Variation in male movement paths during the mating season exhibited by the Timber Rattlesnake (*Crotalus horridus*) in St. Louis County, Missouri

Corey Devin Anderson

**Abstract.** Movement paths during the mating season exhibited by the Timber Rattlesnake (*Crotalus horridus*, (Linnaeus, 1758)) in St. Louis County, Missouri, were determined by radiotelemetry and examined to test two hypotheses: 1) that males make long, linear movements over several days that bring them into contact with females and 2) that mating season movements by males increase displacement distances from overwintering dens. Analysis of mating season movement paths indicated that movement steps associated with mating behaviors occurred over a period lasting from days to weeks, with most males making a series of steps over various distances. Analysis of movement direction with descriptive circular statistics indicated that, for most paths, component movement vectors were not oriented in the same direction and were not oriented in the direction of the female. When mating season movements commenced, all males were located at distances from dens that exceeded the average nearest-neighbor distance between dens, but when males were located with females, over half were found at locations within the average nearest-neighbor distance. The results demonstrate that male movement steps during the mating season occur over longer periods of time and over a greater variety of distances and directions than had been previously hypothesized for this species; such movement steps may increase or decrease net displacement distances from resident dens.

Key words: mate searching, consort, circular statistics, telemetry, pitviper, snake

**Introduction**

Male snakes often move long distances, over short time frames, to find receptive females during the mating season. For some species, long-distance movements may increase mating success (Madsen et al., 1993) and, in regions where snakes overwinter communally and show high inter-annual fidelity to the same den location, long-distance movements may also promote mating events between males and females from distantly spaced dens (Blouin-Demers and Weatherhead, 2002; Anderson, 2010; Weyer et al., 2014). However, few studies have examined male mating-season movement paths in explicit detail. Existing studies have sought to generalize the topology of observed paths for their study species (e.g., Coupe, 2002) or to determine movement strategies that resulted in relatively high mating success (e.g., Duvall and Schuett, 1997). Sometimes, notable long-distance movements during the mating season exhibited by one or a few males are mentioned in radiotelemetry studies of snakes (e.g., Reinert and Zappalorti, 1998), but data on variation in male movement patterns is either unavailable or understated.

In the present study, I examined variation in male mating-season movement-paths exhibited by the Timber Rattlesnake (*Crotalus horridus*, (Linnaeus, 1758)) in western St. Louis County, Missouri. I tested the assertion that paths are straight (comprised of a series of movement steps over 1-3 days), which bring them into contact with a female (Coupe, 2002), and the hypothesis that male mate-searching movement paths increase net displacement distances from resident overwintering dens. I also discuss the implications of the observed movement paths for various hypotheses that have been proposed to explain path topologies.
Materials and Methods

Study site and field sampling methods

Data used in this study were collected between 2000 and 2004 in and around the Tyson Research Center in western St. Louis County, Missouri. The study area is approximately 2000 acres (809 hectares), situated between Interstate 44/Route 66 and the Meramec River (Figure 1). The southern section of the study area contains large tracts of limestone-dolomite glade that have been invaded by the Eastern Red Cedar (*Juniperus virginiana*) due to wildfire suppression. This “cedar glade” is intermixed with large stands of oak-hickory forest that stretch north to the bottomlands of the Meramec River floodplain. Major habitat classes (upland forest, bottomland forest, prairie, and field) used by the Timber Rattlesnake were perforated by a variety of natural and human-maintained landscape features (including streams, bluffs, roads, buildings, and fences).

Because the Timber Rattlesnake is difficult to observe, a prolonged sampling period and multifaceted search strategy were needed to locate dens, to identify den residents, and to follow individuals. When encountered, individuals were captured using a snake stick or snake tongs and were transported in a covered, ventilated bucket to a secure location for processing. During processing all rattlesnakes were handled with the hook and tube method. Individuals were sexed via cloacal probing. Live animals were given a unique scale clip on their tail and a passive integrated transponder (PIT) tag was implanted midbody, under the first row of dorsal scales (right side).

A total of 29 timber rattlesnakes (15 males) were temporarily housed to implant radio transmitters (Holohil Systems Ltd., Beck, Ontario, Canada; models: SI-2T and AI-3T) using a variant of the body-cavity implantation procedure of Reinert and Cundall (1982). All surgeries were conducted by the staff surgeon at the Washington University School of Medicine, in a sterile surgical facility using an anesthesia machine with isoflurane gas (Anderson and Talcott, 2006).

