Implications of movement patterns for gene flow in black rat snakes (*Elaphe obsoleta*)

Gabriel Blouin-Demers and Patrick J. Weatherhead

Abstract: Gene flow is fundamental to evolutionary processes but knowledge about movements of individuals and their offspring necessary for gene flow is scant. We investigated potential ecological components of genetic connectivity within a population of black rat snakes (*Elaphe obsoleta*) by radio-tracking 82 individuals. Because adult black rat snakes are highly faithful to their hibernaculum, gene flow between hibernaculum populations has to occur through mating between members of different hibernacula or through juvenile dispersal. The present study was the first to assess the spatial dispersion of a complete network of hibernacula. The mean distance between the nearest-neighbour hibernacula was 811 m, which was less than the mean distances that reproductive males and females were found from their hibernacula during the mating season. Estimates of maximum distances individuals were from their hibernacula during the mating season indicated that, on average, a female was likely to mate with males that came from two hibernacula away from the female's own hibernaculum. Both males and females appeared to contribute actively to gene flow by moving more and increasing their distance from their hibernacula during the mating season. In addition, on average, females nested closer to a hibernaculum other than the one they attended, thereby potentially increasing the likelihood that their offspring would join hibernacula other than their mothers'. Thus, spatial and movement patterns of male and female black rat snakes are consistent with genetic evidence of extensive out-breeding among local hibernaculum populations.

Résumé : Le flux des gènes est un processus fondamental de l'évolution, mais l'influence des déplacements d'individus et de leur progéniture sur le flux des gènes est encore mal connue. Nous avons étudié les composantes écologiques potentielles de la connectivité génétique au sein d'une population de serpents ratiers (*Elaphe obsoleta*) en suivant 82 individus par radiotélémétrie. Parce que les serpents ratiers adultes sont très fidèles à leur hibernacle, le flux des gènes entre les populations des divers hibernacles doit se faire par accouplement entre membres d'hibernacles différents ou par dispersion des jeunes. Cette étude est la première à évaluer la dispersion spatiale d'un réseau complet d'hibernacles. La distance moyenne entre un hibernacle et l'hibernacle voisin le plus proche a été estimée à 811 m, une distance inférieure à la distance moyenne dont les reproducteurs, mâles et femelles, s'éloignent de leur hibernacle au cours de la saison de la reproduction. L'estimation des distances maximales entre un individu et son hibernacle au cours de la saison de la reproduction indique qu'en moyenne une femelle a tendance à s'accoupler avec des mâles provenant d'une distance égale à celle de deux hibernacles du sien. Les mâles et les femelles contribuent activement au flux des gènes en se déplaçant davantage et en s'éloignant plus de leur hibernacle au cours de la saison de la reproduction. De plus, les femelles nichent en moyenne plus près d'un hibernacle autre que le leur et, de ce fait, augmentent sans doute la probabilité que leur progéniture adopte un hibernacle différent du leur. Les patrons spatiaux et les déplacements des mâles et des femelles du serpent ratier s'accordent avec l'hypothèse d'une importante part d'exogamie chez les populations des hibernacles locaux.

[Traduit par la Rédaction]

Introduction

The extent of gene flow between populations is a consequence of the movement of individuals, their progeny, or their gametes. Although fundamental to most ecological and evolutionary processes, interest in gene flow has increased because of its relevance to conservation (Burke 1994; Gottelli et al. 1994; Avise 1995). As habitats become more fragmented, many natural populations become smaller and more isolated. In the absence of gene flow, small populations are at risk of inbreeding depression and genetic drift, factors that can contribute to local extinction (Madsen et al. 1996; Konuma et al. 2000).

Gene flow is intrinsically difficult to measure (Slatkin 1987). Originally, researchers relied on movement data (direct methods) to measure the extent of gene flow between populations. More recently, researchers have used genetic markers (indirect methods) to infer the extent of gene flow

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G. Blouin-Demers[1,2] and P.J. Weatherhead.[3] Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada.

[1]Corresponding author (e-mail: gblouin@uottawa.ca).
[2]Present address: Department of Biology, University of Ottawa, 150 Louis Pasteur, Ottawa, ON K1N 6N5, Canada.
[3]Present address: Program in Ecology, Evolution and Conservation Biology, University of Illinois, Shelford Vivarium, 606 East Healey Street, Champaign, IL 61820, U.S.A.

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necessary to have produced the observed genetic patterns (King and Lawson 1995; Lawson and King 1996; Garcia de Leon et al. 1997; Castella et al. 2000). Many researchers have advocated the integration of direct and indirect measures to obtain a thorough understanding of the underlying mechanisms of gene flow (Slatkin 1985; Avise 1995; Gomez et al. 1997). Data from direct observational studies of movement patterns, however, continue to accumulate at a much slower rate than data from indirect studies, even though direct assessment of movements among populations remains the only valid approach for the interpretation of gene flow in an ecological context (Bossart and Prowell 1998; Bohonak 1999). Moreover, there is often disagreement between the amount of gene flow inferred from movement data and the amount inferred from genetic data (Koenig et al. 1996). Our general goal in this study was to evaluate the implications of movement patterns for gene flow between hibernaculum populations of black rat snakes (*Elaphe obsoleta*) in Ontario, and to assess whether inferences from these direct observations were consistent with those previously derived using indirect approaches.

Black rat snakes hibernate in groups of 10–60 individuals (largely adults) in traditional underground hibernacula (Blouin-Demers et al. 2000*a*). If communal hibernacula are spatially dispersed in relation to the movements of snakes, hibernaculum populations could be genetically isolated from one another (Gregory 1982). Analyses using two different genetic markers, however, found no evidence of genetic structuring among black rat snake hibernaculum populations separated by less than 6 km (Prior et al. 1997; Lougheed et al. 1999). Although genetic neighbourhoods were found to be large for black rat snakes, gene flow was viscous at scales of 15–50 km (Lougheed et al. 1999). These analyses indicate that black rat snakes have more extensive gene flow than has been found in several other snake species (Gibbs and Weatherhead 2001), and suggest that there is regular exchange of individuals or their genes between hibernaculum populations separated by a few kilometres. Consistent with what has been suggested for other snakes (Gregory 1982, 1984), Prior et al. (2001) showed that fidelity to hibernacula was approximately 98% in Ontario black rat snakes, with the few exceptions to complete fidelity involving individuals moving between adjacent hibernacula. Thus, gene flow between hibernaculum populations of black rat snakes must occur primarily through mating between members of different hibernacula and (or) by juvenile dispersal. Our goal was to assess the spatial dispersion of a network of communal black rat snake hibernacula relative to the distances moved by individual snakes. The first step toward this goal was to locate all the communal hibernacula within a defined study area to quantify their spatial dispersion. The second step toward our goal was to evaluate the potential for genetic mixing through mating between populations from different hibernacula.

