Behavioral Interactions Between Juvenile Nine-banded Armadillos (Dasypus novemcinctus) in Staged Encounters

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ABSTRACT.—Juvenile nine-banded armadillos (Dasypus novemcinctus) discriminate between the scents of siblings and nonsiblings, spending more time near and investigating more often the scent of a sibling. In the present experiment we examined whether the ability to make such distinctions generates behavioral differences in how juveniles interact with one another. Two juveniles (siblings or nonsiblings) were placed in an arena and the ensuing interactions were videotaped. Contrary to expectation, juvenile armadillos showed no evidence of behavioral discrimination between siblings and nonsiblings. All interactions were highly amicable and aggressive behavior was almost completely absent. The absence of behavioral discrimination between juvenile armadillos may be due to (1) the lack of an appropriate context for discrimination in our experiments; (2) ontogenetic considerations such that only older animals actually exhibit discrimination, or (3) low genetic variability in our population that makes discrimination less important.

INTRODUCTION
Assessing the functional significance of signals has lagged behind analyses of the potential information in those signals. For example, there are far more studies documenting individual variation in a signal (and thus the potential for individual recognition) than studies showing that such information is actually used by receivers (reviews in Beecher, 1982; Halpin, 1986). The same problem has beset studies of kin recognition. Many investigators have shown that recognition signatures covary with kinship, but far fewer have shown that such variation is used in generating behavioral discrimination between different types of individuals (Holmes, 1988; Barnard, 1990; Blaustein and Porter, 1990; Grafen, 1990; Blaustein et al., 1991; Gamboa et al., 1991; Halpin, 1991).

Kin discrimination in nine-banded armadillos is of particular interest because females give birth to litters of genetically identical quadruplets (Newman and Patterson, 1910; Patterson, 1913; Storrs and Williams, 1968; Prodöhl et al., 1996). In a previous study, we showed that juvenile (i.e., young of the year) nine-banded armadillos, presented in an arena with pads containing scent from a sibling and a nonsibling, spent more time near and investigated more often the pad containing scent from their sibling (Loughry and McDonough, 1994). Given that result, our next question was to ask whether olfactory discrimination is manifested behaviorally in interactions between juvenile siblings and nonsiblings.

There are few reports of interactions between juvenile armadillos in the wild (see McBee and Baker, 1982), so it is difficult to make predictions about how they should behave toward one another. Anecdotal observations of littermates suggest that siblings are highly affiliative, foraging and sleeping together through much of their first summer (pers. observ.; Galbreath, 1982; McBee and Baker, 1982). Such behavior is also consistent with the results of our scent discrimination experiments (Loughry and McDonough, 1994). We have rarely observed natural encounters between juvenile nonsiblings, nor do we know of any published

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accounts of such interactions. However, older animals generally avoid one another, interacting aggressively when they do come into contact (except during mating, see McDonough, 1992, 1994, 1997). These older animals are unlikely to be siblings (Prodöhl et al., 1996) so, if juveniles behave similarly, we would expect nonsiblings to avoid one another as much as possible and behave aggressively when forced to interact. Conversely, siblings should be highly affiliative and interact amicably. We tested these predictions by staging encounters between sibling and nonsibling juvenile armadillos.

**METHODS**

The experiments were conducted during July of 1994 and from 23 June–28 July 1995 at the Tall Timbers Research Station near Tallahassee, Florida, where we have been observing the armadillo population since 1992 (Loughry and McDonough, 1996; McDonough and Loughry, 1997). Juvenile nine-banded armadillos typically are born in March or April and first emerge from their natal burrows in May or June (pers. observ.; McBee and Baker, 1982). Thus, the juveniles used in our experiments were presumably between 80–120 days old (S. McPhee, pers. comm.).

The basic experimental protocol followed that of Loughry and McDonough (1994). Juvenile armadillos were captured during regular censuses on the Tall Timbers property. Once caught, they were housed individually in plastic tubs. Juveniles were held for no more than 48 h and then were released at the point of capture. During captivity juveniles were given access to water but were not fed. Individuals from the same litter were identified by the same criteria as in Loughry and McDonough (1994), *i.e.*, they were the same size and sex and were caught in the same area at the same time (average distance between littermates = 54.8 m, see Prodöhl et al., 1996). Designation of juveniles as littermates was confirmed genetically (Prodöhl et al., 1996). To avoid problems with testing potential half-sibs, individuals in nonsibling tests came from litters that were widely separated (>1 km) from one another.

Interactions between siblings and nonsiblings were staged in a 2.44-m square plywood arena. The walls of the arena were 1.22 m high and the floor was covered in linoleum. The linoleum had alternating 23-cm squares of black and white, which provided a grid for determination of the proximity between interactants. The arena was located outdoors and had no roof. Trials in 1994 (*n* = 2) were staged in the same arena used by Loughry and McDonough (1994) for scent discrimination tests between juvenile armadillos. While this arena was slightly smaller than that used in 1995, trials from 1994 are included here because we could detect no differences in the behavior of juveniles in the two testing chambers.

