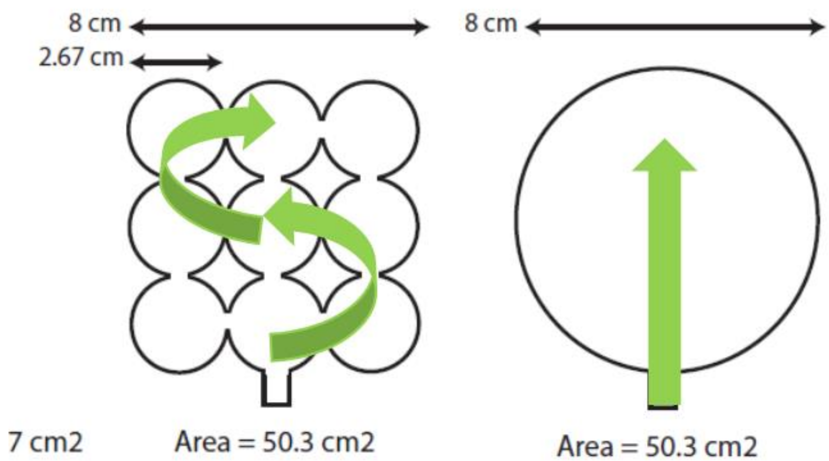
Protocol for data collection and image analysis to understand the interplay of nest architecture and colony size on collective food distribution in Argentine ant colonies.

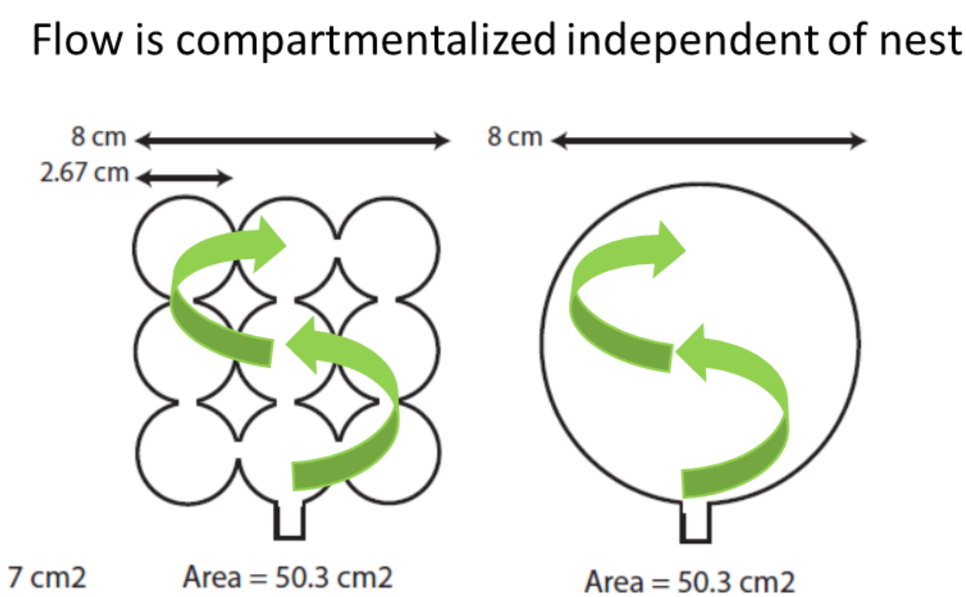
1. Hypotheses:

   A. Nest compartmentalization affects the rate of food intake and distribution in an ant colony

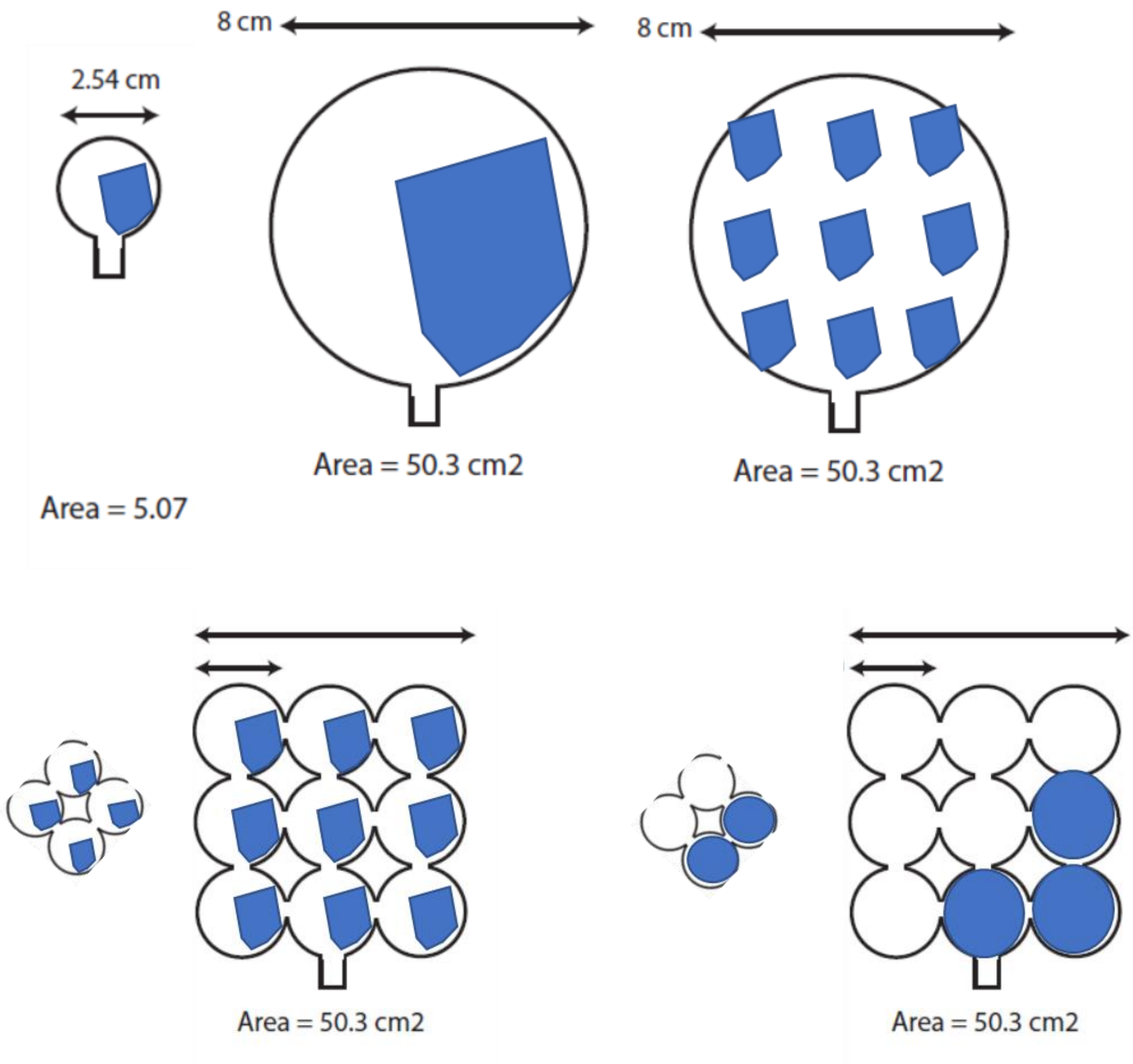
      Prediction1: Food distribution is dependent on and varies with nest architecture



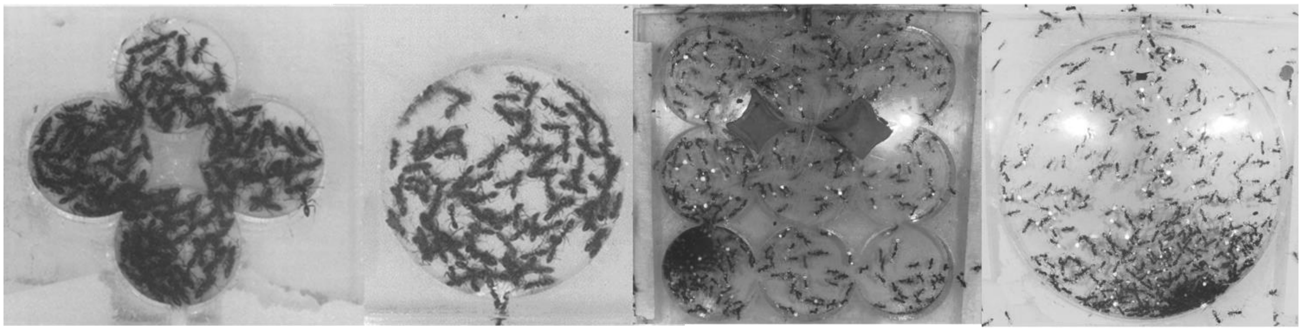
      Prediction 2: Food distribution is independent of and does not vary with nest architecture



B. How does group size affect the rate of food intake and distribution



2. Materials and methods



Small Chambered (SC) | **Small Unchambered (SU)** | **Big Chambered (BC)** | **Big Unchambered (BU)**

Image 1: The different nest shapes and sizes used to carry out the experiment.