The likelihood of mating between members of different hibernacula depends on the movement of individuals during the mating season in relation to their hibernacula. We had three specific objectives: (1) determination of the timing and duration of the mating season; (2) characterisation of the movement patterns of males, reproductive females, and nonreproductive females and the potential for genetic mixing allowed by these patterns; data on the movement patterns of nonreproductive females are included as a basis of comparison for the movement patterns of reproductive females; and (3) quantification of actual mating between members of different hibernacula. In meeting these objectives, we tested the following predictions. First, individual black rat snakes should regularly move distances in excess of the distance between hibernacula during the mating season, so the activity ranges of snakes from different hibernacula broadly overlap. Second, males and reproductive females should be most active during the mating season, reflecting their efforts at encountering mates. Third, mating between members of different hibernacula, or opportunities for such mating, should be common.

Although our eventual goal is also to evaluate the contribution of juvenile dispersal to gene flow between hibernaculum populations, our present goal in this regard is more modest. If hatchling black rat snakes regularly join the hibernacula of their mothers, they would not contribute to gene flow substantially. For example, in some rattlesnakes, the young are born very close to their mother's hibernaculum and they use scent tracks left by their mother or other rattlesnakes to locate the local hibernaculum (Reinert and Zappalorti 1988*a*, 1988*b*; Martin 1992). Although black rat snakes are oviparous, as opposed to viviparous like rattlesnakes, it is possible that female black rat snakes deposit their eggs close to their hibernaculum, thus making it likely that neonates join the maternal hibernaculum. Our last objective was to evaluate the potential for juvenile dispersal to contribute to gene flow by determining the location of a female's nest relative to both her own and other local hibernacula. We predicted that females regularly nest remote from their own hibernaculum, so their offspring often have the potential of joining other neighbouring hibernacula. We should note that presently nothing is known about how juvenile black rat snakes locate communal hibernacula, but demographic analyses indicate that juveniles rarely join hibernacula until a least 3 or 4 years of age (G. Blouin-Demers and P.J. Weatherhead, unpublished data).

Materials and methods

This study was conducted from April 1996 to October 2000 at the Queen's University Biological Station (QUBS; 44°34′N, 76°19′W), approximately 40 km north of Kingston, Ontario, Canada. The study area along the northwestern shore of Lake Opinicon is approximately 9.5 × 2.5 km (1923 ha, of which 1533 ha is not open water) and encompasses QUBS (Fig. 1). The habitat within the study area is composed primarily of mixed and deciduous forest, with numerous openings created by beaver ponds, small hayfields, wetlands, and rocky outcrops (Blouin-Demers and Weatherhead 2001). Because human alteration of the habitat there has been limited and rat snake natural habitat is therefore continuous, the spatial patterns we document for this population should represent the natural state for black rat snakes.

We captured black rat snakes both during their spring egress, by erecting fences around traditional and communal hibernacula (Blouin-Demers et al. 2000*a*), and opportunistically, when snakes were encountered while we were conducting fieldwork (Prior et al. 2001). Communal hibernacula were located by implanting radio transmitters (see below) in

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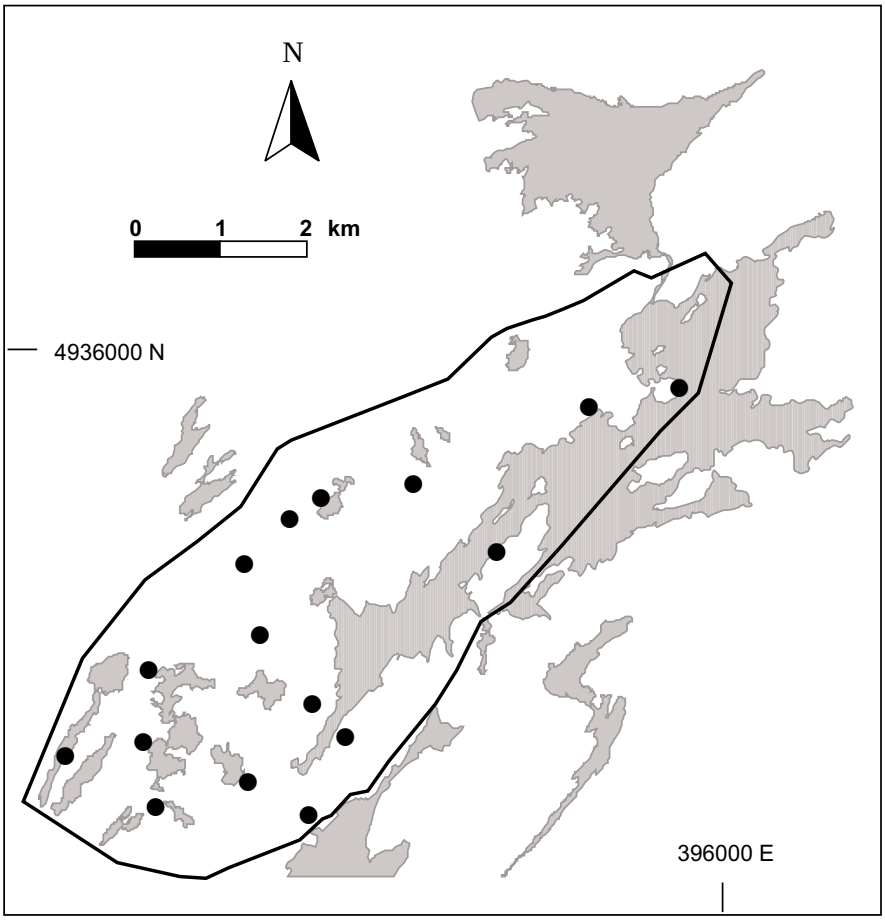
Fig. 1. Map of the study area along the northwestern shore of Lake Opinicon in eastern Ontario where black rat snakes were radio-tracked from 1996 to 2000. The major lakes and the extent of the study area are depicted. Solid circles indicate the location of the 16 known communal hibernacula. Coordinates are given in the Universal Transverse Mercator system (Zone 18, North American Datum of 1983).



