Antlion Herding Patterns with Interrupted Communication Pathways

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Research Question and Hypothesis

Research Question:

Through what communication pathways (pheromonal communication, mechanical means, or innate preferences) do antlion larvae maintain group organization, measured by pit depth, width, and nearest neighbor, and what might this tell us about the antlion's evolutionary history?

Hypothesis:

Antlions likely have an intelligent mode of communication, therefore interruptions in the environment (removal of trails, introduction of physical obstacles, fictional pits) will impact their nesting patterns, whereas they wouldn't if the primary regulator were cannibalism and reclusion behavior (hiding under sand in over-dense areas).

Overview

The question of how antlion spatial patterns, such as pit depth, width, and nearest neighbor, as well as group behavior vary with respect to spatial constraints and interruptions in possible communication pathways was examined through the procedure. This research expands on a previous study that investigated antlions in habitats of, sometimes, extremely small size. It found that antlions, as groups, tend to have fewer and smaller pits on the surface in smaller areas, to maintain fair food-collection densities. This follow-up study aimed to identify the regularity of antlions' surface distributions, and whether this regularity is maintained if trails are removed or the environment is constricted with barriers. It was determined that antlions regularize their settlement patterns through a couple of innate tendencies: they prefer being on borders when possible, to, in a group, use all of the area, and distancing themselves from the raised sand around other pits. These wouldn't have happened in a system reliant on trail density or pheromones (because with trail erasure, regularity was maintained) or in a system reliant on cannibalism. This experiment tested these specific anti-competitive behaviors, building on our previous results, which showed antlions hiding under sand when the population got too dense.

Fig 1: A 24 × 24 trial, with three obstacles introduced into the container

Fig 2: A 3cm antlion pit

Background Information

To design the experiment and understand the underlying behaviors that might affect it, extensive background research was required—specifically on the spatial distribution patterns of antlions. First, a previous study analyzing the spatial patterning and structure of termite mounds in an African savanna was examined to better understand the procedure of the experiment. This study examined how different termite colonies in the African savanna positioned themselves concerning one another and uncovered that termite mounds maintain relatively constant distance from one another, creating uniform hexagons of termite mounds through the savannah. Furthermore, this study uncovered that termite mounds must maintain a constant distance from each other to prevent conflict between termite colonies, limiting the species' success. These results helped guide and shape the study by providing insight into the possible intraspecies competition that could result from close antlion contact, leading to the prediction that antlions would have to space themselves to prevent competition for food. Lastly, this study determined that a change in available space could affect the spatial patterns of termites as well as their behavior, which was later used in designing the conducted experiment.

Next, several studies regarding the anatomy and behavior of antlions were examined to better understand the insects. These studies determined that antlions stay in their larva form, during which they make pits, for 6-8 weeks and develop slower when exposed to less food. This helped determine the timeline of the experiment and determine the intervals at which the antlions would be fed, as to keep results consistent the antlions would have to be the same throughout the course of the experiment, which would require the participating antlions to be fed less to stay in their larva stage to make pits. Furthermore, these studies examined terms such as pit depth and width as well as the feeding patterns and behaviors of antlions, which became crucial areas of study throughout the experiment, as this determined that pit depth and width can signify the dominance and success of antlion settlement. This helped determine dependent variables to examine over the course of the study. Finally, these studies claimed that antlions tend to cannibalize each other in times of food shortage and significant competition. This provided another dependent variable to track over time and examine as the size decreased, as cannibalized antlions were unsuccessfully metabolized and evident in pits.

Next, a series of studies about antlion dispersal pattern called the "Doughnut theory" were examined to better understand the current scientific knowledge surrounding antlion dispersal patterns. These papers determined that antlions naturally position themselves in a "doughnut," in which a ring of antlions circle a center point or food source to limit competition for ants, as each antlion has equal access to the food source. This study also concluded that when antlions are introduced one by one the same results occur, which confirmed that the procedure could introduce one antlion at a time without interfering with results and spatial patterns, helping further perfect and standardize the procedure, as well as provide a better understanding of antlions behavior patterns. These studies provided a better understanding of antlion settlement patterns and gave a guideline for what to expect as trials continued. These studies also provided scientific procedures that could be tested and confirmed throughout the experiment, allowing for a source to cross-check results and procedures to perfect the procedure of the experiment.

