



## Trophallaxis: the functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae)

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### Abstract

Trophallaxis is a complex social fluid exchange emblematic of social insects and of ants in particular. Trophallaxis behaviors are present in approximately half of all ant genera, distributed over 11 subfamilies. Across biological life, intra- and inter-species exchanged fluids tend to occur in only the most fitness-relevant behavioral contexts, typically transmitting endogenously produced molecules adapted to exert influence on the receiver's physiology or behavior. Despite this, many aspects of trophallaxis remain poorly understood, such as the prevalence of the different forms of trophallaxis, the components transmitted, their roles in colony physiology and how these behaviors have evolved. With this review, we define the forms of trophallaxis observed in ants and bring together current knowledge on the mechanics of trophallaxis, the contents of the fluids transmitted, the contexts in which trophallaxis occurs and the roles these behaviors play in colony life. We identify six contexts where trophallaxis occurs: nourishment, short- and long-term decision making, immune defense, social maintenance, aggression, and inoculation and maintenance of the gut microbiota. Though many ideas have been put forth on the evolution of trophallaxis, our analyses support the idea that stomodeal trophallaxis has become a fixed aspect of colony life primarily in species that drink liquid food and, further, that the adoption of this behavior was key for some lineages in establishing ecological dominance. Trophallaxis plays a vital role in colony physiology, nutrient distribution, and communication, and doing so underscores the necessity of understanding this complicated suite of behaviors in all of its manifestations.

**Key words:** Formicidae, evolution, development, superorganism, social life, fluid exchange, ants, behavior, larva, nutrition, sharing, trophobiont, honeydew, EFN, eusocial, development, review.

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### Introduction

Trophallaxis is a social fluid-exchange behavior observed in many social insects (Fig. 1; LEBOEUF 2020), earwigs (FALK & al. 2014), burying beetles (CAPODEANU-NÄGLER 2018), birds, and mammals (ROSENBLATT 2003), mostly in the context of parental care. Exchanged fluids are battlegrounds for evolutionary conflict, both within and across species (BERNT & WALKER 1999, LIU & KUBLI 2003, PERRY & al. 2013, NAKADERA & al. 2014). The passage of internally stored fluids between conspecifics tends to occur in only critically fitness-relevant behavioral contexts like mate choice or parental care (BERNT & WALKER 1999, PERRY & al. 2013, CAPODEANU-NÄGLER 2018). Inter-species fluid exchanges tend to occur only in obligate adaptive mutualistic or parasitic relationships (VÖLKL 1997, AKINO & al. 1999).

Based on historical perspectives and current knowledge of ant biology and behavior, in this review we define trophallaxis as the direct ingestion by one individual of

material excreted, secreted or regurgitated by another (detailed in Box 1). Given this definition, a suite of odd behaviors in the animal kingdom can be considered forms of trophallaxis: mating-associated behaviors, drinking of tears (HAGA & al. 2010), and even lactation – the defining behavior of mammals – with milk as a form of trophal-lactic fluid.

Ants show an incredible diversity of trophallaxis behaviors. Over the last centuries trophallaxis has come to symbolize the collective and altruistic nature of social insects (SLEIGH 2002). Though trophallaxis includes a suite of related behaviors in ants (Fig. 2, Box 1), its most frequently observed form involves the regurgitation and passage of fluid mouth-to-mouth from one individual to another (Fig. 1). This simple exchange, when performed routinely within colonies, enables a rich and informative social circulatory system that transmits not only exog-



Fig. 1: Adult-to-adult stomodeal trophallaxis between *Camponotus sanctus* (Formicinae) workers. The two workers in the center engage in stomodeal trophallaxis in the colony nest.

enous nutrients, but also endogenous signals that can bring about complex social coordination (LEBOEUF 2020) (see section The mechanics of trophallaxis, contents of trophallactic fluids, and proximal modulators). A means of communication to transmit bioactive molecules across the superorganism over a private communication channel may have been instrumental in the development of super-organismality, at least in some lineages.