previously unmarked snakes captured opportunistically during the active season and following these snakes to their hibernacula. All captured snakes were brought back to the laboratory where they were measured (snout-to-vent length (SVL)) to the nearest 0.5 cm by running a flexible measuring tape along their body. We weighed the snakes to the nearest 1 g using spring scales. We also determined the sex of each snake by gently probing the cloaca for the presence of hemipenes using a clean probe. Finally, all snakes were permanently identified by subcutaneous injection of a passive integrated transponder tag (Anitech Identification Systems Inc., Markham, Ontario).

From all the animals we captured, we selected a subset of sexually mature animals to be implanted with radio transmitters (model SI-2T, Holohil Systems Inc., Carp, Ontario; 8.6 g and 20-month battery life at 20°C). Our choice of animals was dictated by sex and size. The snakes had to be large enough to bear the transmitter (all transmitters represented less than 2.5% of the individual's body mass), and our aim was to track equal numbers of males, nonreproductive females, and reproductive females. We used isoflurane delivered via a precision vaporiser to anaesthetise snakes (Blouin-Demers et al. 2000*b*). Our surgical technique was a slight modification of that of Reinert and Cundall (1982). Snakes were kept under observation for 3 days following surgery and then released at their point of capture. After release, we located snakes, on average, every 48 h (except in inclement weather) using a radiotelemetry receiver (TRX-2000S, Wildlife Materials Inc., Carbondale, Illinois). All animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care, and our experimental procedures were approved by the Carleton University Animal Care Committee. Each snake location was mapped with a Global Positioning System unit giving submetre accuracy in the field (Pathfinder, Trimble Navigation Ltd., Sunnyvale, California), using the Universal Transverse Mercator system (North American Datum of 1983). The coordinates obtained with this system were used to generate home ranges and to calculate all the distances reported herein.

From April 1996 to October 2000, we radio-tracked 82 black rat snakes (29 males and 53 females) for periods ranging from 16 days to 48 months (mean = 11.2 ± 1.2 months, median = 10.4 months). The reproductive status of females was assessed in June by externally palpating the oviducts for the presence of eggs and confirmed in July by nesting activity. Twenty-two of the females were reproductive for part of the period in which they were radio-tracked. The locations of their nests were determined by following the females until

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oviposition and confirmed by excavation (Blouin-Demers and Weatherhead 2000). Twenty-two females and 11 males were followed over more than one active season, so we have data for 127 snake years (24 reproductive-female years, 57 nonreproductive-female years, and 46 male years).

Analyses

We calculated our success at locating new hibernacula each year as the number of previously unknown hibernacula located in that year divided by the number of snakes of unknown hibernaculum membership (at the time of capture) implanted during that year that survived until hibernation. This proportion was then expressed as a percentage. For example, if in a given year we implanted nine snakes of unknown hibernaculum membership (and seven survived until hibernation), and five went to previously unknown hibernacula and the other two went to known hibernacula (they had previously eluded capture at emergence from those hibernacula), we considered our success to be 71%. For sites at which we trapped snakes at least 2 years in a row, the number of snakes attending each hibernaculum was calculated using a simple Lincoln–Peterson index.

All statistical analyses were performed on data (distance away from hibernaculum, home-range area, etc.) averaged for each individual. If data were collected from the same individual in multiple years, we averaged the data for all years. Pooling data for an individual across years was possible because both our data and the data of Weatherhead and Hoysak (1989) indicated that individual snakes occupy the same general area each year. Thus, each individual was only represented once in all analyses, except for the nine females that were tracked both when reproductive and when nonreproductive, in which case they were included twice in the analyses (once for when reproductive and once for when nonreproductive).

For analyses of individual movement patterns during the mating season, we only included data for a snake in a given year if we knew its hibernaculum and if we had followed it for the whole mating season that year. Thus, for these analyses we had data from 50 black rat snakes (36 females and 14 males), representing 84 snake years (18 reproductive-female years, 37 nonreproductive-female years, and 29 male years). For each individual each year, we calculated the mean, minimum, and maximum distance away from their hibernaculum during the mating season and then averaged these values across individuals to yield data for 16 reproductive females, 29 nonreproductive females, and 14 males.

We used two methods to calculate the home ranges of black rat snakes. First, we calculated the 100% minimum convex polygon (MCP). The 100% MCP is the area of the convex polygon encompassing all the points where an individual was located and is the home-range metric most often reported. The MCP method is appealing because of its simplicity, but it has two disadvantages (Anderson 1982). First, it often has a sample size bias whereby the estimated size of the home range increases with the number of observed positions. Second, the home range is constrained to be a convex polygon and this can be an unreasonable assumption. It is often desirable to exclude outlying points from a MCP to prevent a few extreme, and possibly atypical, locations contributing a large additional area. To this end, a percentage of the points lying most closely to the harmonic mean centre of the range may be used, a common one being 95%. Therefore, we also calculated the 95% MCP. We excluded from the calculations of the MCPs the commute between a snake's hibernaculum and its home range. Commuting was recognised as rectilinear movements from and to the hibernaculum, through areas that were never revisited during the remainder of the active season. Including this commute in the home-range calculations would have artificially increased the living area used by black rat snakes. Although black rat snakes are terrestrial (Blouin-Demers and Weatherhead 2001), they are wide-ranging and do not hesitate to cross water to reach terra firma. Therefore, many MCPs contained open water. We removed open-water areas from the area of each MCP so that each MCP represents a "dry-land" home range. We only calculated home ranges in a given year for individuals that were followed for at least June, July, and August, because this is when most activity occurs in Ontario. Forty-five individuals (10 males and 35 females) representing 67 snake years (18 reproductive-female years, 32 nonreproductive-female years, and 17 male years) met this criterion. For each individual each year, we calculated the area of the 100 and 95% MCPs and the distance from the individual's hibernaculum to the closest point of its 100 and 95% MCPs. We then averaged these values across individuals to yield data for 16 reproductive females, 27 nonreproductive females, and 10 males. Because of the tendency of black rat snakes to remain at a given site for several days and to revisit sites regularly, the average total number of locations used by an individual over a complete active season was relatively small (Table 2). Therefore, it was not possible to generate reliable home-range estimates for the mating season only because this represented too few locations and, thus, we only present home-range estimates for the whole active season.