The smaller circular and clove nests housed 111 workers with the queen and the bigger circular and 9 chambered nests housed 1000 workers with queen. Each colony was acclimatized for two days before being video recorded on the third day. A total of 35 colonies were collected from different locations in Temescal Canyon and parts of UCLA campus. Each colony's collection date and location of collection along with the number of brood in each colony and date of video recording has been entered in an excel sheet "JulieNitika – Colony Collection Data" in the storage unit (Storage:\Nitika\Ants)

Each colony was video recorded before feeding for about 10 minutes and images stored as the "prefluor" videos. Each of these colonies was provided sugar water with Fluoroscein that could be visualized through the abdomens of Argentine ants and extracted from images under a green filter (see image below). We used the red channel to extract location of food as the pixels above mean + x standard deviation as pixels with food.

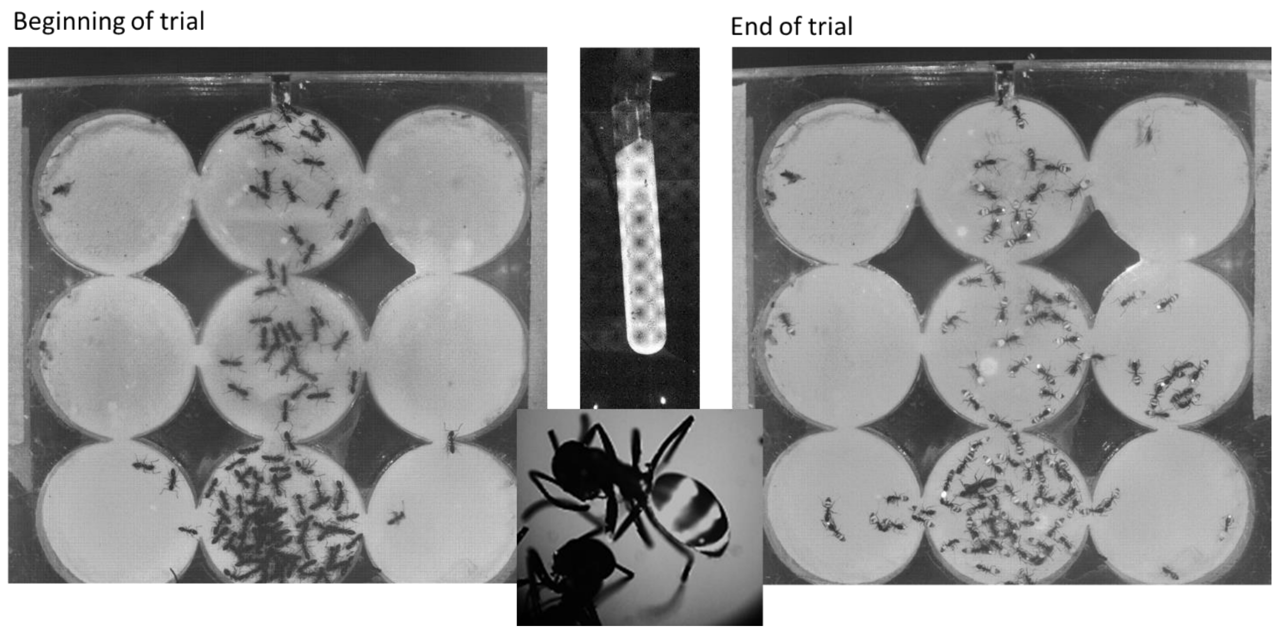


Image 2: The same colony before and after being fed fluorescent sugar water distributed through trophallaxis within the colony and used to extract the location of food at each timepoint through image analysis in MATLAB.

The location of ants themselves can also be extracted at different timepoints within the colony using the red channel in the images as the pixels below mean - x standard deviation as pixels with ants (Image 3).

