Final Report:
Diet and Colony Structure of Two Emerging Invasive Pest Ants

Background

California is a hub of trade, both globally and domestically. As a consequence, non-native organisms are frequently introduced to the state and, on occasion, become widespread and damaging invasive species. As these populations grow unchecked, they can become colonists of both residential and commercial structures. In California, many of our most obvious structural pests have originated in this way. Prominent examples include the German cockroach (*Blatella germanica*), the American cockroach (*Periplaneta americana*), the Oriental cockroach (*Blatta orientalis*), the black rat (e.g. roof rat or house rat: *Rattus rattus*), the brown rat (e.g. sewer rat or Norway rat: *Rattus norvegicus*), and the Argentine ant (*Linepithema humile*). Numerous other introduced species are significant agricultural pests (e.g. some fruit flies, and many moth and beetle larvae) and disease vectors (e.g. several species of mosquitoes).

Controlling the impact and spread of newly introduced species is challenging, particularly because the growth of knowledge about these organisms is generally slow. In the domain of structural pest control, this creates a major barrier to the discovery and advancement of tools and strategies for pest control. Here, we report the results of our research on the basic biology of two relatively unstudied introduced ants that are spreading in California: the brown rover ant (*Brachymyrmex patagonicus*) and the Moorish sneaking ant (*Cardiocondyla mauritanica*).

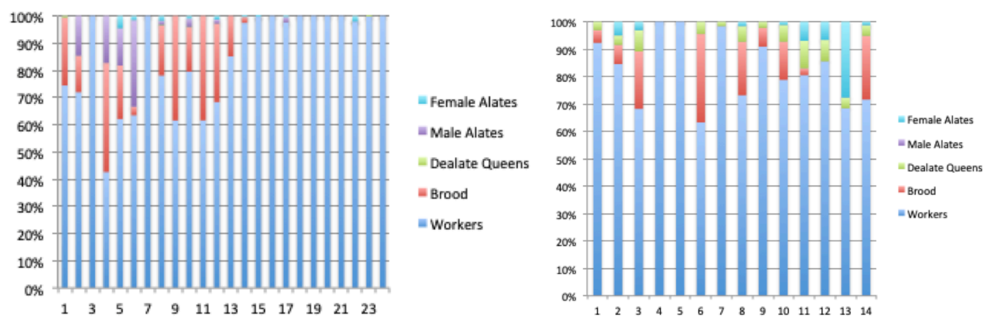
Colony composition and structure

Composition. Understanding the composition of ant colonies is essential for understanding how they behave, their capacity for ecological dominance, and their rates and patterns of spread. Some ant colonies, called **monogyne**, possess only a single queen. Monogyne colonies typically grow at a moderate rate and can produce new queens that disperse long distances to initiate new colonies far from their natal locations. **Polygyne** colonies, on the other hand, possess multiple queens, which allows the colony to grow at a rapid pace and attain high population densities. Under normal circumstances, polygyne colonies spread only locally, as new queens remain within their natal colony. However, human activities, such as the transport of plants or garbage, can move viable propagules and introduce colony fragments to widely distributed locations.

To understand the colony composition of the introduced rover ants and sneaking ants, we excavated and censused colonies of both species in northern and southern

California (Figure 1). In the brown rover ant (*Brachymyrmex*), we very rarely collected a reproductive (dealate) queen, and when we did, we never found more than one, suggesting that this species has a monogyne reproductive structure (n=24 colonies). In contrast, in colonies of the sneaking ant (*Cardiocondyla*), we commonly found multiple queens (mean queen number=6.7 per colony; n=14 colonies), indicating a polygyne reproductive system. In addition, in both species, we commonly found unmated (winged alate) queens, often in large numbers, indicating a capacity for both dispersal and colony growth.

Figure 1. The composition of colonies of the brown rover ant (left; n = 24 colonies) and the sneaking ant (right; n = 14 colonies).