All analyses were conducted with JMP version 3.2 (SAS Institute Inc. 1997) and ArcView Geographic Information System version 3.0 (Environmental Systems Research Institute 1996) equipped with the Animal Movement Extension version 1.1 (Hooge and Eichenlaub 1997) on a Macintosh desktop computer. We inspected box plots to determine if the assumptions of normality and homogeneity of variance were upheld, and applied appropriate transformations when the assumptions were violated. When no transformation allowed our data to meet these assumptions, we used nonparametric tests. Significance of statistical tests was accepted at α = 0.05. All means are reported ±1 SE.

Results

Spatial dispersion of hibernacula

We located 14 communal hibernacula within our study area by implanting radio transmitters in 27 snakes (from 3 to 8 per year) of unknown hibernaculum membership (previously never captured at a hibernaculum). Two other hibernacula had been located in 1982 as part of another radiotelemetry study (Weatherhead and Hoysak 1989). Our success at locating new hibernacula decreased significantly, both as a function of study year (r = –0.907, $F_{[1,3]}$ = 13.89, p = 0.034) and as a function of the total number of hibernacula known within the study area (r = –0.948, $F_{[1,3]}$ = 26.65, p = 0.014). The regression equation for the latter analysis indicated that our success would equal 0% when 17.7 communal hibernacula

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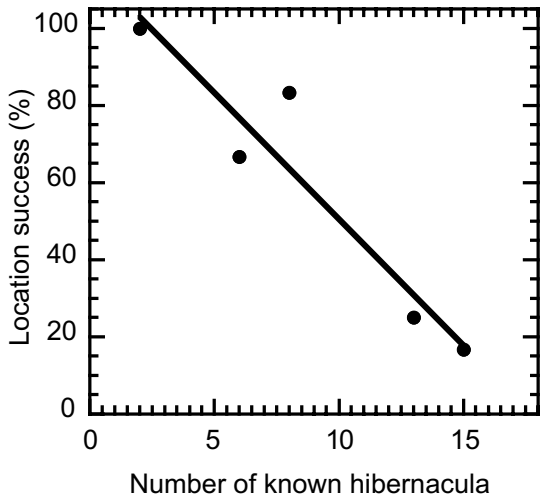
Table 1. Distances relevant to potential gene flow between black rat snake hibernaculum populations in eastern Ontario.

Distance from	*N*	Distance (m) Mean ± 1SE	Maximum	Minimum
Hibernaculum to closest hibernaculum	16	811 ± 60	1255	431
Mating location to mating male's hibernaculum	6	877 ± 161	1520	452
Mating location to mating female's hibernaculum	7	490 ± 87	707	83
Mating male's hibernaculum to mating female's hibernaculum	3	999 ± 531	1813	0
Female's nest to female's hibernaculum	21	1025 ± 176	4031	218

Note: Inter-hibernaculum distances are based on 16 hibernacula in the 9.5 × 2.5 km study area. Distances for mating are based on 11 mating events observed over 5 years, and nesting distances come from 23 females that were radio-tracked to their nest sites.

Fig. 2. Percent success at locating previously unknown communal hibernacula of black rat snakes in eastern Ontario as a function of the number of known hibernacula within the study area.



were known within the study area (Fig. 2). Therefore, our data suggest that we are missing only one or two communal hibernacula within the study area. For each of the 16 known hibernacula, we calculated the distance to the nearest neighbouring hibernaculum. The mean distance to the nearest neighbour was 811 ± 60 m, and no hibernaculum was farther than 1255 m from its nearest neighbour (Table 1).

Mating patterns

From 1996 to 2000, we relocated the 82 radio-tracked snakes a total of 5657 times and we witnessed 11 mating events. Of the 22 individuals involved, we knew the hibernaculum membership (the individual was captured at its hibernaculum at least once during the study) for 6 males and 7 females. Males were significantly farther than females from their hibernaculum when found mating ($t_{[11]}$ = 2.20, p = 0.049; Table 1). The hibernaculum membership of both members of a mating pair was known on only three occasions. One mating event involved two members of the same hibernaculum and the other two mating events involved individuals from different hibernacula. The average distance between the hibernacula of the two members of a mating pair was 999 m (Table 1). The earliest recorded mating event was 20 May and the latest 23 June. Recognising that the exact timing of the mating season may vary from year to year depending on weather patterns, we considered the mating season in our study population to be from 15 May to 30 June.

Movement patterns

Few mating events were witnessed, owing to the secretive nature of black rat snakes (black rat snakes were visible to us on only 30% of the relocations; Blouin-Demers and Weatherhead 2001). Thus, we wished to evaluate the potential for mating between members of different hibernacula based on movement patterns. Each time a snake was relocated during the mating season (15 May – 30 June), we calculated the distance it was from its hibernaculum. We contrasted the mean, maximum, and minimum distances away from the hibernaculum for males, nonreproductive females, and reproductive females. Because of numerous outliers (which are biologically significant in the context of gene flow), the assumption of normality could not be satisfied by any transformation. Thus, we used Kruskal–Wallis tests to determine if the reproductive groups differed in the distances they were from the hibernaculum during the mating season. These tests indicated that snakes in the three reproductive groups differed in mean distance ($\chi^2_{[2]}$ = 8.91, p = 0.012), maximum distance ($\chi^2_{[2]}$ = 9.70, p = 0.008), and minimum distance ($\chi^2_{[2]}$ = 14.08, p = 0.001) from the hibernaculum during the mating season. Post-hoc tests revealed that reproductive females were significantly farther from their hibernacula, on average, than nonreproductive females, that males had significantly greater maximum distances from their hibernacula than both reproductive females and nonreproductive females, and that nonreproductive females had significantly shorter minimum distances from their hibernacula than reproductive females and males (Fig. 3).