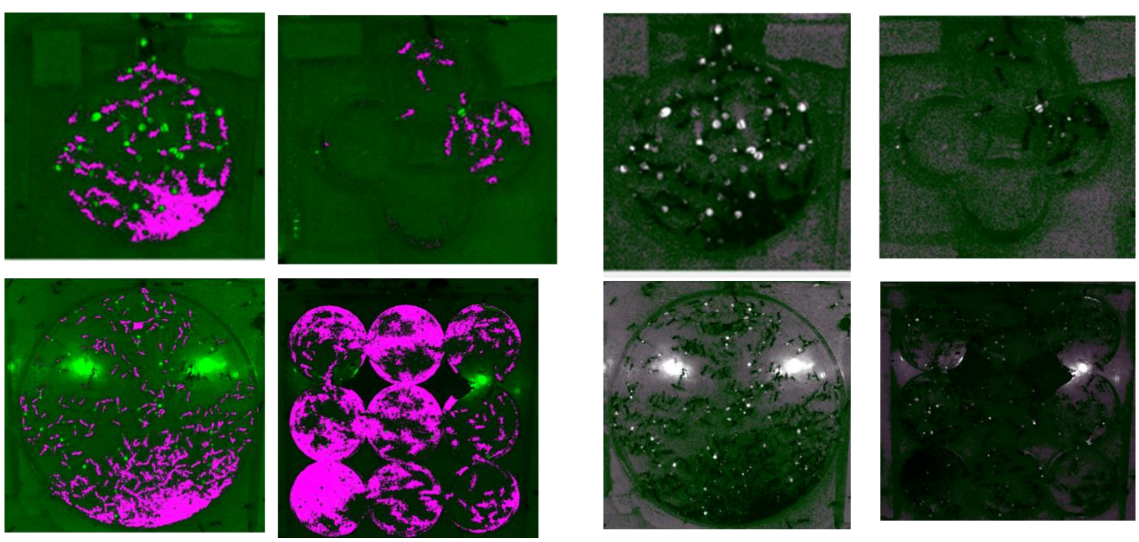
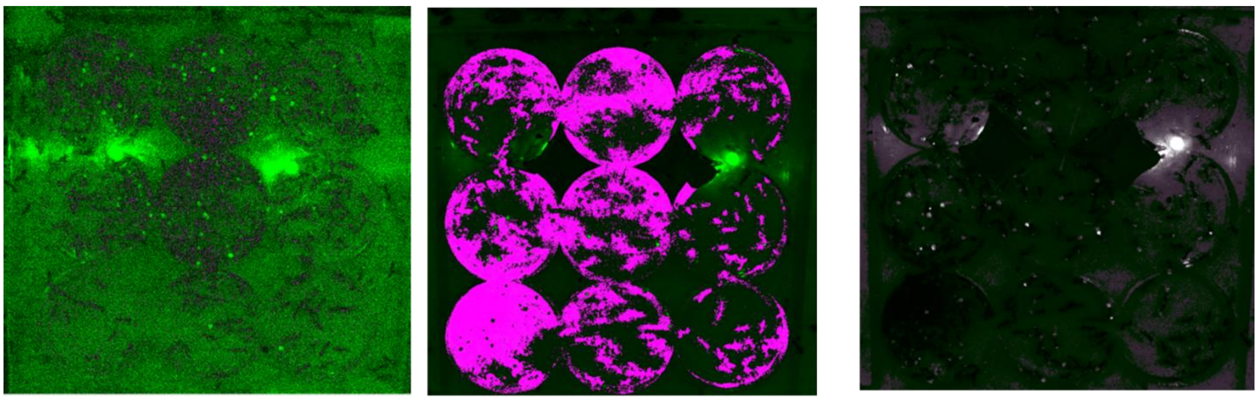


Image 3: The location of ants extracted using the red channel of images can be viewed in purple (left) and the fluorescent fool in the abdomens of ants as well as being exchanged via trophallaxis can be viewed in white pixels (right).

NOTE: I identified some issues that can be troubleshot by playing around with the standard deviation in the threshold being set in the MATLAB code to identify ants and food. Troubleshooting is a trade-off between false positives and false negatives and varies across videos (see examples in Image 4 below).