Snakes with radio transmitters were released at their capture location and homing techniques were used to make visual locations during daylight hours. Our goal was obtain one location per day, but this was not always feasible for all individuals (e.g., due to inclement weather). If males with radio transmitters were found near (<1 m) a female and behavioral indicators were consistent with sexual activity (i.e., precopulatory shedding by females, mate guarding behavior by males, courting by males, or coitus) they were considered to be engaged in a mating consort.

During the first four years of the study (2000 to 2003), all individuals found in mating consorts with focal rattlesnakes were captured (if accessible). Because marking and identifying individuals required disturbing mating pairs, only paths and distances to the first mating female were considered (i.e., when males mated with more than one female during the mating season). In 2004, consorts were not disturbed, but when accessible, individuals were scanned in situ with a PIT tag reader.

For each location, a survey grade GPS (Trimble Navigation Limited, Sunnyvale, California, USA; Figure 1. Representative mating-season movement-paths exhibited by the Timber Rattlesnake (*Crotalus horridus*) at the Tyson Research Center in St. Louis County, Missouri. B2000 (purple) exhibited the straightest path and most steps were oriented in the general direction of the consort location. In contrast, C2001 (orange) exhibited the second most tortuous path, with many steps not oriented in the direction of the consort location. The path exhibited by E2001 (blue) was relatively straight, but included a movement step in the opposite direction of the consort location.
model: GeoExplorer III) was used to log approximately 25 points, which were later differentially corrected and averaged for maximum precision [using United States Geological Survey (USGS) and Continuously Operating Reference Stations (CORS) base files with the GPS Pathfinder Office (Trimble, 1999) software program]. The accuracy of corrected locations was inspected by overlaying the locations on an aerial photograph and cross checking each location with the description (and/or measurements) from the corresponding field data. Inaccurate locations were retaken, eliminated, or estimated using the data sheets.

Analysis of movement paths

The mating season for the Timber Rattlesnake begins explosively in the late summer and then declines into the fall (Brown, 1993; Alridge and Duvall, 2002). The exact timing of the mating season usually varies from region to region, from year to year at the same location, as well as among individuals from the same location within the same year (Martin, 1992; Martin et al., 2008). Hence, it is difficult (and probably inadvisable) to set annual dates for the beginning and end of the mating season. Nevertheless, for each individual in our study, reasonably accurate starting points for movement paths could be determined by close observation of behavior in the field combined with a posteriori inspection of movement paths and net-displacement plots. Locations were traced backwards from the first location where a female was found to the last location in an individual’s range from which the movement pattern was initiated and “normal behaviors” (e.g., ambushing and basking) observed during the foraging season ceased. Several males exhibited movement patterns consistent with mate searching behavior, but were never located in proximity to a female; for the purposes of the present study, these movement paths were not included in the analysis.

To minimize the effects of human interference on observed paths, males who were captured and implanted with radio transmitters during the mating season were not included in the analysis. For males that mated in more than one year, I used paths from the first year in which males were tracked for one complete active season (rather than using consort data from years in which males were captured in the late spring or early summer).

Locations were converted into time sequenced vector paths using the program extension Hawth’s Tools (Beyer, 2004) in ArcGIS 9.1 (ESRI, 2005). Hawth’s Tools was also used to generate movement parameters (e.g., step length and movement bearing) for each movement step in a path.

Because movement paths represent observations made on the same individual serially, they are dependent on each other, and a test of significance cannot be made. Therefore only descriptive circular statistics (Batschelet, 1981) were compiled for individual paths. Descriptive statistics included: range (the length of the smallest arc that contains all sample points), mean (angle of the sample), median (angle of the sample), straightness index (r), and homeward component (u). Homeward component was used to determine whether movement steps were oriented in the direction of the consort. To determine whether males that moved frequently also moved in many directions, VassarStats (Lowry, 2015) was used to calculate and test the significance of the Spearman rank-order correlation coefficient between the number of movement steps exhibited by each male and path tortuosity (measured as one minus the straightness index).

Lastly, to determine whether mating-season movements increase net displacement distances from resident dens, the Wilcoxon signed-ranks test was used to test whether Euclidean distances from dens to mating locations were greater than Euclidean distances from dens to starting locations in the beginning of the mating season. I used the one-sample sign test to assess whether Euclidean distances from dens to consorts and Euclidean distances from dens to start locations exceeded the average nearest-neighbor distance between dens.

