A Gut Feeling: Do Gut Microbiomes Help Invasive Species Rapidly Acclimate? 
Proposal for the Shipley-Skinner Reserve – Riverside County Endowment
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Introduction: Ever since the Red Imported Fire ant Solenopsis invicta and the Argentine ant Linepithema humile arrived in the United States, we have been trying to estimate their impacts and potential for further invasion. These aggressive species have spread beyond their predicted invasive range, into climates and habitat distinct from their native range. Using only traditional factors of native climate and habitat, current prediction models have under-predicted S. invicta’s realized invasive range (Fitzpatrick, Weltzin, Sanders, & Dunn, 2007). Several explanations for this highly successful and ongoing expansion have been proposed. For example, fire ants have an affinity for disturbed habitat and a generalist diet, potentially facilitating the invasion of habitats outside of their native geo-temporal niche. Here, we propose to investigate the possibility that invasive ant species’ rapid command of new environments may stem from their gut’s ability to quickly adapt to a novel microbial environment. This study will track the rates of change in bacterial diversity in worker ants, comparing invasive species, their native competitors, and native non-competitors.

The Shipley-Skinner reserve is uniquely important for native ant species because of location of the protected area, and the variety of habitats it encases, particularly the sage scrub habitat. A study found that inland sage scrub habitat may be the best habitat in California for ant diversity, because its unique climate supports many native ants while deterring domineering invasive ants that depend more on coastal and/or urban moisture (Staubus et al., 2015) Furthermore, our Mediterranean climate in general supports high biodiversity relative to its size (Staubus et al., 2015). Protected inland sage scrub communities are critical, as invasive grassland and human settlements have reduced the prehistoric levels of sage scrub land by 90% and continue to threaten what remains (Staubus et al., 2015). The reserve is therefore also uniquely important to our study, because these protected areas allow us to study native species away from disturbed habitat. Furthermore, because the reserve is close to urbanized areas, we expect to find invasive ant species nearby, helping us to control for location effects.

Background: Invasive ants impact humans directly, but also cause ecological damage by replacing native ant species. Studies show a direct connection between native California ants being replaced by invasive ants and lower native plant fitness. For instance, L. humile reduced floral visitation rates, floral visitor diversity, bee visitation, and increase pollen limitation in native California plants. These effects directly reduce seed set, genetic diversity, and therefore the fitness of our native plant species (Hanna et al., 2015). Similarly, the threatened California endemic Coast Barrel Cactus (Ferocactus viridescens) had significantly lower seed and fruit production when visited by L. humile, then when visited by a California native ant, Crematogaster californica (LeVan, Hung, McCann, Ludka, & Holway, 2014). These impacts may be stronger when native plants are in competition with invasive species that rely on wind pollination or self pollination (e.g. invasive grasses) (Hanna et al., 2015).

Invasive species can affect other trophic levels as well. For example, S. invicta is known to kill mammals, birds, and reptiles, including poultry, lizards, snakes, and ground-nesting birds (Allen, Epperson, & Garmestani, 2004). Invasive ants also indirectly harm reptiles through competition, such as L. humile replacing the California endemic Coastal Horned Lizard’s food source, native harvester ants (Suarez, Richmond, & Case, 2000). They dramatically reduce native ant diversity, which can have cascading effects on other plants and animals. L. humile invasions in particular remove native ant species that provide key seed-dispersing services to native plants, and because L. humile is ineffective at seed dispersal, this disrupts the population dynamics of native plants (Carney, Byerley, & Holway,
While invasive ant species reduce native species, they also benefit other invasive species, for example by tending invasive scale insects and aphids. This invasive mutualism with plant parasites has been shown to change forest plant and arthropod communities, which then recover after removal of the invasive species (O’Dowd, Green, & Lake, 2003).