Underdetection in noisy videos | Overdetection in clear videos | Faint fluorescence

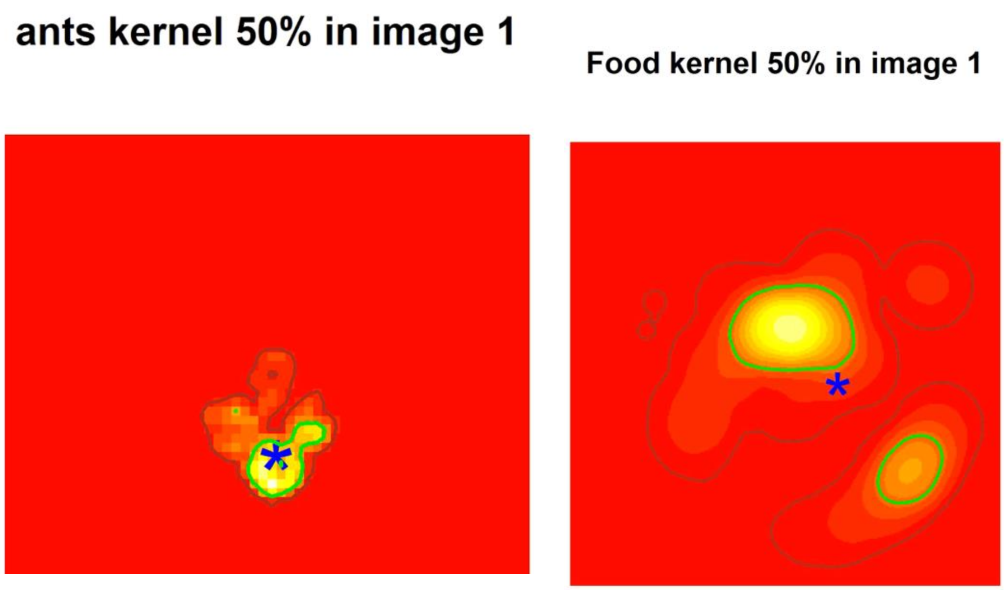
Image 4: Some variation in video quality and accompanying issues that needed troubleshooting.

We dealt with some of the issues like lighting reflection creating blind spots in the images by incorporating a code that will allow hand-drawn polygons with blind spots to be excluded from the image analysis, so they are not mistaken for fluorescent food. The code also allows to differentiate between different nest shaped that were filmed by asking for the number of wells in a selected video, which would be 1 for circular nests, 4 for clover nests and 9 for the big 9-chambered nests and can be hand-drawn in MATLAB code (Ver5_20210227_NitikaTroubleshotProcessTifFolder.m) when prompted.

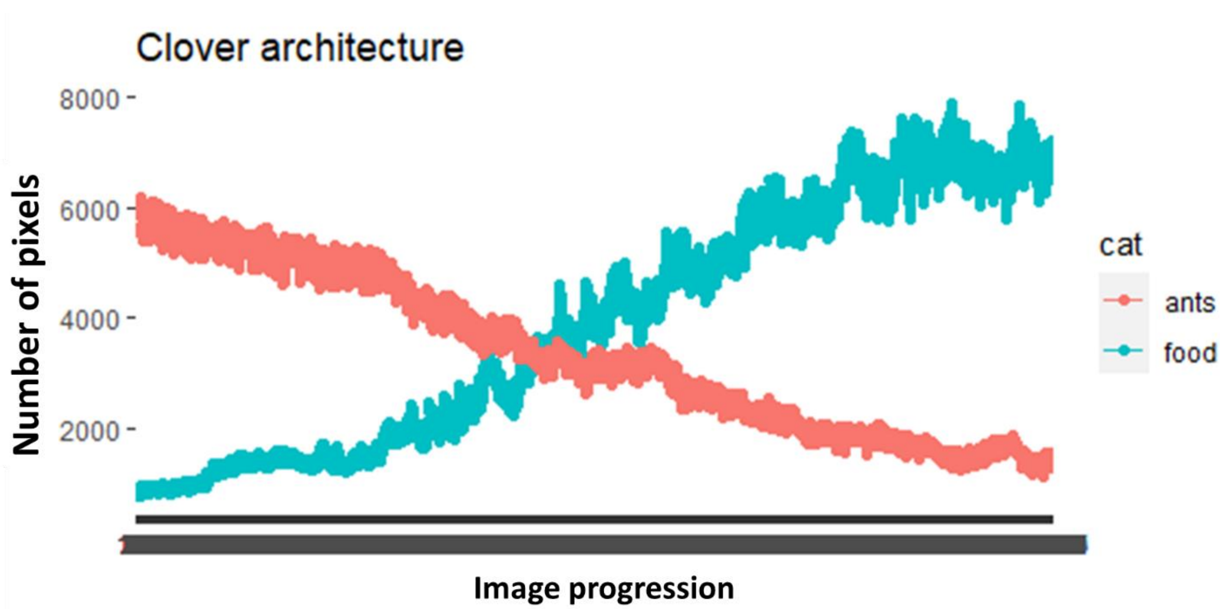
To avoid slow processing and huge files with coordinates for negative space, I extracted only the pixels that contained ants and food in the progressive images which grow exponentially as the food gets distributed within the colony.

