open to hunting that occurred before capture.
METHODS

We captured deer in drop nets and rocket nets baited with corn from January 2003 to April 2003. Our capture objectives were to place radiocollars on 100 males in each study area to monitor dispersal and survival, but the bait attracted diverse groups of deer, and we often netted mixed groups containing males, females, and juveniles of both sexes. Use of drop and rocket nets permitted capture of multiple deer at one time but did not guarantee capture of all deer near the net. Capture activities occurred after the rut but before spring fawning season when some juveniles emigrate from their natal ranges (Holzenbein and Marchinton 1992, Rosenberry et al. 1999, Long 2005). When attaching ear tags (Temple Tag, Ltd., Temple, TX), we collected a tissue sample from each ear using an ear punch. We used one ear punch for each deer and cleaned it thoroughly with 95% ethanol before use on another animal. We placed clipped tissue in whirl-packs for transport to the laboratory, where we stored samples at −80°C until DNA isolation.

We isolated total DNA for each individual from the earclip tissue, using the Qiagen DNeasy Tissue Kit (Qiagen Genomics Incorporated, Bothell, WA), following manufacturer’s protocols. We amplified 10 microsatellite loci individually using polymerase chain reaction (PCR): BM6438, BM6506, BM848, K, N, O, OarFCB193, P, Q, and R (Anderson et al. 2002). We attempted loci D, 4208, and ILSTS011 (Anderson et al. 2002) but were unable to obtain consistent allele-sized calls. We performed PCR using the HotStarTaq™ Mastermix kit (Qiagen Genomics Incorporated) following manufacturer’s protocols with 50–150 ng DNA in 10 μL of total volume with a 0.2–μM final concentration for each primer (Shaw et al. 2006). Thermal cycling conditions included an initial denaturation at 95°C for 15 minutes, followed by 40 cycles PCR, 50 seconds at 95°C, 50 seconds at 60°C, and 85 seconds at 72°C, followed by a final extension at 72°C for 30 minutes (Shaw et al. 2006). We fluorescently labeled forward PCR primers (Qiagen Genomics Incorporated) and electrophoresed PCR products on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) using a 30-cm capillary and POP5 polymer. We included the Genescan® Rox500 (Applied Biosystems) internal molecular weight standards in every lane and sized products using GeneScan Analysis Software Version 3.7 (Applied Biosystems). To verify genotyping accuracy, we rescored approximately 25% of individuals at each locus.

To characterize genetic diversity, we estimated observed heterozygosity, expected heterozygosity (Nei 1987), and allelic diversity. We used exact probability tests to evaluate conformance to Hardy–Weinberg and linkage equilibria. We obtained unbiased estimators of exact significance probabilities using the Markov-chain algorithm described by Guo and Thompson (1992), as implemented in the computer program GENEPOP (Raymond and Rousset 1995), using a dememorization of 10,000 per 100 batches and 1,000 iterations. Although BM6438 and BM6506 map to the same bovine chromosome (Anderson et al. 2002), we found no evidence of linkage disequilibria among any pair of loci in our analysis.

We used the computer program CERVUS Version 3.0 (Marshall et al. 1998) to generate critical log-likelihood (ΔLOD) scores to assign maternity at different levels of statistical confidence, based on simulations (Marshall et al. 1998). The number of candidate mothers strongly affects the power of CERVUS to assign maternity. In our study, estimated preharvest female deer densities were 7 deer/km² in Armstrong County and 5 deer/km² in Centre County (C. S. Rosenberry, Pennsylvania Game Commission, unpublished data). Given that a female and fawn are unlikely to disperse >1.6 km in the interval between birth and our sampling, we estimated our candidate number of females to be those expected within 2.56 km², that is, 18 deer/km² and 13 deer/km² for Armstrong and Centre counties, respectively. We determined the critical ΔLOD score using the following simulation parameters: 10,000 cycles, 18 candidates for Armstrong County and 13 candidate parents for Centre County, proportion of candidates sampled (depending on no. of F in capture group and no. of candidate mothers for the study area), 0.989 proportion of loci typed (based on empirical data), and 0.01 proportion of loci mistyped (the default). We calculated allele frequencies from each study area separately (ad only) in the CERVUS maternity analyses. We performed maternity assignments at 95% and 80% confidence levels, which represent stringent and relaxed conditions for maternity assignment, respectively.

