Literature Review 3: Annotated Bibliography

Note: I organized my annotated bibliography in order from the oldest publishing to the most recent publishing. I decided to organize it this way so that I could see a timeline of the research conducted and see how each article builds off of each other / added to the topic. There is a total of 10 articles used in this document.

Article Citation:

Weber, N.A. (1972). The fungus-culturing behavior of ants. *American Zoologist, 12*(3), 577-587. https://doi.org/10.1093/icb/12.3.577.

Article Type: Secondary

Article Summary:

Ants in the tribe Attini cultivate fungus "gardens" within their nests. Attini colonies are started when a fertilized female ant from an existing colony takes a piece of the fungal garden and "plants" it at a new nest site, after which she lays eggs. Worker ants help take care of the garden according to labour division; minima (smallest ants) tend to the garden directly through behaviours of licking whereas media (medium ants) help provide nutrients for the garden by bringing leaf clippings to the nest. In this review, the authors main objective was to describe what behaviours help the ants maintain the health of their gardens by observing how they keep their gardens from becoming contaminated. The method of how research conducted was not explicitly stated, however, most of the references come from the author's personal research, suggesting it is a review of the research they had conducted up until the point of publishing.

Weber's span of studies from 1941-1972 compared and contrasted different species within the tribe Attini. It was found that there are four main common behaviours that protect gardens from contamination. The first is the act of grooming, where ants constantly lick themselves and each other. Future research may look into whether or not the saliva of these ants is anti-bacterial or anti-fungal. The second was the act of minima ants cleaning contaminants off the leaf pieces that media ants bring to the nest. The third was the act of ants adding their excretions to both the leaf cuttings and the fungal garden. The fourth was the act of "planting" pieces of the fungal garden onto the leaf cuttings. These findings suggest that although the specifics of the behaviours can differ between different species, general fungal cultivating behaviours are a trait unique to Attini.

Article Contribution:

This specific article of Weber's was important to include in my literature review because it provides a review of the foundational knowledge and studies that are required to understand fungal-cultivating behaviours. It also gave some insight to how the Attini caste system operates, which gave me additional foundational knowledge for the other papers in this annotated bibliography. The article is also important to this field of study because it synthesizes previous findings of these of fungal-cultivating behaviours in tribe Attini by comparing and contrasting behaviours across different species.

Article Citation:

Currie, C.R., & Stuart, A.E. (2001). Weeding and grooming of pathogens in agriculture by ants. *The Royal Society of London Series B, 268*, 1033-1039. https://doi.org/10.1098/rspb.2001.1605.

Article Type: Primary

Article Summary:

Previous studies about fungiculture behaviour have found that a mutualistic relationship exists between leaf-cutter ants and the fungal gardens they cultivate. Researchers of this study sought to understand how ants protect their gardens from pathogens, specifically from *Trichoderma viride*, a generalist pathogen, and *Escovopsis*, a specialist pathogen. The study looked at how these fungal contaminants are removed through behaviours of weeding (physical removal of contaminants using mandibles) and grooming (removal of contaminants by collecting spores in the mouth and disposing them as pellets). To address this, the researchers exposed ant colonies to three different treatments (five colonies per treatment), including a water (control) treatment, a *Trichoderma viride* treatment, and a *Escovopsis* treatment. Behaviours were observed using video equipment that recorded every 3 hours for a total time of 72 hours. The behaviours observed included the number of ants found on top of the garden and the number of ants exhibiting grooming at the time of recording. Weeding behaviour was determined by weighing pellets that accumulated throughout the experiment. The elimination of the pathogens was determined by sampling each garden for *Trichoderma viride* and *Escovopsis* at 150 hours post-treatment.

The researchers found that grooming behaviours and pellet weighs were increased in colonies exposed to pathogens compared to colonies exposed to the control. It was also found that colonies exposed to *Escovopsis* had significantly more ants on top of the garden than in control colonies. Overall, the colonies had higher responses to *Escovopsis*, indicating that while ants use the same behaviours and tactics to dispose of generalist and specialist pathogens, they are more successful at protecting their gardens from generalist pathogens. There may be other mechanisms that ants use to protect their gardens, so the researchers suggest future studies into other types of fungicultural behaviours.

Article Contribution:

This article by Currie is prevalent in modern research on ant fungicultural behaviour, with a high citation number of 327. It advances knowledge in the field by studying the topic of pathogen defence in ant fungiculture, which was a topic that previously had little investigation. The article also aligns with the results found in previous research about what behaviours ants exhibit to keep their gardens cleanly (such as weeding and grooming behaviour).