Once the data is extracted in batches of 500 (designed for my less powerful laptop), the coordinates of ants and food in separate csv files can be used to see the progression of food in the colony and the spatial location can be used to visualize the spread of food through kernel density estimation heatmaps and area quantification using multiple measures like ratio of food:ants pixels over time, distance between food and ant core areas .

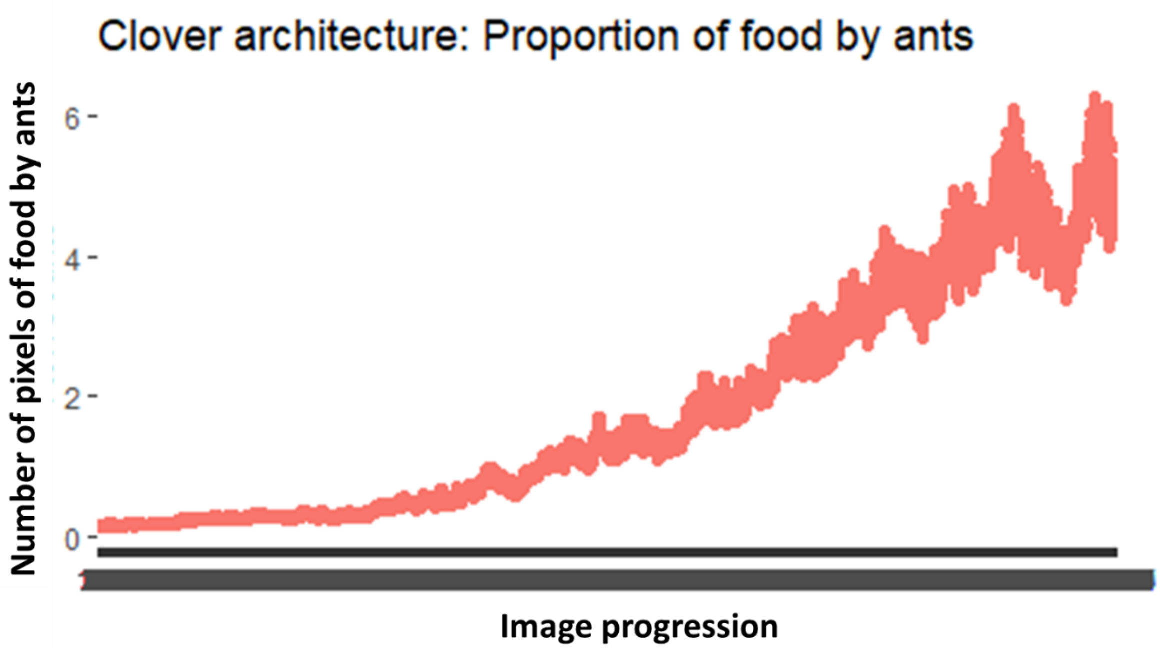
A)