Finally, to effectively conduct a follow-up examination of the nations spatial patterns and distributions the previous year's research and results was thoroughly examined. This examination helped provide information on the most effective procedure, materials, and dependent variables to measure, as the previous year's notes were used to improve upon the preexisting examination process. Along with this the examination of the previous years teachers clearly illustrated that the rate of cannibalism and the average pit depth and width were correlated with the size of the enclosure of the antlions, as the rate of cannibalism increased as the enclosure decreased in size while the pit depth and width decreased as the enclosure decreased in size. This distinction helped illustrate the natural patterns of the organisms and allowed for the clear development of a follow-up experiment, as the examination of the initial trial developed a clear natural pattern that could be examined through the introduction of other various environmental stimuli. Based on this analysis, the emergent property of the antlions distribution was clearly to arrange themselves in an organized fashion however the antlions lacked a known effective mode of communication, as prior research revealed that insect larvae lack secretion glands for communication and proper vocal anatomy. The lack of a mode of communication but the presence of a clear spatial pattern lead to the development of the question of how the organisms were able to arrange themselves in such an intricate pattern and prompted the follow-up study to examine how the organisms were able to distribute in such an organized fashion by either identifying a mode of communication or determining that the distribution was due to simple mathematics.

Materials

A square 24"×24" plastic container was filled with sand and used to house antlions during each trial, the plastic container was adjusted to a 12×12 container using a plastic barrier between trials. Between trials, 20 6-inch diameter circular plastic containers were used to house the antlions and were also filled with sand. In order to house the antlions 3 50-pound bags (150 pounds in total) were used to fill the individual 6-inch containers and the trial boxes. A total of 20 antlions were used throughout all trials in order to properly collect data, along with this a 3-foot string was used to create a grid system that housed the antlions. Furthermore, a ruler (with Centimeters), a sharpie, and 20 toothpicks were needed to properly determine the position and qualities of each pit. A small plastic cup 2 inches in diameter, a brush to flatten antlion trails, and several rocks were also needed to serve as obstacles and disruptors towards the antlions communication patterns. Finally, an ample supply of ants was needed to feed the antlions throughout the study, as well as a sieve to properly find, move, and collect the antlions.

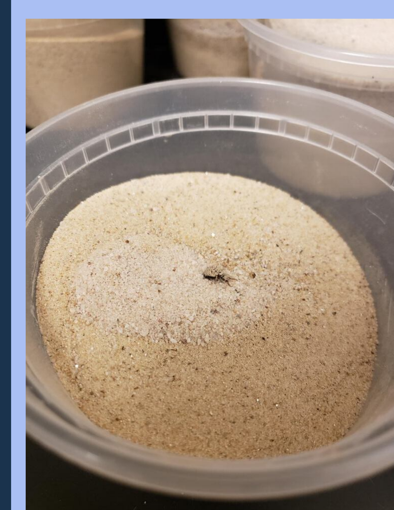


Fig 3: An antlion in the inter-trial holding containers with native sand

Fig 4: An image depicting the distribution of fake pits along the 24 × 24 trial, showing the 12 fake pits.

Fig 5: An image depicting the 12 × 12 trial with the introduction of an obstacle, showing how the 24 × 24 trials and 12 × 12 trials were separated.

Methods

The materials were first purchased. 24 16oz deli containers were filled with 2.5 inches of play sand and one antlion was placed in each container. Every week, each plastic container (i.e. each antlion pit) was given a small cricket The crickets were purchased from a pet supply store.

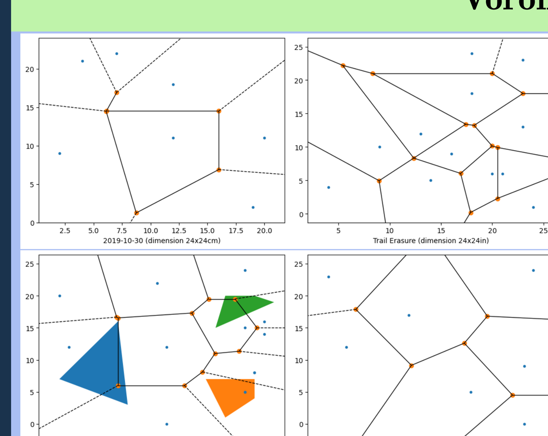
The remaining sand (100lbs) was spread into a 24" × 24" plastic container at a depth of at least 2 inches. A meter stick and a pen was used to make one-inch separated marks on the vertical and horizontal axes of the box so the antlions' pits' locations could be observed.