Trials between juveniles were videotaped from above the arena using a JVC GR-60 VHS-C camcorder. All trials were run in the afternoon, from approximately 1200–1700 h. In the morning, before a trial, each animal had a small piece of differently colored reflective tape glued to its carapace to facilitate the identification of individuals during the trial. To start a test, two juveniles were placed in the arena simultaneously (but at opposite ends of the arena) and given 5 min to acclimate to their surroundings. Taping began after this period but only after both juveniles exhibited consistent movement around the arena. We could detect no differences in the behaviors exhibited during the acclimation period and during the subsequent trial, so it is unlikely that discrimination only occurred at the onset of a trial. Each trial lasted 20 min, with the exception of the two in 1994, which lasted 15 min. Some individuals (*n* = 13) were tested twice, once with a sibling and once with a nonsibling. Juveniles were given at least 30 min between successive trials and we attempted to counterbalance the order of trials for members of the same litter (*i.e.*, if one juvenile was tested first with a sibling, we tried to test the next juvenile from the same litter with a nonsibling
Table 1.—Mean (± se) values for behavioral components of interactions between armadillo siblings and nonsiblings

<table>
<thead>
<tr>
<th></th>
<th>Nonsiblings</th>
<th>Siblings</th>
<th>P*</th>
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<tbody>
<tr>
<td>Amicable encounters/min</td>
<td>1.61 (0.15)</td>
<td>1.70 (0.96)</td>
<td>0.85</td>
</tr>
<tr>
<td>Total amicable/min</td>
<td>3.14 (0.33)</td>
<td>2.84 (0.55)</td>
<td>0.64</td>
</tr>
<tr>
<td>% amicable</td>
<td>19.90 (3.49)</td>
<td>14.53 (2.96)</td>
<td>0.36</td>
</tr>
<tr>
<td>Aggressive encounters/min</td>
<td>0.01 (0.01)</td>
<td>0.005 (0.004)</td>
<td>0.51</td>
</tr>
<tr>
<td>% aggressive</td>
<td>0.11 (0.08)</td>
<td>0.02 (0.02)</td>
<td>0.46</td>
</tr>
<tr>
<td>Proximity (cm)</td>
<td>108.05 (6.84)</td>
<td>114.97 (6.66)</td>
<td>0.53</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>11</td>
<td></td>
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</table>

* Mann-Whitney U tests

first). We also attempted to avoid using the same juvenile in the same test more than once (e.g., testing two members of the same litter against the same nonsibling), but this was not always possible because of limitations in the number of animals available at a particular time (n = 4 animals used twice instead of once). That same constraint also precluded matching a juvenile with a nonsibling of the same sex in all tests. However, analysis of same-sex (n = 10) vs. mixed-sex (n = 7) nonsibling dyads revealed no differences in behavior (using the behaviors listed in Table 1, Mann-Whitney U test, all P > 0.25), so the data from both groups are pooled in our analyses. In addition, there were no differences between male-male and female-female interactions (Mann-Whitney U tests; nonsiblings, all P > 0.12; siblings, all P > 0.22), so those data were pooled also. Between trials, the floor of the test arena was washed, once with water and again with isopropyl alcohol to remove any residual odors from the interactants in the previous test.

The resulting videotaped trials were decoded by an observer who did not know the identity of the interactants in each trial. We calculated proximity between juveniles by recording the distance (i.e., number of squares) between the approximate midbody of each interactant at 10-sec intervals for the duration of the trial and then averaging these to produce a single proximity score for each trial. Behavioral interactions between juveniles were classified as either aggressive or amicable. Aggressive behavior has been described by McDonough (1994). In our tests, we observed only one type of this behavior, “kicking”, in which one animal moved its hind leg forcefully in a sideways motion at the other individual (see McDonough, 1994). We recorded the number and duration of all such aggressive interactions during a trial. Amicable behavior included three behaviors involving apparent olfactory investigation of the other individual, but that varied in the location of the area investigated. These behaviors included contact that was nose-to-nose, nose-to-body (one or both juveniles appeared to sniff the ventral surface of the other), and nose-to-anus. We considered these behaviors amicable because observations of older animals in the wild suggested that individuals rarely tolerate such close contact with another individual (pers. observ.; McDonough, 1994). The final amicable behavior was “climbing”, in which one juvenile placed its front claws on the back of the other and began climbing on to the top of the second animal’s carapace. We recorded four measures of amicable behavior: as with aggressive behavior, we recorded (1) the number and (2) the duration of amicable encounters that occurred during a trial. However, because an amicable encounter could contain a series of several different behaviors, we also recorded (3) the total number of amicable behaviors performed during each encounter. Finally, for each trial we calculated (4) the percentage of different amicable behaviors performed by dividing the number of observations of each
TABLE 2.—Mean (± se) values for behaviors exhibited by individual juvenile armadillos (n = 13) exposed to both a sibling and a nonsibling