Results

Eleven of 15 males that received radio transmitters were observed in a total of 21 consorts between 2000 and 2004, with 10 of these 11 males meeting study criteria (outlined above). However, 1 of these 10 males (hereafter denoted by their alphabetical ID and sample year, where “a” indicates that this was the first of several consorts in a given year) was only located one time between 14 July and 18 July; hence, only data on displacement distances and movement period for this male (T2004) were included in the data analysis.

Males initiated searching behaviors between 07 July and 03 August; males were located with females between 18 July and 11 August. Parameters measured for the nine sample paths varied considerably among individuals (Table 1). The number of search days ranged from 5 to 24 (median = 12). For all paths, the maximum movement distance between locations (counting only locations over successive days) ranged
between 234 m and 1274 m, and the minimum distance between locations (ibid.) ranged between 10 m and 320 m. Three different males moved over 1000 m in a single day (B2000, C2001, and Y2004a). For some individuals (A2001a, D2001, J2001a) there was a discrepancy between the maximum distance moved over successive locations over successive days (Table 1, “Max1”) and the maximum distances moved over successive locations when the individuals was not located over successive days (Table 1, “Max2”). For example, the maximum distance between successive locations over successive days (“Max1”) equaled 178 m for A2001a. However, the maximum distance between successive locations when A2001a was not located over successive days (“Max2”) was 388 m; in this case, two days were missing between successive locations.

Descriptive circular statistics for qualifying movement paths also varied considerably among individuals (Table 2). In general, males that made a large number of movements tended to move in many directions ($r_s = 0.65$, $P = 0.05$). With the exception of B2000 (Figure 1), most paths were not straight (i.e., $r << 1$) and were not oriented in the direction of the consort location (i.e., $v << 1$).

For 6 out of the 10 paths, males ended up closer to their resident den than where they started, while for the remaining four paths males made extensive movements away from their resident den. Euclidean distances from dens to consorts ($n = 10$; median = 636 m; interquartile range = 1167 m) were not significantly greater than Euclidean distances from dens to start locations (median = 1065.5; interquartile range = 295 m). Euclidean distances from dens to consorts did not significantly exceed the average nearest-neighbor distance (= 520 m) between dens ($n = 6$); however, Euclidean distances from dens to the starting locations of movement paths did tend to exceed the nearest-neighbor distance between dens ($P = 0.001$).

### Table 1. Movement dates and step distances (m) for male movement paths during the mating season exhibited by the Timber Rattlesnake at the Tyson Research Center between 2000 and 2004. Male ID = alphabetical identification and sample year (“a” indicates that this was the first of several consorts in a given year); # Steps = the number of observed movement steps between locations from start to finish; Min = minimum step distance over successive days, given that the individual moved; Max1= maximum step distance between successive locations over successive days; Max2 = maximum step distance, with the number of days missing in superscript.

<table>
<thead>
<tr>
<th>Male ID</th>
<th>Dates</th>
<th># Steps</th>
<th>Min (m)</th>
<th>Max1 (m)</th>
<th>Max2 (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2001a</td>
<td>02-Aug to 11-Aug</td>
<td>6</td>
<td>10</td>
<td>178</td>
<td>388$^2$</td>
</tr>
<tr>
<td>B2000</td>
<td>03-Aug to 11-Aug</td>
<td>6</td>
<td>94</td>
<td>1049</td>
<td>1049</td>
</tr>
<tr>
<td>C2001</td>
<td>15-July to 08-Aug</td>
<td>18</td>
<td>22</td>
<td>1073</td>
<td>1073</td>
</tr>
<tr>
<td>D2001</td>
<td>17-July to 02-Aug</td>
<td>12</td>
<td>29</td>
<td>234</td>
<td>471$^1$</td>
</tr>
<tr>
<td>E2001</td>
<td>25-July to 30-July</td>
<td>5</td>
<td>320</td>
<td>762</td>
<td>762</td>
</tr>
<tr>
<td>J2001a</td>
<td>16-July to 05-Aug</td>
<td>9</td>
<td>82</td>
<td>417</td>
<td>1138$^3$</td>
</tr>
<tr>
<td>P2004</td>
<td>10-July to 23-July</td>
<td>9</td>
<td>35</td>
<td>731</td>
<td>731</td>
</tr>
<tr>
<td>Y2004a</td>
<td>07-July to 18-July</td>
<td>6</td>
<td>98</td>
<td>1274</td>
<td>1274</td>
</tr>
<tr>
<td>Z2004a</td>
<td>15-July to 21-July</td>
<td>5</td>
<td>106</td>
<td>486</td>
<td>486</td>
</tr>
</tbody>
</table>
by some males. Results underscore the fact that not all long-distance movements promote genetic exchange among den populations; in fact, some long-distance movements appear to decrease mating distances.