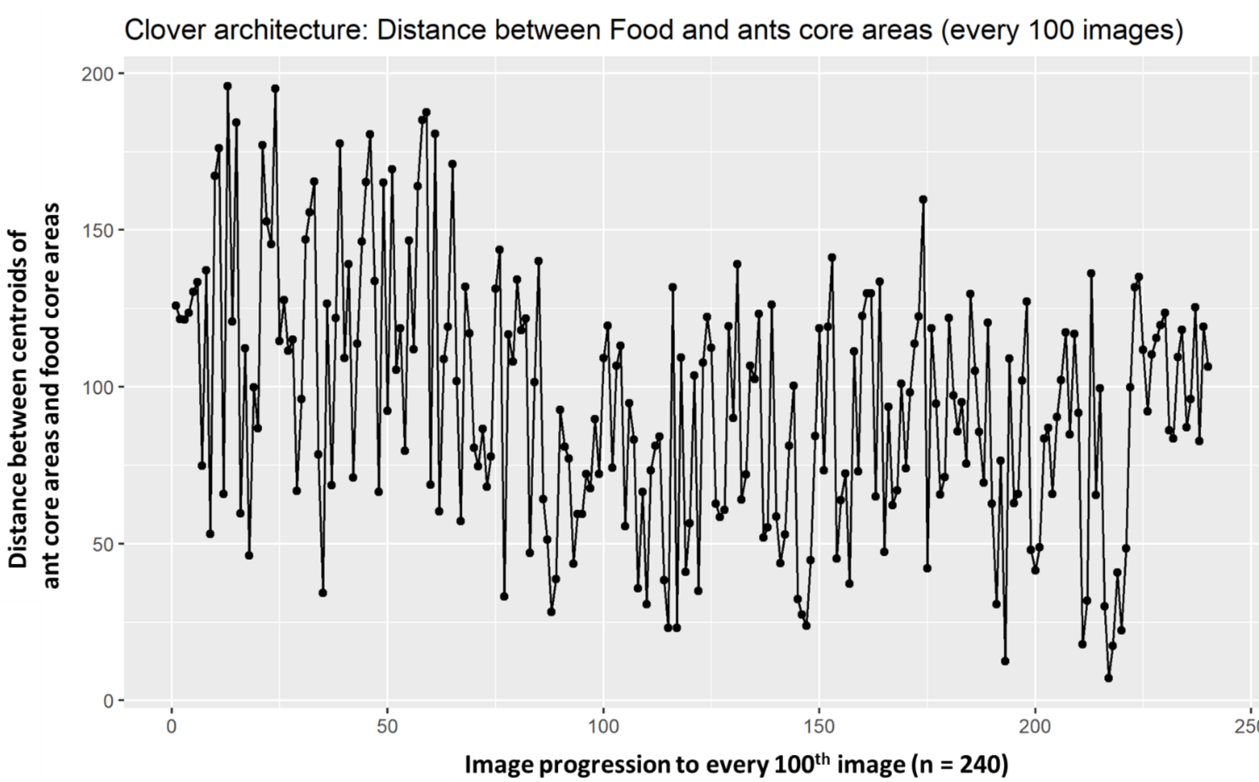
B)



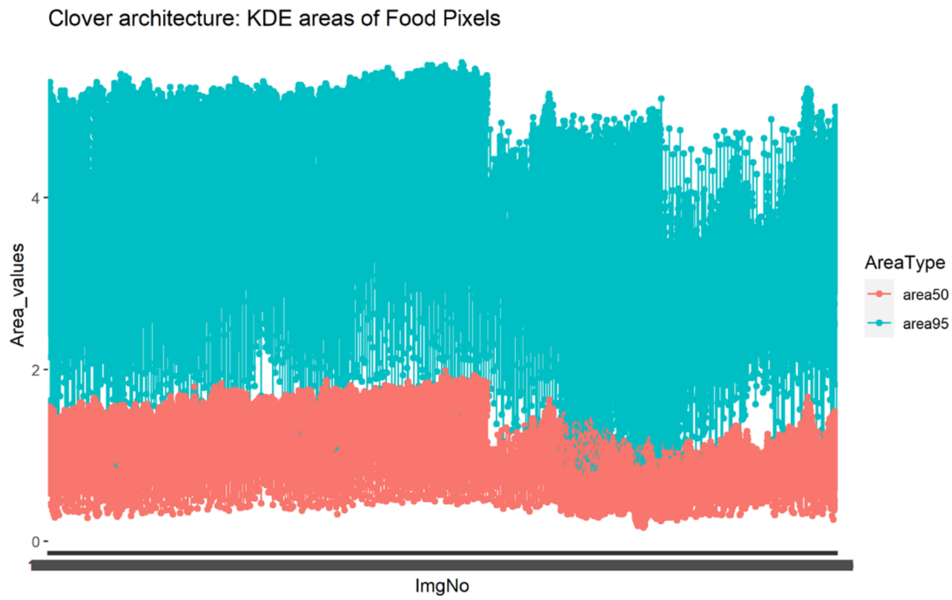
C)