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Nonsiblings</th>
<th>Siblings</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amicable encounters/min</td>
<td>1.46 (0.15)</td>
<td>2.00 (0.31)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total amicable/min</td>
<td>2.68 (0.31)</td>
<td>3.37 (0.45)</td>
<td>0.26</td>
</tr>
<tr>
<td>% amicable</td>
<td>19.91 (4.54)</td>
<td>17.13 (2.22)</td>
<td>0.97</td>
</tr>
<tr>
<td>Aggressive encounters/min</td>
<td>0.02 (0.01)</td>
<td>0.004 (0.004)</td>
<td>0.13</td>
</tr>
<tr>
<td>% aggressive</td>
<td>0.17 (0.10)</td>
<td>0.01 (0.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Proximity (cm)</td>
<td>110.45 (9.11)</td>
<td>108.72 (7.71)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* Wilcoxon tests

behavior by the total number of amicable behaviors observed. Because of some variability in the duration of trials (see above), the number of amicable and aggressive encounters were divided by the total time of the trial to generate rates of interaction per min (this was also done for the total number of amicable behaviors performed). The percentage of time spent in amicable and aggressive encounters during a trial was calculated by dividing the total duration of each type of encounter by the duration of the trial and multiplying by 100.

Data collected from the videotapes were analyzed for differences between sibling vs. nonsibling trials with nonparametric tests using the Statview statistical package (Abacus Concepts, 1992). Although our previous scent experiments generated certain predictions about the outcome of the present experiment, we used two-tailed tests to be conservative. In what follows, means are presented ± se and statistical significance was recognized at P < 0.05.

RESULTS

We used 38 juveniles in a total of 28 tests: 17 between nonsiblings and 11 between siblings. No significant difference in the behavior of siblings vs. nonsiblings was detected (Table 1). To further evaluate sibling vs. nonsibling interactions, we examined the 13 individuals who were tested with both a sibling and a nonsibling. Again, we could find no differences in how these individuals behaved toward siblings vs. nonsiblings (Table 2).

Interactions between siblings and nonsiblings were both characterized by significantly more amicable than aggressive behavior (Table 1; Wilcoxon tests for siblings: P < 0.003 for % amicable vs. % aggressive and for amicable encounters/min vs. aggressive encounters/min; for nonsiblings: P < 0.0003 for the same two comparisons). However, it could be that siblings engaged in different types of amicable behaviors than did nonsiblings. To evaluate this possibility, we compared the percentages of different amicable behaviors performed in the two conditions. There were no significant differences in the types of amicable behaviors employed between siblings and nonsiblings (Table 3). However, climbing was more frequent between nonsiblings than between siblings (Table 3). This was probably because climbing was not observed in five of 11 sibling tests. Climbing was observed in almost all nonsibling tests (15 of 17), but the difference in the incidence of climbing between sibling and nonsibling trials was not significant (Fisher's exact test, P = 0.076).

DISCUSSION

We have previously shown that juvenile nine-banded armadillos are capable of olfactory discrimination between littermates and nonlittermates (Loughry and McDonough, 1994). Given these earlier results, the findings of the present study come as something of a surprise
because, contrary to expectation, juveniles showed no evidence of behavioral discrimination between siblings and nonsiblings. At present, we can identify at least three nonexclusive explanations for our results.

First, it is possible that our experiments did not present an appropriate context for eliciting discrimination (cf. Barnard, 1990; Gamboa et al., 1991; Hokit et al., 1996). Thus, in our experiments, juveniles may have been able to distinguish olfactorily between siblings and nonsiblings, but had little reason to act on this information. Perhaps if we had presented a valuable resource such as food or a refuge (e.g., an artificial burrow), behavioral discrimination would have been more evident. We find this explanation plausible, but unlikely. Juveniles appeared highly affiliative in all our tests and it seems unlikely that provision of some resource would have generated aggression. Indeed, although aggression is common among older armadillos (yearlings and adults, see Denson, 1979; Breece and Dusi, 1985; McDonough, 1994), we have never witnessed an instance of juvenile-juvenile aggression in the wild (although opportunities to observe such interactions are rare). There are even reports of individuals from more than one litter sharing the same burrow (Kalmbach, 1943). Thus, while the lack of behavioral discrimination in our experiments could be due to the unnaturalness of the testing situation, it seems more likely that juveniles are just indiscriminant.