The results of the present study are consistent with a previous analysis of the same data set by Anderson (2010), who reported that out of 12 mating pairs where the resident hibernaculum of both the male and female had been determined, males and females were from the same hibernaculum in 4 cases. Nevertheless, Anderson (2010) found no evidence of restricted gene flow among sample den populations based on an analysis of microsatellite DNA loci, which supports the observation that movements directed away from a male’s hibernaculum may enhance genetic exchange between den populations. Therefore, male mating season movements appear to have countervailing effects on mating distances.

Path topology

Although most males moved substantial distances over the course of the mating season, only one male (B2000) exhibited the quick, straight-line path described in the literature for the Timber Rattlesnake (Coupe, 2002) and other species, such as the Prairie Rattlesnake (Duvall and Schuett, 1997). Instead, most paths covered a variety of distances and directions, lasting over a period of days to weeks. While additional studies are needed to determine how typical these sorts of movement patterns are for *Crotalus horridus*, they appear to invalidate the general assertion that males of this species make long, linear movements over 1-3 days, which bring them into contact with a female (Coupe, 2002).

At the present time, little is known about how males of this species find receptive females. Coupe (2002) suggested that the Timber Rattlesnake might use multiple methods of mate location (separately or in combination) and discussed three hypotheses that might explain how males locate females over long distances: scent trailing, prior experience, and efficient searching. Scent trailing is the most widely assumed hypothesis (Ford, 1986), and is likely used in some manner in the searching process. However, for many species it is not clear whether scent trails are left actively or if males find receptive females who have left odors passively. Some have even suggested that males might follow the trails of other male rattlesnakes to find females (Brown, 1995). While additional experimental work is needed to clarify the sensorial mechanisms by which males locate females over long distances: scent trailing, prior experience, and efficient searching.

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Table 2. Circular statistics for qualifying male mating-season movement-paths exhibited by the Timber Rattlesnake during the mating season at the Tyson Research Center between 2000 and 2004. Male ID = snake initial and year; # Steps = the number of observed steps between locations from start to finish; \( r \) = straightness index (\( r << 1 \) indicates that the path was not straight); \( v \) = homeward component (\( v << 1 \) indicates that movement steps were not oriented in the direction of the consort); range = the length of the smallest arc (in degrees) that contains all sample points; Mean = mean angle of the sample; Median = median angle of the sample.

<table>
<thead>
<tr>
<th>Male ID</th>
<th># Steps</th>
<th>( r )</th>
<th>( v )</th>
<th>Range</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2001a</td>
<td>6</td>
<td>0.446</td>
<td>0.390</td>
<td>228</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>B2000</td>
<td>6</td>
<td>0.863</td>
<td>0.863</td>
<td>89</td>
<td>241</td>
<td>251</td>
</tr>
<tr>
<td>C2001</td>
<td>18</td>
<td>0.100</td>
<td>0.085</td>
<td>296</td>
<td>87</td>
<td>13.5</td>
</tr>
<tr>
<td>D2001</td>
<td>12</td>
<td>0.128</td>
<td>-0.117</td>
<td>276</td>
<td>58</td>
<td>172</td>
</tr>
<tr>
<td>E2001</td>
<td>5</td>
<td>0.527</td>
<td>0.525</td>
<td>87</td>
<td>301</td>
<td>322</td>
</tr>
<tr>
<td>J2001a</td>
<td>9</td>
<td>0.095</td>
<td>0.090</td>
<td>266</td>
<td>102</td>
<td>220</td>
</tr>
<tr>
<td>P2004</td>
<td>9</td>
<td>0.285</td>
<td>0.179</td>
<td>283</td>
<td>189</td>
<td>184</td>
</tr>
<tr>
<td>Y2004a</td>
<td>6</td>
<td>0.439</td>
<td>0.395</td>
<td>233</td>
<td>196</td>
<td>192.5</td>
</tr>
<tr>
<td>Z2004a</td>
<td>5</td>
<td>0.263</td>
<td>-0.869</td>
<td>231</td>
<td>123</td>
<td>143.5</td>
</tr>
</tbody>
</table>
may have left scent trails at smaller spatial scales that males might have followed.