We also calculated the mean distance individuals were from their hibernaculum at 10-day intervals for the duration of the active season (Fig. 4). Males were farthest from their hibernacula at the end of the mating season. Nonreproductive females were farthest from their hibernacula late in the active season. Reproductive females were farthest from their hibernacula during the mating season and during the nesting season. During the mating season, reproductive females were farther from their hibernacula than males, and males were farther from their hibernacula than nonreproductive females. At this time, reproductive females were farther from their hibernacula than the average distance between hibernacula, whereas males were about the same distance from their hibernacula as the average inter-hibernacula distance and nonreproductive females were closer to their hibernacula

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Fig. 3. Mean, maximum, and minimum distance (D) away from the hibernaculum (A) and total distance moved, mean distance moved between relocations, and mean distance moved per move (B) during the mating season (15 May – 30 June) for male (M), nonreproductive-female (NRF), and reproductive-female (RF) black rat snakes radio-tracked from 1996 to 2000 in eastern Ontario. Means with the same letters are not significantly different based on post-hoc tests.

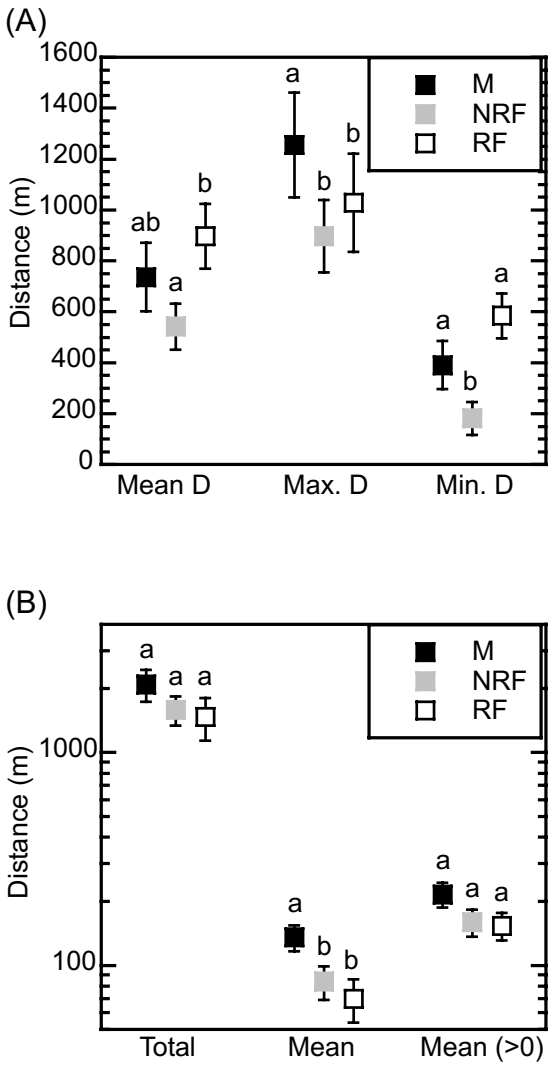
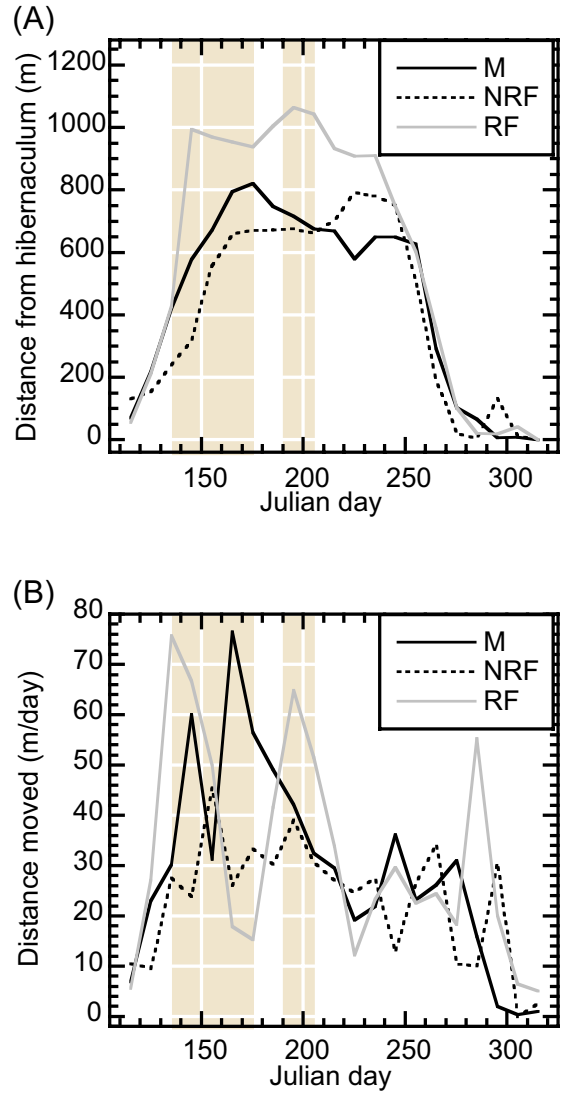


Fig. 4. Mean distance from an individual's hibernaculum (A) and mean distance moved per day (B) at 10-day intervals for male (M), nonreproductive-female (NRF), and reproductive-female (RF) black rat snakes radio-tracked between 1996 and 2000 in eastern Ontario. The shaded areas indicate the mating and nesting seasons.



than the average inter-hibernacula distance. During the nesting season, reproductive females were again farthest from their hibernacula (greater distance than the average inter-hibernacula distance), but males and nonreproductive females were similar distances from their hibernacula (shorter distance than the average inter-hibernacula distance). Later in the active season, females that had nested started coming back towards their hibernacula and the distances from hibernacula were similar among reproductive groups and less than the average inter-hibernacula distance.