Using the grid, each trial was started by distributing a group of antlions in an array shape (the dimensions and populations of which are in a table below), and equally spaced between eachother and the walls, all inserted around the same time. Antlions were transferred between the small containers and the experimental environment by scooping them with a plastic spoon and sifting the sand from the antlion with a sieve. After the first and second days of each two-day trial, the coordinate locations, diameters, and depths of each antlion pit were recorded for later analysis. After each trial, all living antlions were restored to their pits and dead antlions disposed of.

Further trials repeated these same protocols except with modified space restrictions and several methods to disrupt potential communication pathways. Each disruption method was trialled with each space restriction, each trial run over a two day period. There are three different space restrictions and three different disruption methods. The space restrictions are 24" × 24" (the initial box size), 16" × 16", and 12" × 12" (constructed in the original container by cardboard and duct tape barriers). The three disruption methods are "trail erasure," "fake pits," and "artificial obstacles," making for nine trials in total.

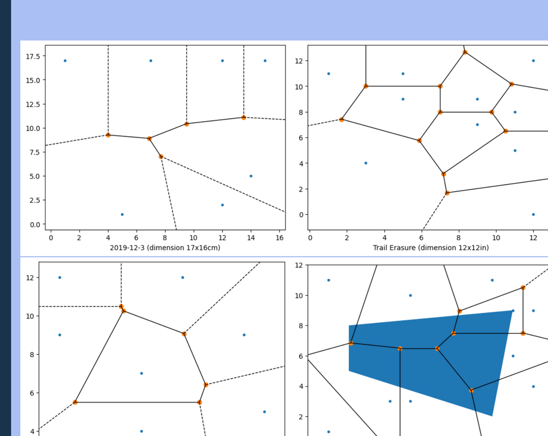
"Trail erasure" will be, once a day, brushing away old trails in the sand which antlions have dug out, in an effort to determine if the reduction of this possible communication pathway will destabilize or change the pit distribution. "Fake pits" will be sand scooped out in an inverse cone to mimic an antlion pit, with two or three placed uniformly randomly once a day, except when it would sit on top of an existing pit. This will show if the antlions are intelligently avoiding pits or if cannibalism creates the patterns that are observed in their distribution. "Artificial obstacles" are small stones or hard plastic barriers with a minimum height of .5in above the sand to determine if antlions are aware of the shape of their settlement region and use that to organize the group.

Voronoi Diagrams



$24x24$ Trials

These Voronoi diagrams, which label the territories of each antlion (an antlion "possesses" a part of the map within its segmented portion if its pit—the blue dot—is closest to that point). By examination of the Voronoi diagrams in conjunction with measurement of the nearest neighbor metric, it was determined that antlions regularize their nesting pattern to fairly allocate food intake. Based on the Voronoi diagrams depicted it can be also concluded that the introduction of fake pits most significantly altered the distribution patterns of the antlions because antlions created fewer pits.



12×12 Trials

In order to correlate environmental factors and spatial distribution of the antlions, four similar trials were conducted after the initial 24 × 24 trial, where the enclosure size was reduced to 12 × 12. The Voronoi diagrams for this subtrial for each of environmental conditions and a 12 × 12 control are shown to the left. Given the fact that the Voronoi area (the area of land that is closest to a given pit) remaIns relatively constant across all trials and subtrials it can effectively be concluded that the antlions have a non-random distribution pattern. Furthermore, since the mode of communication of the antlions seems to be undisturbed by both the introduction of fake pits, obstacles and the removal of trails, it can be concluded that the non-random distribution observed is not due to communication, but rather canabalism, which is the regulating factor in the antlions distribution pattern.