We also assessed the resolving power of the series of loci used in the study, through calculation of average exclusion probabilities, with both parents unknown (based on the combined first parent nonexclusion probability reported in CERVUS). This exclusion probability is the average probability that the set of loci will exclude an unrelated candidate female from maternity of an arbitrary offspring when the genotype of the father is unknown (Marshall et al. 1998).

To prevent false exclusion of parents, we analyzed all homozygotes for these loci with the second allele considered unknown for both counties when using CERVUS Version 3.0 (Marshall et al. 1998), assuming the cause was null or nondetectable alleles, following O’Connor and Shine (2003). We also analyzed maternity with these 3 loci excluded to ensure that confidence in maternity assignments was not overstated because of non-Hardy–Weinberg equilibrium (non-HWE) at these 3 loci (Marshall et al. 1998).

We performed tests of pairwise relatedness as an additional means of evaluating relationships among mother–juvenile pairs and adult females. We used the program KINSHIP Version 1.31 (Goodnight and Queller 1999) to estimate mean relatedness (R; Queller and Goodnight 1989) of offspring to assigned mothers (expected R = 0.5) and offspring to which no mother was assigned (expected R < 0.5 depending on family structure within groups; e.g.,...
expected $R = 0.25$ for half-siblings, $R = 0.125$ for first cousins. We present results as means ± 95% confidence intervals, with normality of relatedness values tested by using the Anderson–Darling normality test as implemented in Minitab Version 15 (Minitab, State College, PA). We also used KINSHIP to test adults for sibling relationships within capture groups because close relationships among adults can hinder CERVUS assignment success (Marshall et al. 1998). To resolve full-sibling relationships above and beyond half-sibling relationship, we set the null hypothesis to half-sibling relationship (Konovalov et al. 2004). To test for homozygotes at these loci when we used all 10 loci for assignment. We based exclusion probabilities on postremoval data.

### Table 1. Loci examined, adult sample size, number of alleles, observed and expected heterozygosities, null allele frequency, and average exclusion probability for one candidate parent in white-tailed deer, as calculated by CERVUS Version 3.0, in Armstrong and Centre counties, Pennsylvania, USA, 2003.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Locus</th>
<th>No. of alleles</th>
<th>Obs</th>
<th>Exp</th>
<th>Null allele frequency</th>
<th>Exclusion probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armstrong</td>
<td>O</td>
<td>49</td>
<td>0.776</td>
<td>0.732</td>
<td>–0.033</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td>BM48a</td>
<td>48</td>
<td>0.354</td>
<td>0.880</td>
<td>0.419</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>49</td>
<td>0.898</td>
<td>0.925</td>
<td>0.008</td>
<td>0.709</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>49</td>
<td>0.816</td>
<td>0.925</td>
<td>0.059</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>49</td>
<td>0.429</td>
<td>0.498</td>
<td>0.070</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>BM438</td>
<td>49</td>
<td>0.857</td>
<td>0.905</td>
<td>0.022</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>OarFCB193</td>
<td>49</td>
<td>0.898</td>
<td>0.883</td>
<td>–0.014</td>
<td>0.597</td>
</tr>
<tr>
<td>Centre</td>
<td>O</td>
<td>28</td>
<td>0.671</td>
<td>0.053</td>
<td>0.020</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>BM48a</td>
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<td>0.357</td>
<td>0.769</td>
<td>0.367</td>
<td>0.436</td>
</tr>
<tr>
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<td>Q</td>
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<td>0.926</td>
<td>0.052</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td>N</td>
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<td>0.643</td>
<td>0.882</td>
<td>0.144</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
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<td>0.685</td>
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<td>0.263</td>
</tr>
<tr>
<td></td>
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<td>0.893</td>
<td>0.894</td>
<td>–0.007</td>
<td>0.602</td>
</tr>
<tr>
<td></td>
<td>OarFCB193</td>
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<td>0.964</td>
<td>0.907</td>
<td>–0.039</td>
<td>0.637</td>
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<tr>
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<td>0.874</td>
<td>0.130</td>
<td>0.609</td>
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<td></td>
<td>P</td>
<td>28</td>
<td>0.536</td>
<td>0.855</td>
<td>0.224</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>27</td>
<td>0.222</td>
<td>0.205</td>
<td>–0.052</td>
<td>0.020</td>
</tr>
</tbody>
</table>