In addition to damaging local ecosystems in their invasive ranges, invasive ants cause substantial economic harm by destroying infrastructure, lowering agricultural production, incurring pest control costs, damaging livestock, and increasing medical costs, causing several billion dollars in annual damage in the U.S. (Bertelsmeier, Luque, Hoffmann, & Courchamp, 2014). In terms of medical costs, both *S. invicta* and *L. humile* are damaging because of their invasion of human habitat. At least 83 people had been killed or have had near death allergic reactions to *S. invicta* stings according to a survey from 1989 (Rhoades, Stafford, & James, 1989). *L. humile* has been shown to be a mechanical vector of human pathogens and parasites in hospital, livestock, and agricultural settings, such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus*, and the worm *Ascaris lumbricoides* (dos Santos, Fonseca, & Sanches, n.d.; Ipinza-Regla, Gonzalez, & Figueroa, n.d.). In terms of agriculture, invasive ants also cause harm by having more effective mutualisms with crop pests than native ants, as described above.

**Justification:** Why study the gut microbiome of invasive ants versus native ants? Previous research on *S. invicta*, suggests that the gut microbiome lacks specialized endosymbionts, and can rapidly assimilate microbes from novel environments (Lee & Hooper-Bui, 2014). This ability may contribute to a species’ invasive potential for two reasons. First, novel microbes in the new environment may be detrimental to specialized endosymbionts. An ant species relying on a specific gut endosymbiont may not be able to maintain its population when exposed to new bacteriophages and competition from new microbes. Second, certain novel microbes in the new environment may be beneficial in protecting the ant itself against local pathogens, including fungi, viruses, and other microbes.

Recent studies exploring ant microbiomes are beginning to show possibly unique characteristics in invasive ant guts versus native ones. The microbiomes of *L. humile* and the other species in this proposed project have not been explored. However, recent papers on *S. invicta* have explored the species’ lack of a consistent microbiome, while most ant microbiome literature focuses on ant symbionts or pathogens. These papers are the basis of our hypothesis; however, they have only confirmed the environmental origin and plasticity of *S. invicta*’s gut microbiome. We plan to add to these conclusions by exploring rates of microbial turnover in *S. invicta*, in addition to describing the unexplored microbiomes and rates of turnover in five other ant species. There is evidence that the larvae of *S. invicta* have completely plastic gut microbiomes, and do not depend on endosymbionts (Lee & Hooper-Bui, 2014). The 4th instar larvae of fire ant colonies raised in lab completely changed their microbiome based on their diet, while workers sampled from far-apart locations had entirely disparate microbiomes (Lee & Hooper-Bui, 2014). Furthermore, antibiotic clearance in 4th instar larvae caused no significant differences in colony mortality, suggesting that fire ants have no essential endosymbionts (Lee & Hooper-Bui, 2014).

Other studies support the premise that the microbiome of an entire *S. invicta* colony is dynamic and solely environmental in origin. One study showed that *S. invicta* colonies from distant locations had completely different bacteria in workers’ guts, and hypothesized that their microbiome was therefore environmentally derived (Ishak et al., 2011). A second study fed specific bacteria species to workers and queens over two weeks, and showed that 30% of workers and 5% of queens acquired that species of bacteria (Jouvenaz, Lord, & Undeen, 1996).

Beyond having a plastic microbiome, one paper showed *S. invicta*'s higher tolerance for bacterial diversity than the native ant species they displaced, *Solenopsis geminata*. *S. invicta* workers and 4th instar larvae had significantly higher ranges of bacterial diversity in their guts, in spite of significantly
lower bacterial diversity in the surrounding soils than the *S. geminata* workers and larvae (Ishak et al., 2011). This paper indicated that this difference may be due to *S. invicta* picking up more mutualistic bacteria from the environment than *S. geminata*.

While there is rich literature on advantages of obligate symbionts in ant gut microbiomes, this study will be the first to test if the ability to rapidly adjust the gut microbiome provides advantages in novel habitat, potentially allowing some species to be more invasive than others. This study will track the rates of change in bacterial diversity and abundance in worker ants, comparing invasive species, their native competitors, and native non-competitors.