Results

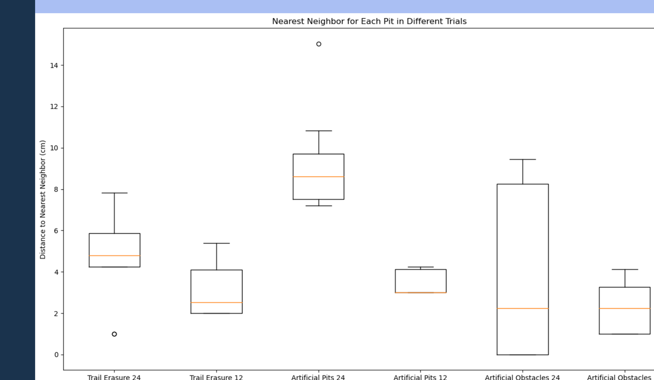


Fig 6: Antlions' pits' depths and widths correlate strongly with square root of trial area, meaning that antlions are aware of how to optimally obtain ants for the group. Bigger dots mean more pits of that size in that trial area

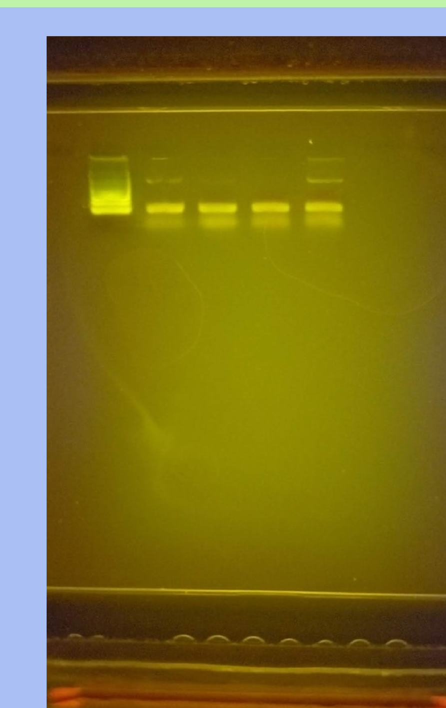


Fig 7: DNA Ladder and PCR tests were used to detect Cytochrome-B gene appearance in a primed DNA mixture from crushed antlions.

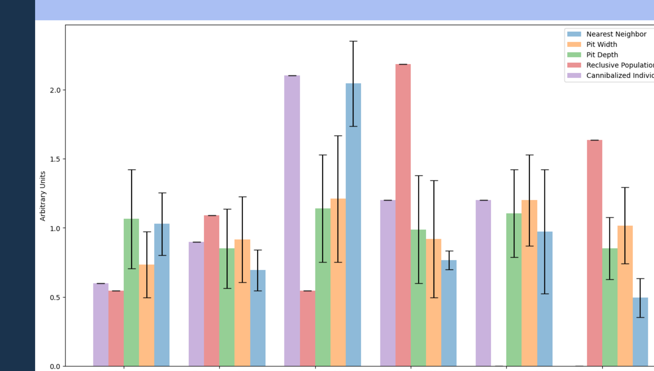


Fig 8: Histogram describing different statistics on a per-trial basis, including a variance measure for each based on how much it varied within the trial. Each statistic was normalized

Trial Size	Date	Introduced	Deaths	Pits formed
33×32	2019-10-16	31	6	9
24×24	2019-10-30	27	3	7
17×16	2019-12-3	19	3	7
17×16	2019-12-5	10	0	3
8×7	2019-12-19	12	4	3
8×7	2019-12-20	5	0	4

Fig 9: Number of Deaths and Pits Successfully Formed in Each Trial/Subtrial

	12 × 12	24 × 24
Artificial Obstacles	0.5807	0.0267
Artificial Pits	0.0151	0.0005
Trail Erasure	0.0257	0.0443

Fig 10: The probability of receiving the given antlion nesting distribution compared to simulated uniform randomness.

Size	Interruption	Reclusive	Dead	Pits formed	Nearest Neighbor (avg)	Width (avg)	Depth (avg)
24 × 24	Trail Erasure	1	2	12	4.69in	2.17cm	2.00cm
12 × 12	Trail Erasure	2	3	10	3.17in	2.70cm	1.60cm
24 × 24	Artificial Pits	1	7	7	9.33in	3.57cm	2.14cm
12 × 12	Artificial Pits	4	4	7	3.50in	2.71cm	1.86cm
24 × 24	Artificial Obstacles	0	4	13	4.45in	3.54cm	2.08cm
12 × 12	Artificial Obstacles	3	0	10	2.26in	3.00cm	1.60cm

Fig 11: The general statistics for each new trial, also portrayed in the histogram, of deaths, reclusivity, and some dimensional statistics.