Colony structure. The spatial size of an ant colony is a fundamental determinant of how it interacts with the surrounding ecosystem. Colonies that occupy larger areas also possess larger numbers of ants. As a result, larger colonies exert a stronger influence on other species in the area – consuming more resources, competing more effectively, and reducing biodiversity. When the surrounding ecosystem is the human-built environment, large colonies are more abundant and noticeable, and are more difficult to control or eradicate. In addition, when insecticidal treatments of large colonies occur at a scale smaller than the colony itself, these treatments may only “punch a hole” in the colony, which is then easily repopulated from the periphery.

The spatial structure of ant colonies exists along a continuum. Spatially restricted colonies that are comprised of only one or few nests are classified as **multicolonial**. At the other extreme, species that form extremely large colonies with no colony boundaries across wide geographic areas are classified as **unicolonial**.

To determine the colony structure of the rover ant and the sneaking ant, we performed behavioral assays that quantified aggression between ants collected from different locations. When ants belong to the same colony, they do not display aggression toward each other, but when ants from different colonies are paired together, they do show aggression.

Surprisingly, across 51 behavioral assays performed for the rover ant, we nearly always saw some level of aggression (mean aggression score = 3.43 ± 0.13, on a scale from 1 – 4), even between colonies that were located only meters apart. This indicates that the rover ant possesses a multicolonial colony structure, which is extremely rare for invasive ant species.

In contrast, we never saw aggression displayed by the sneaking ant (aggression score always = 1) across 50 behavioral assays. Thus, the sneaking ant appears to form widespread supercolonies, similar to that seen in the invasive Argentine ant.

Implications for pest control. These results, in addition to revealing some of the basic biology of these two ant species, also have practical implications for pest control. The rover ant (*Brachymyrmex patagonicus*) appears to be monogyne (single queen colonies) and multicolonial. Efforts to control this species are likely to be successful if the single reproductive queen can be eradicated. However, because the landscape will be occupied by numerous separate colonies, treatments (particularly with insecticidal baits) will need to be widely distributed, as toxicants will only be moved a short distance by workers. Because the rover ant is already widespread and abundant, and a serious pest in many locations, these data are likely to be relevant as pest control professionals customize their approaches to target this species.

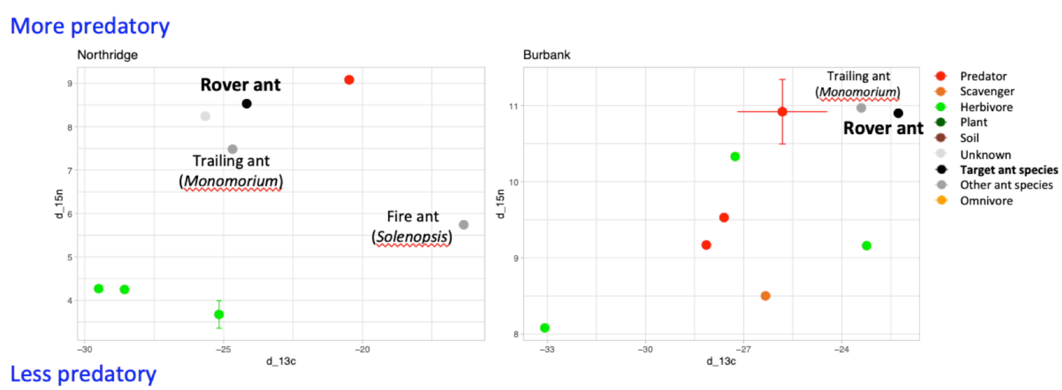
In contrast, the sneaking ant (*Cardiocondyla mauritanica*) has a very different reproductive organization and colony structure. These colonies each have many reproductive queens (polygyne), and thus, will require considerable effort to successfully eradicate. However, because sneaking ants also possess a unicolonial colony structure, workers are likely to distribute insecticides from bait stations across a fairly broad area, thus increasing the efficacy of such approaches. It is also worth noting that this species, true to its common name, was extremely furtive and cryptic in the field, making it difficult to find and collect. The low abundance and relative rarity of this species suggest that it is unlikely to become a significant structural or agricultural pest.