**Objectives:** We will compare the diversity of bacteria in the worker gut of six ant species first in the field, and then over time in common laboratory conditions. Because the microbes encountered in the field will be different from those in lab, we expect to see gradual turnover of bacteria species from the initial field collection, as they are replaced by microbes from the novel lab environment. We predict that the microbiomes of two invasive ants, *S. invicta* and *L. humile*, will exhibit a more rapid turnover of their microbiome and higher bacterial diversity than their native competitors. We also expect to see

<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
<th>Habitat</th>
<th>Diet</th>
<th>Distribution</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Linopithorcordum humile</em></td>
<td>invasive</td>
<td>moist, disturbed</td>
<td>generalist</td>
<td><img src="./image1.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><em>Tapinoma sessile</em></td>
<td>Native, invasive in urban areas</td>
<td>urban, and non-urban in most of North America</td>
<td>generalist</td>
<td><img src="./image2.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><em>Solenopsis invicta</em> (Red imported Fire Ant)</td>
<td>Invasive</td>
<td>disturbed, arid</td>
<td>generalist</td>
<td><img src="./image3.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><em>Solenopsis syriaca</em></td>
<td>Native</td>
<td>arid, tolerates drier habitat than <em>S. invicta</em></td>
<td>generalist</td>
<td><img src="./image4.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><em>Formica franklini</em></td>
<td>Endemic</td>
<td>dry river beds</td>
<td>generalist</td>
<td><img src="./image5.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><em>Veromessor pergandei</em></td>
<td>Endemic</td>
<td>desert, highly tolerant to heat and dry environment</td>
<td>generalist</td>
<td><img src="./image6.png" alt="Image" /></td>
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diversity among the native ant species according to their niche. By pairing phylogenetically close species, this study will also look for correlation between related species in their microbiomes. We also will look for differences between unrelated ants, and ants with unique niches.

**Expected Results:** If invasive ants do prove to have higher gut bacterial diversity tolerance and plasticity, this result may inform new predictive methods for identifying future invasive insects. This research avenue may also suggest novel directions for the development of targeted biocontrol efforts. Beyond exploring the differences between invasive and native ants, these results will also allow us to explore the effects of niche and phylogeny on gut microbiome contents. This study will also contribute to surveying the native species on the Shipley-Skinner reserve, as we will document ant diversity and abundance at collection sites within the reserve. Future directions to explore include comparing the microbiomes of ants in their native range to their microbiomes in their invasive ranges, and exploring seasonal effects on gut microbiome plasticity and diversity. Comparing relative microbe taxon abundance via qPCR can also be used to quantify the changes in the bacteria communities over time on a finer scale.

**Species Selection:** Two invasive species will be used: *S. invicta* and *L. humile*. Four native species will be collected: *Veromessor pergandei*, *Tapinoma sessile*, *Formica francoeuri*, and *Solenopsis xyloni*. We chose to pair each invasive species with a phylogenetically related species, and to include two other native species from southern California that are found in similar habitats to the two invasive species. Comparable traits are critical to examining how competitive species interact with and pick up microbes from their environment and through other interactions. *Tapinoma sessile* (native) has been studied in the past as the most similar native competitor in North America to *L. humile* based on highly similar worker size, morphology, foraging ecology, physical and chemical defenses, diet, nesting preferences, their shared subfamily Dolichoderinae, and their large polygynous, polydomous supercolonies (Buczkowski & Bennett, 2007). *T. sessile* also becomes an urban pest by altering its behavior in the same way as *L. humile* (Buczkowski & Bennett, 2007). In terms of U.S. range, *L. humile* is found mainly in California and most southeastern states (Buczkowski et al. 2004). *T. sessile* is endemic to North America, and its range often overlaps *L. humile* (Holway, 1999; Human & Gordon, 1999).

*S. xyloni* and *S. invicta* are closely related species in the genus *Solenopsis* that can hybridize, and that also compete for food and habitat where they overlap from Florida to southern California (Axen, Wildermuth, & Helms Cahan, 2014). *S. xyloni* occupies some arid environments that *S. invicta* cannot colonize, but they broadly overlap in more moist environments (Chen, Rashid, & Feng, 2014).