Article Citation:

Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J., & Billen, J. (2006). Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *American Association for the Advancement of Science, 311*(5757), 81-83. https://doi.org/10.1126/science.1119744.

Article Type: Secondary

Article Summary:

Attine ants have a mutually beneficial relationship with the fungus that they "farm" inside of their nests. Researchers have found that Attine ants have additional associations with other organisms as well. This includes a negative relationship with pathogenic fungi from the genus *Escovopsis*, which the ants try to protect their fungus gardens from, as well as an additional mutually beneficial relationship with pathogen-inhibiting bacteria from the genus *Pseudonocardia*, which the ants use as a means to prevent the pathogenic fungi from spreading. In this review, the objective of the authors was to describe what anatomical features on Attine ants allow them to interact with *Escovopsis* fungi and *Pseudonocardia* bacteria, as well as the evolution of such features. To do this, the authors collected and summarized the results of their own research on the subject.

Previous research had found that Attine ants have a cavity found just behind their heads, inside of which the *Pseudonocardia* bacteria grows. Also found on the cavity are fovea (eye structures) that connect to exocrine glands (produce and externally release substances such as saliva), which allow the ants to support the bacteria through secretions. Researchers compared this cavity structures on Attine ants to other groups of ants closely related to Attine ants. It was found that these closely related ant groups did not have fovea in their cavities and also did not have *Pseudonocardia* bacteria present in their cavities, suggesting that the presence of such cavities in Attine ants developed out of a coevolutionary relationship with *Pseudonocardia* bacteria. This also suggests that Attine ants have used *Pseudonocardia* bacteria to fight against *Escovopsis*

fungi for millions of years. Due to these long-term co-evolutionary relationships, it appears that specific species of Attine ants are specialized to specific groups of the cultivated fungus, *Escovopsis* fungi, and *Pseudonocardia* bacteria.

Article Contribution:

This article helped advance research on Attine fungus-cultivating ants by reviewing the research that led to conclusions that Attine ants not only co-evolved with their fungal gardens but also with the bacteria that helps them to defend their fungal gardens from harm. I included it in my literature review because it paired nicely with the articles that mentioned the relationship between the ants and bacteria as those articles didn't go into specifics about how the relationship worked (such as the bacteria living inside the exoskeleton cavity). Also, the article paired nicely with another article about Attine evolution by Branstetter.

Article Citation:

Little, A.E.F., Murakami, T., Mueller, U.G., & Currie, C.R. (2006). Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. *Biology Letters, 2*, 12-16. https://doi.org/10.1098/rsbl.2005.0371.

Article Type: Primary

Article Summary:

The health of the fungus gardens that Attine ants cultivate is constantly threatened by pathogens, such as *Escovopsis* fungi. Previous research has found that these pathogens can be lethal to both the ants and their gardens if not controlled. Previous research has also found that the ants participate in an additional mutually beneficial relationship with anti-pathogenic bacteria, which helps them prevent the spread of pathogenic fungus in their gardens. One of the behaviours that Attine ants perform when protecting their gardens is cleaning up ("eating") parasitic fungal spores and turning them into pellets through their infrabuccal pockets (a filtering device that collects particles after they've gone through the mouth). At the time of this study, it was unclear whether or not the bacteria played a role in the production of said pellets. To address this, researchers looked at the microbial environment of the infrabuccal pockets as well as the behaviours associated with pellet-laying. The methods used for this experiment included 40 colonies of Attine ants. In the first experiment, there were treatments of environments with and without *Escovopsis* fungi, which was used to determine if pellet-making was in response to infected gardens or not. The second experiment dissected the pellets that were created to see if there were any microbes present.

The results showed that the colonies exposed to *Ecovopsis* had significantly more pellets than the control. Additionally, the results showed that the pellets did not contain viable *Escovopsis* but did contain bacteria. There were higher amounts of bacteria found in colonies exposed to *Escovopsis* than the control. This suggests that the piling of pellets by the ants is in association with the removal of pathogenic fungi. It also supports previous research that suggested the bacteria in the infrabuccal pockets and pellets help make *Escovopsis* non-viable.

Article Contribution:
This article was important to include in my literature review as it created some of the foundational knowledge about the relationship between Attine ants and bacteria, which helped me to make sense of other articles about this topic that are included later on in this annotated bibliography. Similarly, it was an important contribution to the field as it confirmed that the ants have a relationship with the bacteria and furthermore confirmed that the bacteria is found in the infrabuccal pocket and makes pathogenic fungus non-viable.