ANOVA showed that reproductive groups differed in their frequency of movement between relocations ($F_{[2,56]}$ = 5.18, p = 0.009), where a snake was considered to have moved when it was >2 m from its previous location. Tukey–Kramer honestly significant difference (HSD) tests indicated that males moved significantly more often (66 ± 5%) than nonreproductive females (53 ± 4%) and reproductive females (44 ± 5%). Although males moved longer total distances during the mating season than nonreproductive females and reproductive females, ANOVA on square-root transformed data indicated that the difference was not significant ($F_{[2,58]}$ = 1.38, p = 0.260; Fig. 3). However, ANOVA showed that the mean distance moved between relocations (square-root transformed) did vary across reproductive groups ($F_{[2,55]}$ = 4.30, p = 0.018), and Tukey–Kramer HSD tests indicated that males moved farther between relocations, on average, than nonreproductive females and reproductive females (Fig. 3). When we considered only instances where snakes did move, we again found that males tended to move farther than females, but ANOVA using square-root transformed data revealed that this difference was not significant ($F_{[2,55]}$ = 1.54, p = 0.224; Fig. 3).

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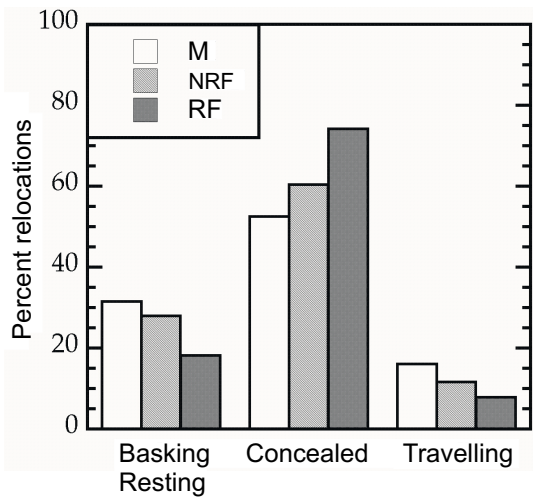
Table 2. Summary statistics for the 100 and 95% minimum convex polygon (MCP) home ranges of black rat snakes radio-tracked from 1996 to 2000 in eastern Ontario.

Home-range variable	Mean ± 1SE	Maximum	Minimum
No. of days tracked	140.1 ± 3.1	206.0	87.0
No. of times located	52.4 ± 1.5	94.0	26.0
No. of unique locations	23.2 ± 0.9	50.0	10.0
Area of the 100% MCP (ha)	18.5 ± 2.5	93.2	1.2
Area of the 95% MCP (ha)	15.3 ± 2.2	89.4	0.8
Distance from hibernaculum to 100% MCP (m)	445.4 ± 85.9	3985.0	0.0
Distance from hibernaculum to 95% MCP (m)	459.7 ± 85.7	3985.0	0.0

Note: Values are based on data for 67 snake years (18 reproductive-female years, 32 nonreproductive-female years, and 17 male years).

Fig. 5. Behaviour exhibited at relocation by male (M), nonreproductive-female (NRF), and reproductive-female (RF) black rat snakes radio-tracked during the mating seasons (15 May – 30 June) of 1996–2000 in eastern Ontario.



Finally, consistent with the movement data, the three reproductive groups exhibited different behaviour at relocation (N = 1523, $\chi^2_{[4]}$ = 41.62, p < 0.001), with males being found to travel more often than members of the other two groups during the mating season (Fig. 5).

We calculated the mean distance travelled per day for 10-day intervals for the duration of the active season (Fig. 4). Nonreproductive females had no defined peak of activity and their movements were similar to the movements of males and reproductive females in the later part of the active season. Reproductive females had three defined peaks of activity. The first peak corresponded to dispersal from hibernacula and the first half of the mating season and was similar in magnitude to that for males. The second peak corresponded to the nesting season and the third peak to the return to hibernacula. Males had one activity peak and it corresponded to the mating season. During the mating season, males and reproductive females moved more than nonreproductive females and, as expected, during the nesting season reproductive females moved more than males and nonreproductive females. The movement data thus provide additional evidence for a spring only mating season for Ontario black rat snakes.

Home ranges

Snakes that we included in the home-range calculations in a given year were radio-tracked for an average of 140 ± 3 days, during which time we relocated snakes an average of 52 ± 2 times (Table 2). However, because of the tendency of black rat snakes to remain in the same location for several days and to revisit exact sites previously occupied (the same rock or the same hole in the same tree), the number of unique locations was about half the number of relocations (mean = 23 ± 1 unique locations; Table 2). ANOVA revealed that males, nonreproductive females, and reproductive females were tracked for the same number of days ($F_{[2,50]}$ = 0.50, p = 0.608) and were relocated the same number of times ($F_{[2,50]}$ = 2.58, p = 0.086). The number of unique locations, however, differed among reproductive groups ($F_{[2,50]}$ = 4.47, p = 0.016). Tukey–Kramer HSD tests indicated that males had more unique locations than females (reproductive and nonreproductive), reflecting their greater tendency to move. Linear regressions indicated that the area (natural-logarithm transformed) of an individual's 100% MCP home range (r = 0.16, $F_{[1,51]}$ = 1.28, p = 0.264) and 95% MCP home range (r = 0.15, $F_{[1,51]}$ = 1.13, p = 0.293) was not a function of the number of days an individual was radio-tracked. However, the area (natural-logarithm transformed) of an individual's 100% MCP home range (r = 0.43, $F_{[1,51]}$ = 11.60, p = 0.001) and 95% MCP home range (r = 0.35, $F_{[1,51]}$ = 6.94, p = 0.011) did increase as a function of the number of times this individual was relocated.

Averaged across all individuals, the 100% MCP home range was 18.5 ± 2.5 ha and the 95% MCP home range was 15.3 ± 2.2 ha (Table 2). There was substantial variation in the area used by individual black rat snakes. Some snakes used areas less than 1 ha, often mostly restricted to the immediate vicinity of a favourite basking tree or an old barn, whereas others covered impressive amounts of ground (up to 93 ha). There was substantial overlap in home ranges within and between males and females. Home ranges of snakes of both sexes from different hibernacula also overlapped broadly. ANOVA showed that the area of the 100% MCP home range (natural-logarithm transformed) did not vary across males, nonreproductive females, and reproductive females ($F_{[2,50]}$ = 2.29, p = 0.112; Fig. 6). However, the area of the 95% MCP home range (natural-logarithm transformed) did vary across reproductive groups ($F_{[2,50]}$ = 3.17, p = 0.049). Tukey–Kramer HSD tests indicated that males had significantly larger 95% MCP home ranges than nonreproductive females (Fig. 6). The commute between the hibernaculum and the home range