a We report initial observed heterozygosities, expected heterozygosities, and null allele frequencies. For maternity allocation, we removed the second allele for homozygotes at these loci when we used all 10 loci for assignment. We based exclusion probabilities on postremoval data.

RESULTS

In Armstrong County, we sampled 42 adult females and 45 juveniles from 22 capture groups that contained ≥1 adult female and ≥1 juvenile. In Centre County, we sampled 25 adult females and 35 juveniles from 18 capture groups containing ≥1 adult female and ≥1 juvenile. Size of the capture groups ranged from 2 deer to 7 deer, with an average of 3.7 deer per group. The average number of adult females per group was 1.7 deer. The ratio of juveniles to adult females within capture groups ranged from 0.4 to 5, with an average of 1.5. To characterize adult allele frequencies, we included individuals captured without juveniles. Therefore, total adult sample size was 50 and 28 in Armstrong and Centre counties, respectively.

Allelic diversity ranged from 3 alleles to 20 alleles among the 10 microsatellite loci (Table 1). BM6438 and BM6506 map to the same bovine chromosome (Anderson et al. 2002); however, we found no evidence of linkage disequilibria among any pair of loci in our analysis. Genotypic frequencies differed from HWE expectations at BM848, BM6506, and P loci ($P < 0.001$) for the Armstrong County population and at BM848 ($P < 0.001$) and P loci ($P = 0.004$) for the Centre County population. All departures from HWE reflected an excess of homozygotes, suggesting presence of null or nonamplifying alleles, as evidenced by estimated null allele frequencies of 13–42% for deviant loci (Table 1). Combined exclusion probabilities for 7-locus and 10-locus data sets were ≥0.99. For both populations, percentage of juveniles for which we could assign maternity
was ≤51%, regardless of number of loci or criteria used for assignment (Table 2). Even using the criteria most likely to assign maternity (10 loci at the 80% confidence level), we assigned maternity to only 23 juveniles (51%) in Armstrong County and 15 juveniles (43%) in Centre County. Both increasing confidence level and decreasing number of loci considered resulted in marginally decreased maternity assignments. Adjusting confidence levels from 80% to 95% decreased assignment frequency by an average of 12%. Adjusting the locus set from 10 loci to 7 loci decreased assignment frequencies by an average of 7%.

Average relatedness for mother–offspring pairs was not different from expected for either Centre (0.41 ± 0.09) or Armstrong counties (0.47 ± 0.05). Average relatedness of nonmother–offspring pairs was 0.03 ± 0.09 and 0.04 ± 0.05 in Centre and Armstrong counties, respectively, consistent with expected values for unrelated individuals (R = 0). Relatedness values were consistent with expected values considering the 7 loci results as well (data not shown).

Program KINSHIP sibling analyses resolved 2 capture groups with a pair of adult half-siblings within the Centre County capture groups. In Armstrong County, we captured 5 groups containing half-siblings and 2 groups containing full-siblings and half-siblings among the adult candidates at the 0.05 significance levels. Type II error rates were high in full-sibling and half-sibling analyses in the 2 counties (0.31–0.34 at 0.05 significance level), indicating that we may have underestimated sibling assignments. However, relatedness and likelihood-ratio analyses produced concordant results, indicating presence of close relatives of the mother did not affect our ability to assign maternity.