Article Citation:

Richard, F., & Errard, C. (2009). Hygienic behavior, liquid-foraging, and trophallaxis in the leaf-cutting ants, *Acromyrmex subterraneus* and *Acromyrmex octospinosus*. *Journal of Insect Science, 9*(63), 1-9. https://doi.org/10.1673/031.009.6301.

Article Type: Primary

Article Summary:

Leafcutter ants grow fungus inside of their nests, which is used as their primary food source. Previous research has found that the ants and their gardens are vulnerable to pathogens, such as *Escovopsis* fungus, and that the ants use mechanical tactics (such as licking their gardens, themselves, and other nestmates) to get rid of *Escovopsis* spores. One of the greatest risks to pathogen infection comes from the large worker ants who forage, as they may be exposed to pathogens while outside. Thus, the researchers hypothesized that large workers who spend time foraging would participate in grooming behaviours more frequently than small non-foraging workers. To study this, the researchers hosted 90 *Acromyrmex subterraneus* workers (60 large, 30 small) in laboratory enclosures consisting of a nest and foraging area. The 90 workers were divided up into a control group, which had no fungus garden, and a group with a fungus garden. Researchers recorded visual observations in 30-minute intervals from 9am-12pm and 2pm-4pm, during which behaviours of mouth-to-mouth contact, grooming, fungus interaction, immobility, and “other” activities were recorded.

The researchers found that in both ant species, large foraging workers spent more time self and nestmate grooming than small non-foraging workers when a fungus garden was present inside the nest. Additionally, it was found that in both species the control groups had decreased grooming compared to the groups with fungus gardens. These findings support the researchers' hypothesis, suggesting that although all workers participate in grooming and sanitation behaviours to some extent, it is a set of behaviours that depends on labour division within the colony. This also suggests that self and nestmate grooming plays a large role in pathogen prevention. The researchers suggest conducting a similar study in the field rather than a laboratory to see if the results differ or remain similar.

Article Contribution:

This article is important to the field because it expands on the previous work of Currie and Little in regards to how ants are able to defend against harmful pathogens. It expands by taking a close look at self and nestmate grooming behaviours rather than at weeding and garden grooming behaviours, which had already been studied by other researchers. I felt it was important to include this article in my literature review because it gives additional insight on how labour division impacts fungus cultivating and foraging behaviours by comparing the grooming of small workers to large workers.

Article Citation:

Abramowski, D., Currie, C.R., & Poulsen, M. (2010). Caste specialization in behavioral defenses against fungus garden parasites in *Acromyrmex octospinosus* leaf-cutting ants. *Insectes Sociaux: International Journal for the Study of Social Arthropods, 58*, 65-75. https://doi.org/10.1007/s00040-010-0117-y.

Article Type: Primary

Article Summary:

Attine ants rely on caste systems (social organization) and labour division to perform different tasks that sustain the entire colony. These labour divisions are especially important to fungi-cultivating behaviours in the ants, which allow them to grow and protect the fungi that they use as a food source from pathogenic *Escovopsis* fungi. The use of glandular chemical compounds and bacterial secretions are used during behaviours of weeding and grooming the fungal garden. The authors of this article hypothesized that minor (small) workers specialized more-so in grooming behaviours whereas major (larger) workers specialized in weeding behaviours. To study this the researchers used four colonies of *Acromyrmex octospinosus* and placed them on petri plates with cultivating fungus and *Escovopsis* spores. The ants were observed five times

and hour for 3-hours in 10-minute observation periods using a stereomicroscope. During each observation period, the number of ants present on the fungus were counted, according to their caste division.

The results of the experiment indicated that minor workers participated in the majority of grooming behaviours, while major workers participated in the majority of weeding behaviours. This supports the researcher's initial hypothesis and suggests that caste specialization plays an important role in the behaviours of attine ants. It is predicted by the authors that minor workers may be more suited for spore removal as their small size may make it easier for them to access the hyphae of the fungus, however, more research needs to be conducted in this area.

Article Contribution:

This article was important to include in my literature review as it connects to the theme of caste specialization throughout my bibliography. Specifically, this article led the way in determining which caste specializations were responsible for which pathogen protecting behaviours, which is a development from previous articles such as "Weeding and grooming of pathogens in agriculture by ants" by Currie et al. in 2001. As you'll see in the articles to follow, this was important to the field as it led the way for more questions about the evolution of caste specializations within Attine ants.