Ants are exceptional in their species-richness and ecological dominance (WARD 2014). Their rapid diversification coincides with the rise of angiosperm plants (MOREAU & al. 2006, MOREAU & BELL 2013, BARDEN & GRIMALDI 2016). Plants provided ants access to carbohydrates through inter-kingdom fluid exchange – directly, through extrafloral nectaries, and indirectly, through honeydew secreted by hemipterans (see section Participants and modes of trophallaxis) – making these nutrient-rich fluids a regular food source (DAVIDSON 1997, COOK & DAVIDSON 2006, LANAN 2014, NELSEN & al. 2018). As all ants are to some extent social and exhibit some form of division of labor, ant species that exploit such liquid food sources need to transport these liquids to nestmates. A minority of ant species transport liquid externally (HÖLLDOBLER 1986, DEJEAN & al. 2005, LŐRINCZI 2014, MAÁK & al. 2017) in behaviors termed pseudotrophallaxis or social-bucket behavior (Fig. 2). The majority of liquid-feeding ant species

have evolved stomodeal trophallaxis – an internalized method of fluid transport (see section The evolution of trophallaxis).

It is in our interest to better understand this behavior given its implications for agriculture, pest control, modern robotics and computing. Trophallaxis behavior is especially relevant for agriculture, due to the tight relationship between many ant species and plants. These ant colonies, typically species that engage in stomodeal trophallaxis, are fundamentally sessile organisms that protect host plants from destructive herbivores. Even if the ant colonies are tending aphids on plants, an overall net benefit to the plant has been demonstrated (STYRSKY & EUBANKS 2007). Trophallaxis is potentially powerful for managing ant pests, as trophallactic fluid reaches every member of a colony, enabling the distribution of poisonous compounds. Finally, the efficiency of trophallaxis to share nutrients and information has inspired human technologies and algorithms (SCHMICKL & CRAILSHEIM 2008, GRÄWER & al. 2017, CARRILLO & al. 2019) particularly in the realms of distributed computing and robotics. Additionally, we estimate that approximately half of all ant species engage in some form of intra-species fluid exchange as part of their behavioral repertoire (Fig. 3). In this review we focus only on trophallaxis in ants, though this behavior clearly occurs in bees, wasps, and termites with parallel participants, modes, mechanics, and contexts.

The diversity of contexts within which trophallaxis occurs suggests that this behavior fulfils many roles (see section The contexts and roles of trophallaxis). Trophallaxis can occur in its most well-known context of nutrition, or in other contexts such as control of larval growth and development, immune defense, nestmate recognition, aggression, or the inoculation and maintenance of the microbiome. Broadly, it remains unclear what information is shared by trophallaxis and to what end.

Available literature on trophallaxis is sparse and mostly consists of observations of stomodeal trophallaxis between adults and correlations with other data while many critical bits of information go unreported. Trophallaxis is rarely reported in formicines and dolichoderine ants due to its omnipresence. The absence of trophallactic behavior is

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Fig. 2: Nutrient transfer between ants. Ants share nutrients in the form of liquid food, solid food and trophic eggs. True trophallaxis requires that an individual directly regurgitates, secretes or excretes material in order to share it without external storage. Stomodeal trophallaxis requires regurgitation of liquid out through the mouthparts of the donor, while proctodeal trophallaxis requires the excretion of liquid from the rectal end of the abdomen of the donor. Pseudotrophallaxis is an external transport of liquid, either on the body (corporal pseudotrophallaxis), between the mandibles (mandibular pseudotrophallaxis), or using tools such as pine needles (tool-mediated pseudotrophallaxis). All forms of trophallaxis and pseudotrophallaxis can occur between ants, between ants and myrmecophiles, between adults, from adult to larva and from larva to adult, but they vary in prevalence. For species that perform each of these behaviors, references are listed in Appendix S1. Solid food and trophic eggs are alternatives to pseudotrophallaxis and trophallaxis as they allow storing and sharing of nutrients between adults and from adults to larvae. Pseudotrophallaxis behaviors marked with an asterisk (\*) can theoretically occur between adults and larvae, but we have found no evidence that they do occur. \*\* In mandibular pseudotrophallaxis, ants could in theory add endogenous compounds to the transported liquid, but to our knowledge, it has never been investigated. \*\*\* The origin of the liquid shared is unclear and could be either the digestive system or various glands. Note that adult-to-larva proctodeal trophallaxis is indicated as a two-step process.