Future studies that integrate both male and female movement paths would be insightful, but these data may be difficult to collect for the Timber Rattlesnake because females do not breed annually (Martin, 1992; Brown, 1993) and the probability of observing a mating event between two radiotransmittered snakes is low when population sizes are relatively large. For example, in the present study, many females were initially captured (and implanted with a radiotransmitter) after they were found in a consort with a radiotransmittered male, but did not breed or were not tracked in subsequent years. In a couple of cases, radiotransmittered females were found in close proximity to radiotransmittered males during the mating season, but these interactions occurred after a prior consort had been disturbed (and, hence, did not fit the study criteria). Obtaining concomitant information about male and female movement patterns during the mating season will likely require larger and longer-term data sets, with less conservative criteria.

Although it has been assumed that proximate olfactory cues play a large role in male mate-searching behaviors, males may also use prior experience in locating receptive females. As the Timber Rattlesnake can potentially live for many years (Brown, 1993; Martin, 1992) and often exhibits home-range fidelity over long timeframes (Reinert, 1993), males that have found females in past years might return to those areas where they have encountered females previously (Coupe, 2002). An example from the present study supporting this idea is male Y, who was initially captured in 2002 near the southern boundary of Tyson with female M. After not exhibiting any mating activity in 2003, male Y returned to the same area in 2004 and mated with two females (in 2005 he returned to the same area again and mated with three females). This pattern seems consistent with the idea that prior experience may have guided this male towards an area where he was successful in finding mates previously.

The final hypotheses presented by Coupe (2002) involved the use of efficient search patterns by males when locating females, as described for the Prairie Rattlesnake by Duvall and Schuett (1997). According to this hypothesis male search strategies might depend on environmental factors that affect the local distribution of females, and depending on how females are distributed, males may locate mates most efficiently by using a particular search pattern. For their sample population, long-distance, straight-line movements may have been most effective for the Prairie Rattlesnake because clusters of females were widely and unpredictably distributed during the mating season. However, in the present study, long-distance, straight-line movements may not have been necessary because female distributions appeared to be clustered predictably in the landscape (Anderson, 2010). Hence, it is possible that quick, straight-line movement patterns in a random direction may not be a successful strategy for locating females in the sampling area examined in the present study.

In the present study, other factors beyond scent trailing, prior experience, and efficient searching may have influenced male movement patterns during the mating season. For example, males may have actively or passively driven other males away from consort females (Martin, 1992; Jellen et al., 2007). If these encounters happened quickly, it may have been assumed that a male had not yet located a female, and this could explain why it took so long for some males to find females. Albeit rare behaviors like combat are extremely difficult to observe, such direct confrontation was never witnessed over six years of intensive sampling. If males were captured and removed from a consort, larger males often appeared quickly; presumably, these larger males would have supplanted the smaller individual if size-determined male-male combat was a major determinant of mating success in the Tyson population. It is possible that male-male combat may be of less importance for populations where males and females are highly dispersed when the mating season begins. For example, males of species that breed in the spring immediately after emergence from dens have been reported to engage in increased fighting, courting, and persuasion (O’Leile et al., 1994; Sexton and Bramble, 1994; Duvall et al., 1992).

In addition to the possibility of male-male agonistic interactions influencing movement paths, one cannot rule out the possibility that some males may have engaged in quick consorts that went undetected between sightings. Nevertheless, it seems unlikely that many consorts were missed because males usually encountered females that were in a pre-shed condition, and often stayed in their proximity for one or more days (until the female shed and became receptive).

Lastly, for species where mating takes place away from dens, variation in movement paths could also be explained by landscape heterogeneity. For example, movement patterns in relatively flat prairie habitat could conceivably differ from patterns observed in the topologically and environmentally complex landscape where the present study was conducted. Likewise,
landscapes may contain both natural features (e.g., rivers, valleys, bluffs) and manmade features (e.g., roads, fences, and field edges) that could affect movements (Sealy, 2002; Anderson and Rosenberg, 2010). Such habitat heterogeneity could result in considerable variation in movement paths, and is likely to be a factor in complex, non-stationary landscapes.

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