Variables

Throughout the experiment the independent variable was the size of the container as well as the type of interruption that was used, which both changed from trial to trial due to intentional manipulation. Furthermore, the dependent variable throughout the experiment was the settlement patterns and behaviors of the antlions (*myrmeleonimmaculatus*), which was quantified through the nearest neighbor calculation, pit depth, pit width, number of cannibalized antlions, and individual settlement positioning determined over the course of several trials. The control trial of the experiment was the 24 × 24 trial with no deliberate interruption, as it shows the spatial patterns and behaviors of the antlions with the most available space and no factors that could impact their standard settlement, limiting the effect of competition on settlement patterns, which qualifies it to be an excellent control group.

Fig 12: An antlion settled in the corder of the 12 × 12 trial, which became a common settlement pattern throughout the first 4 trials.

Fig 13: An image depicting the fake pits that were introduced to the 24 × 24 trial, where a total of 12 fake pits were introduced, each with a pit depth of 5cm and a pit width of 8cm.

Fig 14: Part of the DNA barcoding process for species determination: concentrated mixture after chemicals, centrifuging, and crushing

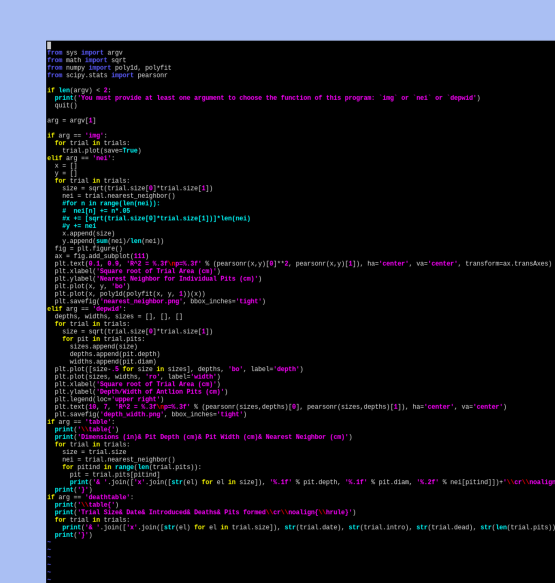


Fig 15: The code used to create Voronoi diagrams with SciPy and Matplotlib

Data Analysis

Antlions organize themselves systematically in response to the environments where they find themselves. Individuals attempt to regularize their own pit locations according to nearby pits and the borders of the living space, and this structure is affirmed by a statistical test on the uniformity of their distribution. Using a Monte Carlo simulation of 10,000 randomly distributed patterns of the same number of pits as observed from each trial, a typical distribution of the distance to the nearest neighbor was determined. The distance to the nearest neighbor is approximately independent for each pit, so a Cramér-von Mises statistical test was applied to the observed distributions, resulting in striking evidence that these pit distributions do not conform to a uniformly random independent distribution of each pit, with all (except one) distributions of antlions having $p < 0.05$.

- As a result of the statistical analysis procedure, during which the settlement patterns of antlions in a given trial were compared to a completely random settlement, a clear correlation was shown between the settlement patterns of the antlions and environmental constraints.
- A clear increase in the reclusive population as trial size decreased was observed in every obstacle condition, as the reclusive population increased from one (24 × 24) to two (12 × 12) during the trail erasure trial. The same results are shown across the trial with fake pits, where the number of reclusive antlions increased from one to four, and the obstacle trial, where the amount of reclusive antlion increased from zero to three.
- The number of reclusive antlions increased by an average of 2.333 antlions, or an increase of 233.3%, which marks quite a significant change.

This change indicates that the introduction of various obstacles and interference in other modes of communication can change the natural settlement patterns of the antlions, as a significantly larger number of reclusive antlions were observed during trials with artificial pits, indicating that the density of pits in a given area affects how antlions settle the territory. This analysis makes biological sense, as by regulating the density of antlions in a given territory the organisms can reduce intraspecific competition, which in turn helps the species reproduce more as a whole, thereby explaining the phenomenon. Along with this, a dramatic change in the cannibalistic nature of the antlions was noticed once various obstacles were introduced, for example.