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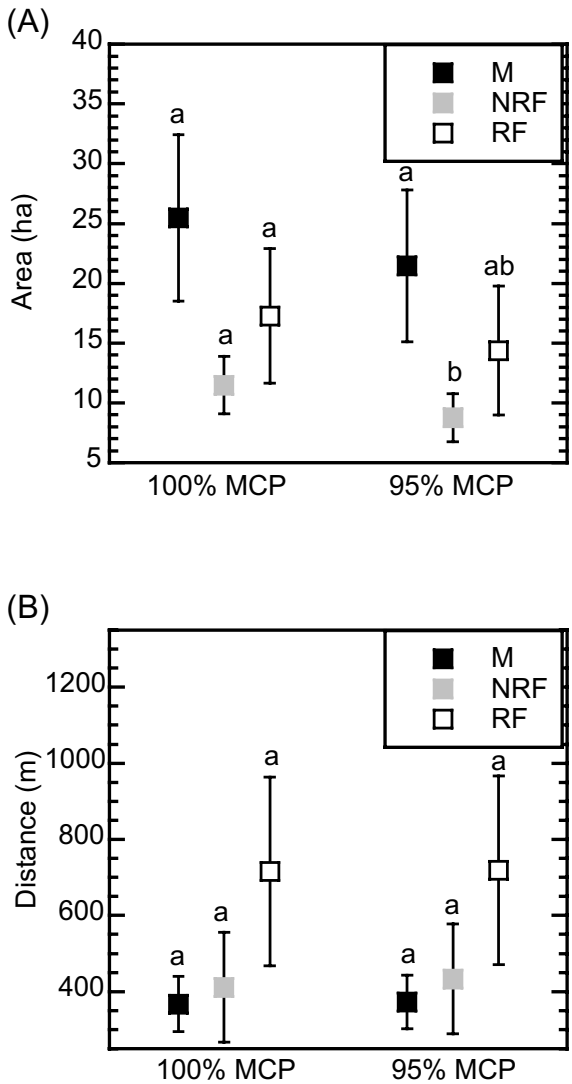
Fig. 6. Areas of the 100 and 95% minimum convex polygon (MCP) home ranges (A) and commuting distances between an individual's hibernaculum and its 100 and 95% MCP home ranges (B) for male (M), nonreproductive-female (NRF), and reproductive-female (RF) black rat snakes radio-tracked between 1996 and 2000 in eastern Ontario. Means with the same letters are not significantly different based on post-hoc tests.



lasted, on average, 7–10 days. Averaged across all individuals, the commute to the 100% MCP home range was 445.4 ± 85.9 m and the commute to the 95% MCP home range was 459.7 ± 85.7 m (Table 2). Once again, however, there was substantial variation in the distance commuted by individual black rat snakes, ranging from 0 m for individuals who's hibernaculum was included within the home range to almost 4 km for one particularly mobile individual. The distance between an individual's hibernaculum and its 100% MCP home range ($F_{[2,48]}$ = 0.49, p = 0.614) or its 95% MCP home range ($F_{[2,48]}$ = 0.46, p = 0.636) did not vary across reproductive groups (Fig. 6).

We trapped snakes 2 years in a row at 10 hibernacula. We used the number of captures of mature animals (1050 mm SVL; Blouin-Demers et al. 2002) in the first and second years of trapping and a Lincoln–Peterson index to estimate the number of mature adults present at each hibernaculum. These analyses indicated that the hibernacula contained an average of 19.1 ± 4.1 mature individuals (range 8–43). Given our estimate of 17.7 hibernacula in our study area, the estimated population is 338 mature individuals. This value is consistent with our capture data. From 1996 to 2000, we captured 484 mature individuals (44.8% female) a total of 961 times within our 1533-ha study area. Some of these individuals presumably hibernate outside the study area, thus accounting for the number of captures exceeding our estimate of the number of snakes using hibernacula within the study area. If we use 400 as the approximate population size, we obtain a mean density of 0.261 mature snakes/ha (0.117 mature females/ha and 0.144 mature males/ha). Thus, the average mature male 100% MCP home range (25.5 ha) would contain an average of 3.0 mature females. Conversely, the average reproductive female 100% MCP (17.3 ha) would contain an average of 2.5 mature males.

Nesting sites

We recorded 23 nesting events by 21 females that we followed with radiotelemetry. The hibernaculum membership was unknown for only two of these females. Females nested, on average, 1025 ± 176 m from their hibernaculum, but as far away as 4031 m (Table 1). Therefore, on average, females nested closer to a hibernaculum other than the one they attended.

Discussion

Our general goal was to evaluate the implications of movement patterns for gene flow between hibernaculum populations of black rat snakes. Specifically, we measured the spatial dispersion of a network of communal hibernacula and then evaluated the potential for gene flow through mating allowed by the movement patterns of black rat snakes. We found that black rat snakes moved extensively in relation to the spatial dispersion of hibernacula and that individuals from different hibernacula had ample opportunity to breed with each other. Males were more mobile than females, and females were more mobile when they were reproductive. Reproductive females also nested far from their hibernacula, increasing the opportunity for their offspring to join hibernacula other than those of their mothers'. Thus, both males and females appear to contribute to the out-breeding of hibernaculum populations.

To our knowledge, the present study was the first to assess the spatial dispersion of a complete network of communal snake hibernacula in a large area. Therefore, it is difficult to evaluate whether our results are typical or atypical of communally hibernating snakes. Some previous studies of snakes involved pairs of neighbouring communal hibernacula. For example, Parker and Brown (1973) studied a pair of mixed-species hibernacula 850 m apart, Lougheed et al. (1999) studied pairs of black rat snake hibernacula, on average, 2380 m apart, and Weatherhead and Hoysak (1989) located two black rat snake hibernacula that were 1200 m apart. Our data suggest that we have located almost all hibernacula within our 1923-ha study area and black rat snake hibernacula tend to be closer together (i.e., ca. 800 m apart) than could have been inferred from the literature. Therefore, our prediction that hibernacula should be close together relative

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to the movements of the snakes, thereby allowing genetic mixing, was supported.