Food preferences in the field and lab

Different ant species vary in their food and nutritional preferences. Homoptera-tending ants often prefer carbohydrate (sugary) food, more predatory ants can prefer more protein-rich (high amino acid) foods, and other species will be more omnivorous. These dietary choices reveal basic information about the biology of the respective species, and are of obvious importance to any bait-based pest control strategy. Here, we examined the trophic ecology of the rover ant and sneaking ant in the field by quantifying stable isotope enrichment, then tested dietary preferences in laboratory choice tests.

Stable isotope analysis. Most of the common elements in nature have forms that differ in mass. These "stable isotopes" are non-radioactive forms of elements that occur naturally in soils and organisms. The ratios of stable isotopes of nitrogen and carbon within the tissues of organisms vary depending on their diet, and are therefore useful for understanding their trophic biology. Specifically, accumulation of the ^{15}N isotope increases with higher trophic position, thus indicating whether the ants are more herbivorous, omnivorous, or carnivorous. In addition, different types of plants use different isotopes of carbon for photosynthesis, so animals that eat these plants have carbon isotope signatures that reveal information about the plants at the base of the food chain.

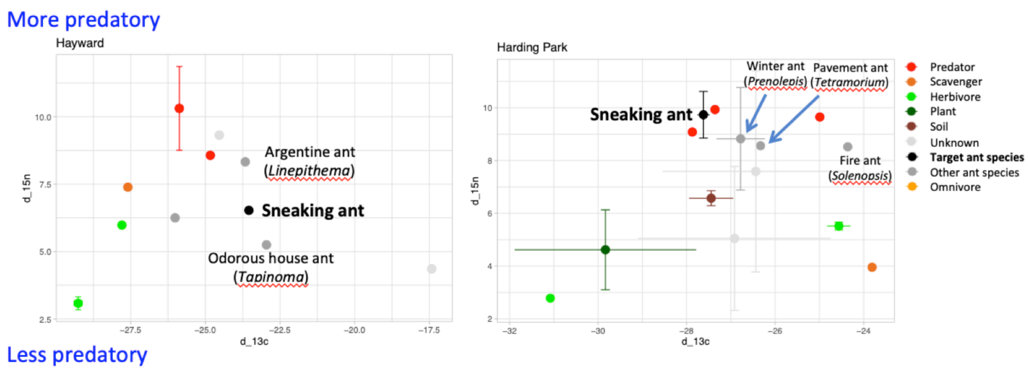
Figure 2. Stable isotope analyses of the brown rover ant. ^{15}N isotope enrichment is shown on the Y-axis. ^{13}C isotope enrichment is shown on the X-axis (but is not informative for the current study).



To estimate the stable isotope ratios, we collected the target ant species as well as other ant species, plants, and known predatory insects to use as references for the target ant species. Samples were analyzed on a CHNOS Elemental Analyzer with an IsoPrime100 mass spectrometer using Pee Dee Belemnite (PDB) as the standard. Our analyses showed that the rover ant had high ^{15}N enrichment (Figure 2), indicating that it occupies a trophic position characteristic of predatory species (or scavenging on dead animals). The sneaking ant showed similarly high ^{15}N enrichment at one site (Harding Park), but was more intermediate at a second site (Hayward)(Figure 3). Compared to other ant species from the same locations, the rover ant and the sneaking ant were equally or more predatory.

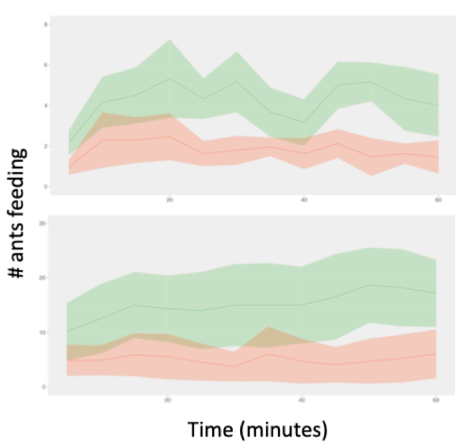
Dietary choice experiments. We next complemented our analyses of diet in the field with laboratory tests of food preference for both species.