Article Citation:

Gerstner, A.T., Poulsen, M., & Currie, C.R. (2010). Recruitment of minor workers for defense against a specialized parasite of Atta leaf-cutting ant fungus gardens. *Ethology Ecology & Evolution, 23*(1), 61-75. https://doi.org/10.1080/03949370.2010.529828.

Article Type: Primary

Article Summary:

Attine ants have a mutualistic relationship with the fungal gardens that they cultivate and play a role in protecting their gardens from harmful pathogens (such as fungi from the genus *Escovopsis*). Previous studies have found that different Attine behaviours associated with *Escovopsis* fungi removal are organized by labour division according to worker ant size. Researchers of this study hypothesized that large worker ants can communicate the location of infected sites on the garden to small workers. To study this, the researcher's used four ant colonies and set-up experiments using dual-chambers. The dual-chamber consisted of one chamber where small workers were present as well as one chamber where small workers were absent, with a tube connecting both chambers together. The first experiment determined whether

the presence of *Escovopsis* increased the movement of small workers to infected garden sites. There were 30 control dual-chambers where no *Escovopsis* was present as well as 30 treatment dual-chambers where *Escovopsis* was present in the chamber without small workers. The second experiment determined if large workers influenced the movement of small workers. Both the control and treatment dual-chambers had *Escovopsis* present in the chamber without small workers, however the control didn't have any large workers present and the treatment had large workers present in both chambers.

The results indicated that small workers are attracted to sites in the garden that are infected by *Escovopsis* fungi. Furthermore, researchers found that although small workers can move on their own accord, the number of small workers moving towards infected sites increased in the presence of large workers. At the time of publishing, the mechanism used by larger workers to recruit smaller workers was unknown. Researchers hypothesize that it may be due to chemical signaling, such as pheromones, and suggest that future research be directed towards this topic.

Article Contribution:

This article helped to advance research about the fungus-cultivating behaviour of Attine ants as it revealed that there is a level of communication occurring between different workers that impacts how they respond to garden infections. This opened up a door for future researchers to conduct research about the communication method (such as whether communication is chemical, visual, etc.). It was an important article to include in my literature review because it highlights to importance of labour division in Attine ants to the functioning of fungus cultivation.

Article Citation:

Branstetter, M.G., Jesovnik, A., Sosa-Calvo, J., Lloyd, M.W., Faircloth, B.C., Brady, S.G., & Schultz, T.R. (2017). Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *The Royal Society of London Series B, 284*, 1-10. http://dx.doi.org/10.1098/rspb.2017.0095.

Article Type: Primary

Article Summary:

Leafcutter ants of the tribe Attini are a monophyletic group (descended from one common ancestor). Previous academia hypothesized that Attini ants arose about 60 million years ago in the tropical regions of South America. Previous researchers also supported the "out-of-the-rainforest" hypothesis, which suggests that these ants and their fungus gardens are Indigenous to tropical habitats in South America and cannot survive elsewhere. The researchers of this article

conducted phylogenetic analyses of Attini ants in order to better understand the evolutionary origin of fungus-cultivating behaviours and to test the hypotheses of previous researchers. A total of 119 ant taxa were included in the study, 78 of which belong to fungus-cultivating species. The researchers used a Hymenoptera (the order of insects consisting of ants, bees, and wasps) RNA library to target ultra-conserved elements (DNA that's identical in two species or more) in the ant taxa, and then used DNA sequencing technology to analyze the data. Furthermore, BEAST (molecular sequencing program) was used to generate a phylogenetic tree and R (statistical computing program) was used to investigate diversification (genetic differences) within the phylogenetic tree.

The results of the different data sets showed nearly identical divergence dates from the last common ancestor of Attini, indicating that fungus-cultivating ants originated approximately 57-61 million years ago, which aligns with previous research. The results also indicated that most Attini ants originated in South America, and still have a large population there to this date. However, it was also found that some clades originated in Middle America with dispersal into South America. Researchers concluded that the results partially support the "out-of-the-rainforest" hypothesis while also highlighting a few exceptions. Based on this evidence, it's suggested that Attina may have evolved fungus-cultivating behaviours due to decrease in prey and increase in fungi, however, this theory requires further research.

Article Contribution:

The work of Branstetter et al. is vital to research about Attini ants as it provides the first evidence that fungal-cultivating behaviours exist outside of tropical South America. This has given a deeper insight and analysis to how these behaviours may have evolved in Attini ants. As such, I included it in my literature review because it provides me with context about ultimate (evolutionary) influences on fungus-cultivating behaviour. This article is also important to the field of entomology in general as it gives insight to how agricultural behaviours may have evolved in other insects, such as bees and termites.