Based on the behaviour of snakes placed together in holding cages, Weatherhead and Hoysak (1989) estimated the mating season for Ontario black rat snakes to occur in mid June. All the mating events recorded by Fitch (1963) in Kansas occurred from 18 April to 28 May. Consistent with these observations, we only observed courtship, combat, or actual mating from mid May to late June in Ontario. We never recorded events of autumn mating in black rat snakes and it thus appears that Ontario black rat snakes only mate in the spring. Males engage in reproductive behaviour each year, whereas females reproduce, on average, every 2–3 years, although some females have been recorded to reproduce 2 years in a row (G. Blouin-Demers, unpublished data). Males seen mating were, on average, the same distance away from their hibernacula as the average distance between hibernacula. However, females seen mating were, on average, approximately half the distance between their hibernacula and the nearest neighbouring hibernaculum.

The potential for out-breeding based on the few actual mating events observed was more limited than that based on individual movement patterns. During the mating season, males tended to move more frequently and longer distances than females. Males also tended to be farther from their hibernaculum than females. The mean maximum distance away from the hibernaculum was greater than the average distance between hibernacula for males, but was about the same for nonreproductive females and reproductive females. Therefore, on average, females that became gravid were encountering males that were from two hibernacula away from their own hibernaculum. If we consider the extreme values for the maximum distances moved from hibernacula during the mating season for males and reproductive females, reproductive females could potentially be encountering males from eight hibernacula away from their own hibernaculum.

The home ranges of black rat snakes in this study were larger than the home ranges calculated for the same population by Weatherhead and Hoysak (1989) (2.56 ha 100% MCP) and larger than the home ranges calculated for black rat snakes in Maryland by Durner and Gates (1993) (9.49 ha 100% MCP). The size of the home ranges also varied widely, possibly in response to variation in habitat quality. Macartney et al. (1988) presented a tabular survey of the home ranges and movements of snakes. For the 11 studies that used radiotelemetry and lasted at least 1 month, the reported 100% MCPs averaged 11.6 ha and ranged from 0.6 to 50.9 ha. The 50.9 ha home range calculation, however, included the commute to and from the hibernacula. If we exclude the studies where the commute was included in the home-range calculations, the area of the mean 100% MCP decreases to 7.7 ha. Thus, black rat snakes appear to have home ranges at the upper end of the spectrum of reported values. Consistent with data reported by Weatherhead and Hoysak (1989), there was both inter and intra-sexual overlap in home ranges. In addition, there was broad overlap between the home ranges of snakes from different hibernacula. In a review of average commuting distances between hibernacula and summer ranges for 13 species, Gregory (1984) reported values averaging 1515 m and ranging from ca. 300 m to 10.7 km. However, if the one study with a mean dispersal distance over 10 km is excluded, the mean distance dispersed decreases to 597 m. Therefore, black rat snakes disperse approximately the same distance to their summer ranges as the other species for which similar data are available. Collectively, our data on the spatial dispersion of black rat snakes relative to their hibernacula support the prediction that the potential for mating between members of different hibernacula is high.

Unlike the situation reported for rattlesnakes (Reinert and Zappalorti 1988*a*, 1988*b*; Martin 1992), where females gave birth close to their own hibernacula, female black rat snakes generally nested farther from their hibernacula than the average distance between hibernacula. Thus, juvenile black rat snakes have the potential to contribute to gene flow by joining a hibernaculum different from their mother's. Determining the extent to which this potential is realised will require documenting juvenile dispersal patterns in the years between hatching and joining a communal hibernaculum.

Our movement data suggest that particularly mobile individuals from hibernacula 8 km apart can come together for mating. Because genetic connectivity between populations can be maintained with as little as one effective migrant per generation (Mills and Allendorf 1996), it is not surprising that Lougheed et al. (1999) found no evidence of genetic structuring for hibernaculum populations less than 6 km apart. However, they did find genetic structuring for hibernaculum populations 15 km apart and evidence consistent with an isolation-by-distance model (Wright 1943). Our movement data show that individuals attending hibernacula separated by 15 km would never come into contact for mating and that eggs would never be laid more than 4 km from the maternal hibernaculum. Our movement data are therefore consistent with the viscosity of gene flow at scales of 15–50 km documented by Lougheed et al. (1999).

In addition to their implications for gene flow, our data on spatial and movement patterns of black rat snakes also have implications for both conservation and sexual selection. In Canada, black rat snakes occur in small isolated populations and have been designated "threatened" by the Committee on the Status of Endangered Wildlife in Canada (Prior and Weatherhead 1998). The main factor hypothesised to be responsible for the declines in Canadian populations is habitat loss and fragmentation. If gene flow among local hibernacula is as extensive as our data suggest, then maintaining the genetic integrity of black rat snakes will require preserving a sufficient buffer of suitable habitat around hibernacula to allow snakes from different hibernacula to come into contact during the mating season.

Male-biased sexual size dimorphism in black rat snakes suggests that males actively compete for access to females (Shine 1978; Blouin-Demers et al. 2002). The greater movement by males, and their larger home ranges relative to females, are consistent with this expectation and suggest that one way males compete is by attempting to encounter as many females as possible during the mating season. Interestingly, however, females appeared not to be passive participants in mating. Rather, reproductive females also became more active during the mating season. This increased activity by females may simply facilitate being found by a male at a time when the snakes are widely dispersed. Alternatively, females could be promoting competition among males and (or) increasing opportunities for multiple mating. Genetic

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analyses of paternity and detailed behavioural observations will be necessary to assess these alternatives. Also, the fact that the average male and female home ranges contained more than one member of the opposite sex suggest that the opportunity exists for multiple mating in black rat snakes. Finally, the important individual variation we documented in home range size and movement patterns suggests that the variance in reproductive success should also be high.

Despite the apparent potential for gene flow through mating, gene flow is only realised if mating occurs and results in offspring. Knowing whether or not members of different hibernacula actually produce offspring together will require performing parentage analyses. Similarly, the distance of nests from the mothers' hibernacula suggested that juveniles can contribute to gene flow by joining a hibernaculum other than their mothers'. However, to determine if gene flow is realised in this fashion, data on actual dispersal by juvenile black rat snakes are required. Given that the movements of black rat snakes fell within the range of reported values for other snake species, we expect that the potential for gene flow between hibernaculum populations documented in this study will also be applicable to many other snake species.

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