Figure 3. Stable isotope analyses of the sneaking ant. ^{15}N isotope enrichment is shown on the Y-axis. ^{13}C isotope enrichment is shown on the X-axis (but is not informative for the current study).



For the rover ant, we collected colonies from the field, and first tested their preference for 10% sucrose versus 5% pure amino acid mixture in a two-way choice test. We recorded the number of ants feeding at the respective foods every five minutes for one hour, and replicated this six times (only one time per day) for each colony. We performed this experiment using three different colonies, but one colony was unresponsive (did not feed), so results are only shown for the two colonies that did exhibit feeding behavior in the lab. In this choice test, the rover ants clearly preferred the sugar solution to the amino acid solution (Figure 4).

Figure 4. Feeding preference of the rover ant in the lab for 10% sucrose (green) versus 5% amino acids (red), every 5 minutes for one hour. Each panel shows the experiment for one ant colony, each replicated six times.



Following this experiment, we performed a dietary experiment with water versus 10% sucrose versus wet cat food as the choices. We introduced wet cat food as a protein source to determine if the avoidance of amino acids in the previous experiment could be overcome by using a more complex protein source (which also includes some lipids and other nutrients). We also introduced a water control in this experiment to control for the potential confounding variable of thirst, since this test now included food presented in both solid and liquid form. Despite the rich and complex protein source represented by the cat food in this experiment, all six rover ant colonies still exhibited a clear and significant preference for the sugar solution in all cases (Figure 5).

Next, we performed a more focused dietary preference study to determine if there is an optimal sugar concentration preferred by rover ants. In a preference test using three different sucrose concentrations (5%, 15%, 25%), we found no significant difference in feeding activity (Figure 6).

Finally, we also performed dietary choice experiments on the sneaking ants (*Cardiocondyla mauritanica*). We first attempted to test water versus 10% sucrose versus cat food, as above, using six laboratory colonies and replicating the one-hour trials ten times per colony. Half of these colonies were unresponsive in the lab setting, sending out no foragers to the food that was presented. The results of the preference experiments for the three colonies that did respond are shown in Figure 7. In all cases, when the sneaking ants did feed, they showed a significant preference for protein over sugar or the water control.

Because the sneaking ant colonies performed poorly in the choice test, and generally appeared inactive and unresponsive in the lab setting, we chose to not pursue the

Figure 5. Feeding preference of the rover ant in the lab for water (purple) versus 10% sucrose (green) versus wet cat food (red), every 5 minutes for one hour. Each panel shows the experiment for one ant colony, each replicated six times.

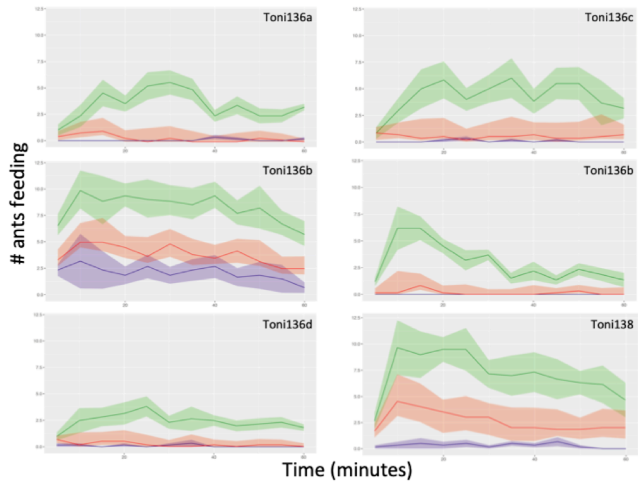


Figure 6. Feeding preference of the rover ant in the lab for sucrose at three different concentrations: 5% (light green), 15% (medium green), and 25% (dark green). Quantified every 5 minutes for one hour. Each panel shows the experiment for one ant colony, each replicated six times.