Second, assuming that our experiments provided an appropriate context and thus that juveniles really are behaviorally indiscriminant, the possibility exists that juveniles possess an ability to distinguish siblings from nonsiblings that is not used until later in life. Other studies have documented such ontogenetic changes in kin discrimination (e.g., Walls, 1991; Blaustein et al., 1993; Holmes, 1994, 1995; Fénéron and Jaisson, 1995). Thus, it is possible that as juveniles reach reproductive maturity and establish their home ranges, they begin to exhibit behavioral discrimination between siblings and nonsiblings, perhaps enabling them to direct aggression toward appropriate targets. However, whether discrimination at a later age is between siblings and nonsiblings or between other, more general categories (e.g., local group member versus nongroup member, male versus female, adult versus juvenile, etc., see Grafen, 1990; Hare, 1992, 1994; Hurst and Barnard, 1995) is currently unknown.

A final explanation concerns the unique history of our population. Armadillos were originally introduced into southern Florida when a few captive animals were accidentally released into the wild during the 1920's (Humphrey, 1974). The population has since spread throughout the state. The legacy of this founder event is predicted to be a reduction in the genetic variability of armadillo populations found in Florida. Preliminary DNA fingerprinting analyses of our population are consistent with this scenario, suggesting the Tall Timbers population may be derived from the founder group (P. Prodöhl and J. Avise, pers. comm.).

### Table 3.—Mean (± SE) percentages of behaviors employed in amicable encounters between armadillo siblings and nonsiblings

<table>
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<tr>
<th></th>
<th>Nonsiblings</th>
<th>Siblings</th>
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<tbody>
<tr>
<td>Nose-nose</td>
<td>26.66 (2.40)</td>
<td>33.11 (5.22)</td>
<td>0.32</td>
</tr>
<tr>
<td>Nose-body</td>
<td>35.98 (1.54)</td>
<td>41.61 (3.69)</td>
<td>0.09</td>
</tr>
<tr>
<td>Nose-anus</td>
<td>27.08 (2.32)</td>
<td>20.07 (3.71)</td>
<td>0.15</td>
</tr>
<tr>
<td>Climbing</td>
<td>10.24 (1.70)</td>
<td>6.12 (2.04)</td>
<td>0.11</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* Mann-Whitney U tests
However, further examination of potential source stocks will be required before any definitive conclusion can be drawn. Juvenile armadillos appear to have highly-tuned discrimination abilities, even to the extent of discriminating between their own scent and that of one of their genetically identical siblings (Loughry and McDonough, 1994). Thus, even though all members of our population may be closely related, the animals still appear capable of discriminating between individuals. However, the high level of genetic relatedness may make acting on this information less important, which would generate the lack of behavioral discrimination reported here.

To conclude, there are three additional points about our study to mention. First, it is important to point out that the experiments reported here are not tests of kin recognition in nine-banded armadillos. Rather, they are an attempt to extend an earlier finding of olfactory discrimination among juveniles to determine if discrimination exists at the behavioral level as well. Tests for kin recognition would require manipulating litters to generate combinations of littermates and nonlittermates reared together and apart to disentangle the effects of rearing association and genetic relatedness on discrimination (e.g., Gamboa et al., 1991). However, our results do provide some insight for anyone planning such a study. Our data on interactions between nonsiblings represent the case where behavioral discrimination in kin recognition tests is expected to be most apparent (i.e., between nonsiblings reared apart; cf. Holmes and Sherman, 1982). The fact that no evidence of such discrimination was obtained in our tests suggests that demonstrating kin recognition among juvenile nine-banded armadillos may be a difficult task.

Second, one could argue that our results are questionable because of the small sample sizes involved. However, the smaller sample size in our tests was for trials involving siblings (n = 11 vs. n = 17 for nonsiblings). Thus, if we were to increase our sample size, we would primarily need to test more siblings. Given the data we report here, these siblings would have to be extraordinarily affiliative and have extremely high rates of amicable interactions to result in a statistically significant difference between the behavior of siblings and nonsiblings. The present results suggest that this is unlikely to occur and thus it seems doubtful that increasing our sample size would dramatically alter our findings.

Finally, it has been widely argued that genetic relatedness is the key to the distribution of altruism and the evolution of sociality (e.g., Hamilton, 1964; Wilson, 1975). Nine-banded armadillos appear to violate such assertions: juveniles act equally amicably with siblings and nonsiblings and, despite the high degree of relatedness among siblings (and, in our population, among all group members), armadillos appear to be primarily asocial (see Clark, 1951; Galbreath, 1982; McBee and Baker, 1982; Herbst and Redford, 1991). Armadillos thus represent a potential paradox in the evolution of social behavior (see also Walls and Blau-stein, 1995). The forces producing such a deviation from expected patterns remain to be discovered.

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Literature Cited


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