For the 2 follow-up analyses, we considered 38 juveniles, assigned follow-up groups with 10 loci at the 80% confidence level using CERVUS, to have been captured with their mothers. Both male and female juveniles were as likely to be captured with their mothers as without their mothers (Armstrong: M, $\chi^2_{1,0.05} = 0.391$, $P = 0.532$; F, $\chi^2_{1,0.05} = 0.182$, $P = 0.670$; Centre: M, $\chi^2_{1,0.05} = 0.000$, $P = 1.000$; F, $\chi^2_{1,0.05} = 1.316$, $P = 0.251$). Also, capture with their mothers did not differ by sex of juvenile within study areas (Armstrong: $\chi^2_{1,0.05} = 0.551$, $P = 0.458$; Centre: $\chi^2_{1,0.05} = 0.614$, $P = 0.433$). Some capture groups contained more fawns than adult females within the group could biologically produce. Limiting analyses to capture groups that most represent family groups (i.e., one ad F with 1–3 juv) did not increase predictability to a useful level. Twenty capture groups containing 33 juveniles fit our definition of a potential family group. Overall, predictability of maternities based on common capture of family groups was 35% but highly variable within study sites and potential family group size.

**DISCUSSION**

Common capture was a poor indicator of mother–offspring relationships in our study. Even with a relaxed criterion of maternity assignment, ≤51% of fawns were assigned to females for both study populations. Consistent and complementary results of maternity assignment were provided from likelihood-ratio and relatedness approaches, suggesting that a substantial number of juveniles did not have a biological mother in their capture group.

There are several, although nonexclusive, potential explanations for lack of mother–fawn associations in our capture groups, including harvest and behavioral factors associated with capture. Harvest removals of adult females (or fawns) would obviously disrupt family groups on our study areas before capture. Within our study areas, harvest rates of adult females ranged from 33% to 39% based on adult female harvests and population estimates (C. S. Rosenberry, unpublished data). Adult females that lose fawns and fawns that lose mothers may continue to associate with their social group or with other deer (Thomas et al. 1965), decreasing the incidence of mother–offspring pairs. This is difficult to evaluate because we were unable to secure samples from harvested individuals. We did detect several capture groups containing female relatives, indicating that some social groups were represented. Attempts to capture deer before hunting seasons or in areas with less-intense adult-female harvests may improve chances of capturing mothers and fawns together. However, capture attempts before hunting seasons may be less effective because fawns do not regularly associate with their mothers until 4 months of age (Schwede et al. 1994). Furthermore, many capture methods require animals to respond to bait, which may be more likely in winter after green vegetation and mast are no longer available.

A combination of behavioral and logistical factors did not allow capture of all deer observed around nets, possibly separating some family groups. Factors affecting our ability to capture all deer present included observability of deer in forested areas surrounding trap sites, willingness of deer to approach bait, number of deer we could safely handle, and age and sex of deer under the net. Thus, we were unable to capture every deer seen or to verify that we captured all deer in the vicinity of the site.

Habitat characteristics and age of fawns may also influence reliability of common capture as an indicator of relatedness. In open habitats, multiple family groups may congregate in open areas to feed, whereas, in more forested habitats, single-family groups consisting of an adult female and her fawns and her yearling female offspring are most common (Hirth 1977). On our hunted study areas with >50% forest

<table>
<thead>
<tr>
<th>Study area</th>
<th>Assigned maternities using 10 loci</th>
<th>Assigned maternities using 7 loci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assigned No.</td>
<td>%</td>
</tr>
<tr>
<td>Armstrong</td>
<td>95</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>23</td>
</tr>
<tr>
<td>Centre</td>
<td>95</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>15</td>
</tr>
</tbody>
</table>
cover, common capture did not reliably predict mother–offspring relationships. Age of fawns at capture may also affect strength of mother–offspring association. As white-tailed deer fawns mature, they associate less often with family members (Schwede et al. 1994), thus increasing the chance of observing an animal apart from close relatives.

Management Implications

Overall, conditions we experienced reflect reality of many field efforts and occur in other studies of free-ranging deer. Consequently, our results likely represent conditions found in deer studies occurring outside enclosures. Common capture of mothers and offspring appears unlikely in many real-world situations. Reasons for this include harvest removals before capture, capturing a subset of deer around nets, and maturing behavior of fawns. For these reasons, we recommend genetic identification of family relationships to improve reliability of conclusions from behavioral studies.

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LITERATURE CITED


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