Clover architecture: Proportion of food by ants
Number of pixels of food by ants
6
4
2
0
Image progression

D)


Clover architecture: Distance between Food and ants core areas (every 100 images)
Distance between centroids of
ant core areas and food core areas
200
150
100
50
0
0
50
100
150
200
250
Image progression to every 100th image (n = 240)

E)



F)

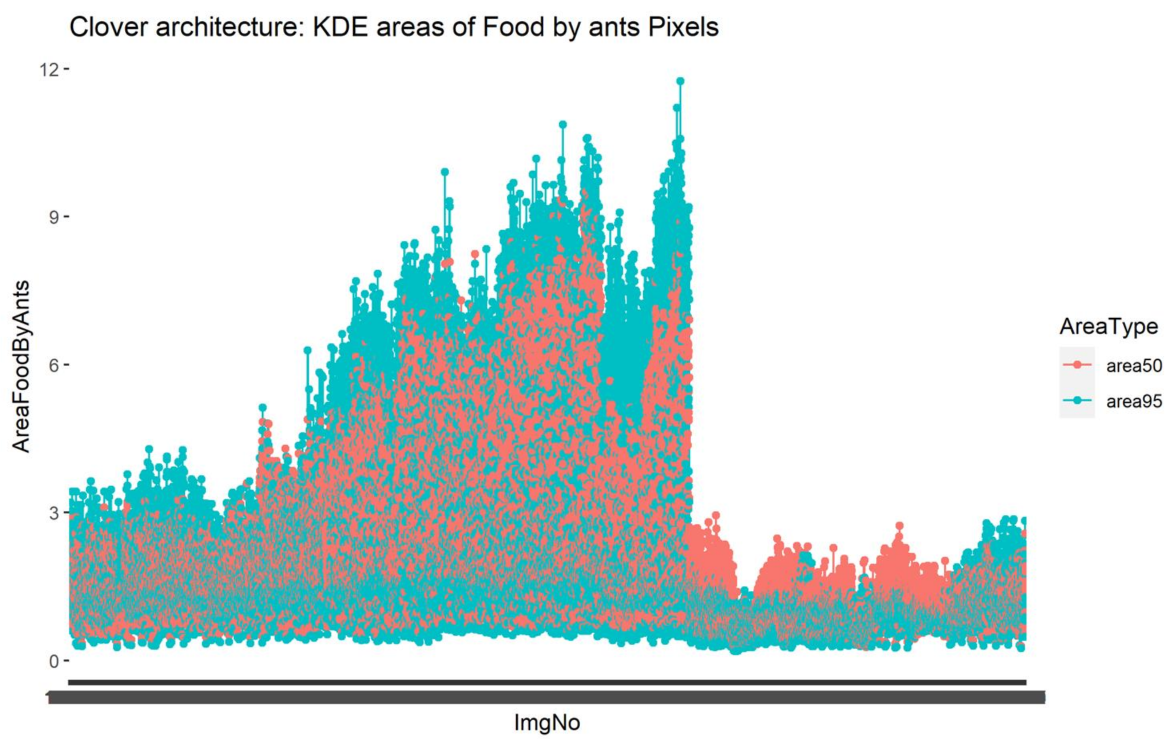


Image 5: Different measures used to understand the spatial distribution of food in Argentine ant colonies over time. A) Heatmaps created using kernel density estimation. B) Number of ant and food pixels over time. C) Proportion of food pixels by ant pixels over time. D) Euclidean distance between food and ant 50% core areas progressed for every 100th image

throughout the video recording. E) KDE areas (both 50% and 95% use) of food flow over time. F) Ratio of KDE (50% and 95%) areas of food pixels by ant pixels over time.

Future work and questions:

1. Do ant clusters act as structure or barriers similar to nest walls?
2. Distance of food and ant core area centroids from the nest entrance to use as a proxy for location.
3. Distance of food and ant core area centroids from the queen.
4. Proportion of area/chambers occupied – using number of kernel peaks to quantify or variance?
5. Null models assuming random movement to quantify probability of being fed in different architectures?
6. More creative ideas for spatial analysis for ways to quantify the differences between compartmentalized and uncompartmentalized nests? (e.g. number of turns an ant must take to exit/enter)