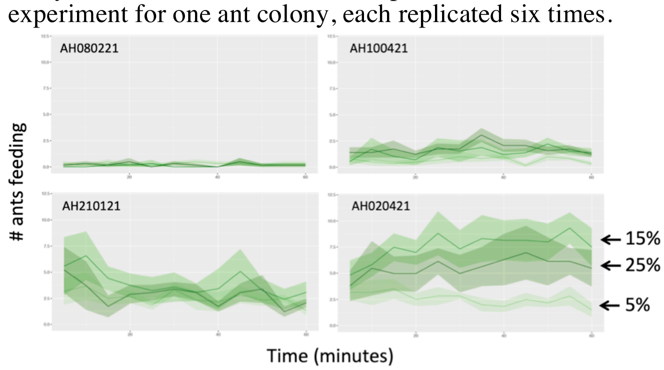
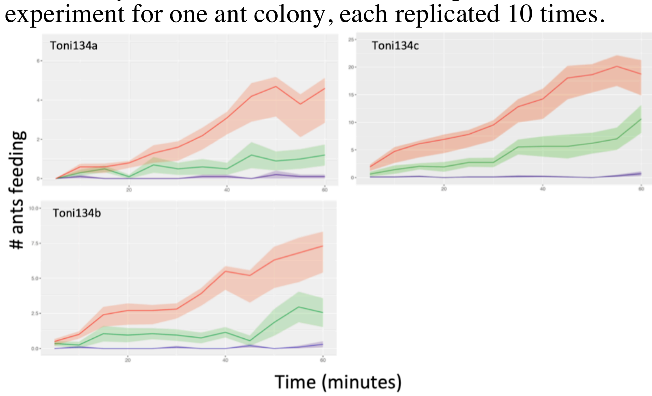


Figure 7. Feeding preference of the sneaking ant for water (purple) versus 10% sucrose (green) versus wet cat food (red), every 5 minutes for one hour. Each panel shows the experiment for one ant colony, each replicated 10 times.



dietary choice experiments further with this species. As their common name suggests, the sneaking ant was extremely furtive and shy in the lab setting, and we concluded that it would be difficult to perform highly replicated, robust dietary choice experiments for a range of different comparisons for this species.

Overall conclusions

The brown rover ant (*Brachymyrmex patagonicus*) and the sneaking ant (*Cardiocondyla mauritanica*) are both introduced ants with similar histories, and both appear to be spreading in California. However, our studies of their basic biology reveals that these are two very different species in nearly every other respect.

The rover ant is already a serious structural pest in some parts of the United States (especially Arizona and parts of southern California). We report here that colonies appear to be monogyne (single queen) and multicolonial (spatially restricted colonies), which has implications for treatment and control. Efforts to control this species are likely to be successful if the single reproductive queen within each colony can be eradicated. However, because the landscape will be occupied by numerous separate colonies, treatments (particularly with insecticidal baits) will need to be widely distributed, as insecticides will not be moved by workers any appreciable distance. Although we found that rover ants in the field occupy a relatively predatory trophic position, our laboratory experiments revealed that they have a much stronger preference for sugar baits when given the opportunity. This difference between the lab and the field probably indicates that, although sugar resources are strongly preferred by rover ants, such food is rare in the field.

In contrast to the rover ant, the sneaking ant (*Cardiocondyla mauritanica*) did not appear to be abundant at any of our study sites, and displayed furtive behavior when kept as lab colonies. Although this species has been widely introduced and appears to be spreading in its introduced range, it appears unlikely to make the transition to becoming a damaging invasive species, and is unlikely to be a common target for pest control. Nevertheless, if it becomes necessary to implement treatment and control measures for this species, the use of insecticidal baits should be successful, as the unicolonial colony structure will allow workers to distribute the toxicants across multiple different nesting locations. However, prolonged treatments may be required to eradicate all of the reproductive queens in each colony, to ensure that surviving propagules are not viable. Finally, protein-based baits will likely perform better than sugar-based baits, as this species occupied a relatively predatory trophic position in the field and displayed a significant preference for protein-based food in our limited lab experiments.