- It was noted in the previous study that the number of cannibalized antlions increased by an average of 13.9785% when the trial size was reduced with no obstacles or communication interference.
- This differs from the trend in cannibalism noted throughout the following trials, as the data clearly shows how the rate of cannibalism tended to decrease across various trials.

This indicates that the antlions' cannibalistic nature is affected by environmental conditions such as changes in terrain and other obstacles, which could be a response to an interrupted communication pattern that results in a closer settlement, which intern would increase the contact between each antlion, thereby leading to more cannibalism. Finally, along with a trend in reclusivity and cannibalism, a trend was noticed in the average pit depth across all trials, as it decreased as trial size decreased throughout the study, regardless of the obstacles introduced (Fig 6). This indicates that the size of pits made by antlions is independent of the mechanisms that govern how they settle, as the study was able to alter all of the settlement patterns of the antlions through the introduction of obstacles except for the average pit depth, which maintained a constant pattern through the introduction of obstacles and the original trial from a previous year. This indicates that the average pit depth does not depend on their settlement pattern of the antlion community as a whole, but rather is dependent on factors such as time and available resources.

Several patterns were also observed in whole antlion group distributions.

- The average nearest neighbor remained relatively constant across all trials and environmental conditions, except for when a series of fake pits were introduced to the environment, as the average nearest neighbor ranged from 2.1-3.9 (Fig 8) for all trials except the 24 × 24 trial with fake pits, where the average nearest neighbor increased to 8.7.
- The nearest neighbor, in the fake pits trial, decreased by about 5.5 inches, which differed from the trail erasure where the nearest neighbor decreased by about 1.95 inches, and the trial where obstacles were introduced, during which nearest neighbor remains constant.
- The antlions maintain a non-random distribution pattern across all trial sizes and environmental conditions, as the antlions near the middle of the habitat maintain equidistance from their neighbors, a principle that is evidenced by the centermost pit in each Voronoi diagram.
- Voronoi diagrams may also show how the antlions tend to maintain semi-constant territory size across both changes in environment and changes in habitat size, as the average territory occupied by each antlion does not change significantly past a certain maximum density of pits. This strategy reduces cannibalism and competition.

Based on the settlement patterns of the antlions on both, an individual and group scale several conclusions can be drawn about the environment's impact on the settlement patterns of the insect along with the mechanisms that antlions use to settle in a non-random pattern.

- One of the most interesting patterns in the data was the impact of fake pits on the antlions settlement, as when fake pits were introduced to the environment the rate of cannibalism and reclusivity among the antlion population increased significantly, as shown by Fig 6.
- Antlions tended to space themselves away from fake pits when settling, represented by an abnormally high nearest neighbor metric (between real pits) during this trial, as shown by Fig 6.
- This indicates that the settlement pattern of the antlions is highly dependent on the existence of pits around them because the fake pits trial showed that raised ground and deformities in the environment allow antlions to decide on their nesting location.
- This may indicate, in part, mechanical rather than cognitive nesting mechanisms.
- Along with this the increase in reclusivity and cannibalism observed during this trial also indicates that antlions, as individuals, attempt to reduce the surface density of pits through extreme behavior.

Conclusion

Based on the trends observed throughout the study it can be concluded that antlions are dependent on a number of environmental conditions when they settle. This is shown through the various effects that introducing various environmental conditions had on their antlions settlement, as introducing fake pits into the habitat significantly increased the antlions tendency for extreme behavior and caused the standard distribution pattern they follow to be altered the most, as shown by Graph 2, which illustrates how the most extreme values for pit depth, nearest neighbor, and cannibalism occurred when fake pits were introduced to the enclosure. Along with this, the data suggests that antlions are also dependent on the presence of antlions trials in an area, as the trials where trails were erased also slightly altered the settlement patterns of the antlions. Finally, it can be concluded that obstructions such as rocks have a minimal effect on the antlions distribution patterns, as the trial with the introduction of rocks and obstacles did not result in any extreme behavior from the antlion population. With these patterns in mind, it can be concluded that antlions do not have a method of communication, as their settlement patterns were disturbed by normal environmental conditions. Despite this, it can also be concluded that antlions distribute in a non-random way in an enclosure, as shown by the aforementioned statistical analysis, thereby indicating that antlions rely on several environmental and local indicators to determine where to settle, such as the density of pits in a given region and the prevalence of trials near a given territory.