Article Citation:

Calheiros, A.C., Ronque, M.U.V., & Oliveira, P.S. (2019). Social organization and subcaste specialization in the leaf-cutting ant *Acromyrmex subterraneus* (Formicidae: Myrmicinae). *Journal of Insect Behaviour, 32,* 267-280. https://doi.org/10.1007/s10905-019-09729-6.

Article Type: Primary

Article Summary:

Ants in the tribe Attini cultivate fungus gardens within their nests, which they are highly reliant on as a food source. Death of the fungus garden leads to death of the ant colony, making the task of protecting the garden from harmful pathogens and other microorganisms vital. Previous research has shown that the different tasks required for garden maintenance are split up between the ant caste system (different sizes of worker ants), with smaller ants attending to the garden internally and larger ants foraging for sustenance for the garden externally. The same set of behaviours was predicted in the hypothesis for this article. To determine this, the researchers created an ethogram to observe and record *Acromyrmex subterraneus* behaviour. The ethogram recorded data on small, medium, and larger workers and included the following categories: brood care, foraging, fungus care, grooming, worker interaction, locomotion, stationary, and other activities. The study was conducted on a total of three *A. subterraneus* colonies, who were housed in artificial nests in laboratory consisting of a foraging chamber, and fungus garden chamber, and a chamber where the ants could expulse their waste. The study went on for seven weeks, during which researchers would spend seven hours a day completing qualitative observations.

The results of the ethogram showed that the categories of foraging, fungus care, and brood care had significant differences in activity based on labour division, with larger workers exhibiting more foraging behaviours and smaller workers exhibiting more fungus and brood care behaviours. The results also indicated that large and medium workers behave more similarly to one another than small workers. These results are in support of previous research as well as the author's hypothesis. The authors suggest further research on labour division amongst different species of Attini ants.

Article Contribution:

This article is important Attini fungus-cultivating research as it provides an ethogram of observed behaviours according to labour division, which future academics could replicate in their own research. I included the article in my literature review because it highlights the vitality of labour division to the success of Attini fungal gardens as well as how said labour division impacts behaviour. I also chose to include it because it is a more recent article that references the research conducted by many of the author's and articles that are mentioned earlier in this document, such as Richard, Currie, and Abramowksi.

Article Citation:

Muratore, I.B., & Traniello, J.F.A. (2020). Fungus-growing ants: Models for the integrative analysis of cognition and brain evolution. *Frontiers in Behavioural Neuroscience, 14*, 1-5. https://doi.org/10.3389/fnbeh.2020.599234.

Article Type: Secondary

Article Summary:

There are high amounts of variation between Attine worker ants, from their differences in morphology to their differences in labour roles within their colony. Such divisions include minima workers, who are the smallest of the colony, media workers, who are mid-sized, and maxima workers, who are amongst the largest of the colony. These divisions allow Attine ants to participate in different types of labour that benefit their fungal gardens, such as providing plant tissue for their gardens, controlling infection of their gardens, and managing waste. The goal of this article was to assess the social complexities and caste systems of Attine ants and how it has impacted their behavioural and cognitive evolution. This was written as a systematic review that looked at a range of different research on the subject, spanning from the 1970's to 2020.

Minima workers mainly work on weeding and grooming their fungal gardens, which likely requires sensory inputs other than vision. Media workers tend to leave the nest to collect plant clippings to "feed" their fungal gardens, a task that requires leg-mandible coordination. These workers are also able to assess the quality of the leaves they cut, suggesting that learning is involved in this foraging process. Maxima workers are mainly involved in defence of the nest, and as such rely on close-range vision and pheromone detection. In general, larger workers have larger brains, which the authors suggest may have correlation with the increased brain-processing required for learning (media workers) and visual acuity (maxima workers). Overall, genetic influences (such as epigenetics) have an impact on the gene expression in Attine ants, which then impacts phenotypes and behaviour.

Article Contribution:

This article was important to include in my literature review because it goes into detail about both the proximate and ultimate impacts on Attine ant behaviour. For example, it discusses the use of pheromones while also delving into the impact that epigenetics has on labour division and fungal-cultivating behaviour. It was also important to my literature review and is an important review article for this topic of study as it outlines previous work that suggests how Attine ants may learn some of their fungal-cultivating behaviours, which provides a good foundation for future research.