Both *F. francoeuri* and *V. pergandei* are endemic to southern California (Bolton, 1995; Davidson, 1978). *F. francoeuri* now overlaps with *L. humile* across much of its range, and relies upon similar food sources (e.g., tending aphids). Another endemic species to southern California, *V. pergandei* is specially adapted to extreme arid environments, with a seed-based diet (Davidson, 1978). While the diet differs from the other focal species, *V. pergandei* is a commonly displaced species in some of the agricultural zones that have been invaded by *S. invicta* and *L. humile*. We predict that the tolerance to extreme heat and a seed-based diet may lead to a unique gut microbiome.

As this study proceeds, we may adjust the focal species, potentially adding species, based on ant diversity on the reserve. Species with differentiated niches and previously unclassified gut microbiomes are our primary focus. For example, if a colony of slave-maker ants (genus *Polyergus*) with a diverse group of kidnapped worker species are found, we could investigate how genetic origin affects a worker’s gut microbiome within identical abiotic and social environments. *Polyergus breviceps* is native to California, and obligately steals workers from other species of ants (often multiple species at once) because it has no worker caste (Trager, 2013). According to antwiki.org, *P. breviceps* has been found...
once in Southern California close to the reserve by Lake Hemet, and we will seek this species in the riparian areas of the reserve.

**Research Plan:** Workers from at least six focal species (Table 1) will be collected from the field and maintained in lab for 8 weeks. About 1000 workers per colony will be collected; keeping over three times the number of workers needed for the microbiome analyses will both allow for ongoing social interactions throughout the experiment and offset worker mortality rates. Five colonies will be sampled per species. Three workers will be sampled everyday from each colony (in lab), however; only workers collected at seven time points will be dissected and have their microbiomes sequenced via MiSeq. Briefly, each individual will be uniquely barcoded, and a region of the 16S gene will be sequenced following the protocols for non-culture based next generation sequencing analysis described by Engel et al. (2015). Workers sampled on days in between these dates will be frozen. These samples can then be later used to clarify the timeline of microbiome change if necessary. An extra set of 3 ants per day will be sampled in case our results warrant qPCR analysis.

These six species were chosen for this study based on their availability according to our preliminary surveys as well as sample locations identified on Antweb.org and Formiciculture.com. Two to three localities will be identified for each species, with two to three distinct colonies sampled per species. In the case of *L. humile* and *T. sessile*, we will sample from at least two distinct supercolonies. After preliminary surveys of ant diversity in the reserve, all colonies will be sampled within 3 days of one another to avoid seasonal variables. The day of the collection will be day zero for that colony, and the first three ant samples for that colony will be placed in ethanol in the field. The remaining workers will be housed in ant cages, separated by species and colony, and fed a uniform diet of egg, sucrose, and agarose for 8 weeks following their collection date.

**Budget:** The Purcell lab at the University of California Riverside will cover costs that exceed this budget. The gas budget is based on current gas prices, trips to survey for species and collection sites, and the collections themselves. The budget for ant cages is based on 30 solid bottom mice cages, one for each colony. Fluon will be applied to the cages to prevent ants from escaping. About 600 samples are estimated for this project, and MiSeq allows for about 380 samples to be sequenced. Three MiSeq applications are requested to allow room to revisit samples and for supplementary species we may find on the reserve.
References Cited:

http://doi.org/10.1111/een.12100

http://doi.org/10.1007/s10531-014-0794-3


http://doi.org/10.1007/s10530-007-9179-9

http://doi.org/10.1007/s00442-003-1200-0

http://doi.org/10.1371/journal.pone.0096842

http://doi.org/10.1086/283294


http://doi.org/10.1111/j.1466-8238.2006.00258.x

http://doi.org/10.1890/14-0542.1


http://doi.org/10.1007/s000400050127