### Box 1: Defining trophallaxis.

The term trophallaxis was introduced by William Morton WHEELER (1918) for social insects as an alternative to the less accurate and more complicated “oecotrophobiosis.” Trophallaxis comes from the Greek “tropho” which means “nourishment” and “allaxis” which means “to exchange.” WHEELER (1918) described evolution of trophallaxis as being [... originally developed as a mutual trophic relation between the mother insect and her larval brood. [Trophallaxis] has expanded with the growth of the colony like an ever-widening vortex till it involves, first, all the adults as well as the brood and therefore the entire colony; second, a great number of species of alien insects that have managed to get a foothold in the nest as scavengers i.e., other species of ants (social parasitism); third, alien social insects, that live outside the nest and are “milked” by the ants (trophobiosis); fourth, alien insects, predators or parasites (symphily); and, fifth, certain plants which are visited or sometimes partly inhabited by the ants (phytophilia.) While compelling, this description is not so much a definition as an “ever-widening vortex” that depicts only social insects, while mammals, birds, amphibians and fish also engage in a similar behavior (ROSENBLATT 2003, BUCKLEY & al. 2010, FURNESS & CAPELLINI 2019). LE MASNE (1953), further broadened the domain of trophallaxis beyond nourishment, associating this behavior with communication. Simply considering only ants and associates, trophallaxis events can involve a variety of participants and can involve fluids emerging from different body parts (LE MASNE 1953). Thus, the definition of trophallaxis has evolved and diversified with use.

Given the importance of trophallaxis behavior for the ecological roles and colony physiology of social insects, we need clear nomenclature and a framework to classify the different forms and roles of trophallaxis to precisely study this behavior. In this review, we disentangle and regroup the vocabulary used in the literature and we clarify definitions of the forms of trophallaxis that have been reported.

This leads us to define trophallaxis not only for social insects, but in general, as the direct ingestion by one individual of material excreted, secreted or regurgitated by another. This definition includes many potential actors and means of exchange and does not presume the precise function of the exchange, though the term ingestion does indicate a nutritive transfer by the involvement of the receiver’s digestive system. The majority of trophallactic events involve liquid transmission and we will routinely refer to trophallactic fluid, though some trophallaxis events involve internally processed, regurgitated, semi-solid material (LE MASNE 1953). As trophallactic regurgitates can vary wildly in viscosity, we view the solidity of the material as less critical in the definition than the fact that the material was internal to the body and is regurgitated, secreted or excreted to another individual.

The major defining characteristic of trophallaxis relative to pseudotrophallaxis is that in true trophallaxis, transmitted fluids are stored internally by the donor in advance of the transmission event, allowing for endogenous components to be transmitted (Fig. 2). This key distinction provides a powerful and private channel for individuals to pass bioactive molecules over the social network and thus alter the physiology of recipients. Also embedded in this definition is an element of consent, where the behavior is purposefully performed by both donor and receiver.

rarely reported, though this information is critical to our understanding of how colonies coordinate without trophallactic behavior. Most reporting on trophallaxis only refers to adult-to-adult trophallaxis, most probably

because adult-to-larva or larva-to-adult interactions are much more difficult to observe. In order to better enable future studies on the evolution and utility of this behavior, we encourage myrmecologists to report both positive and

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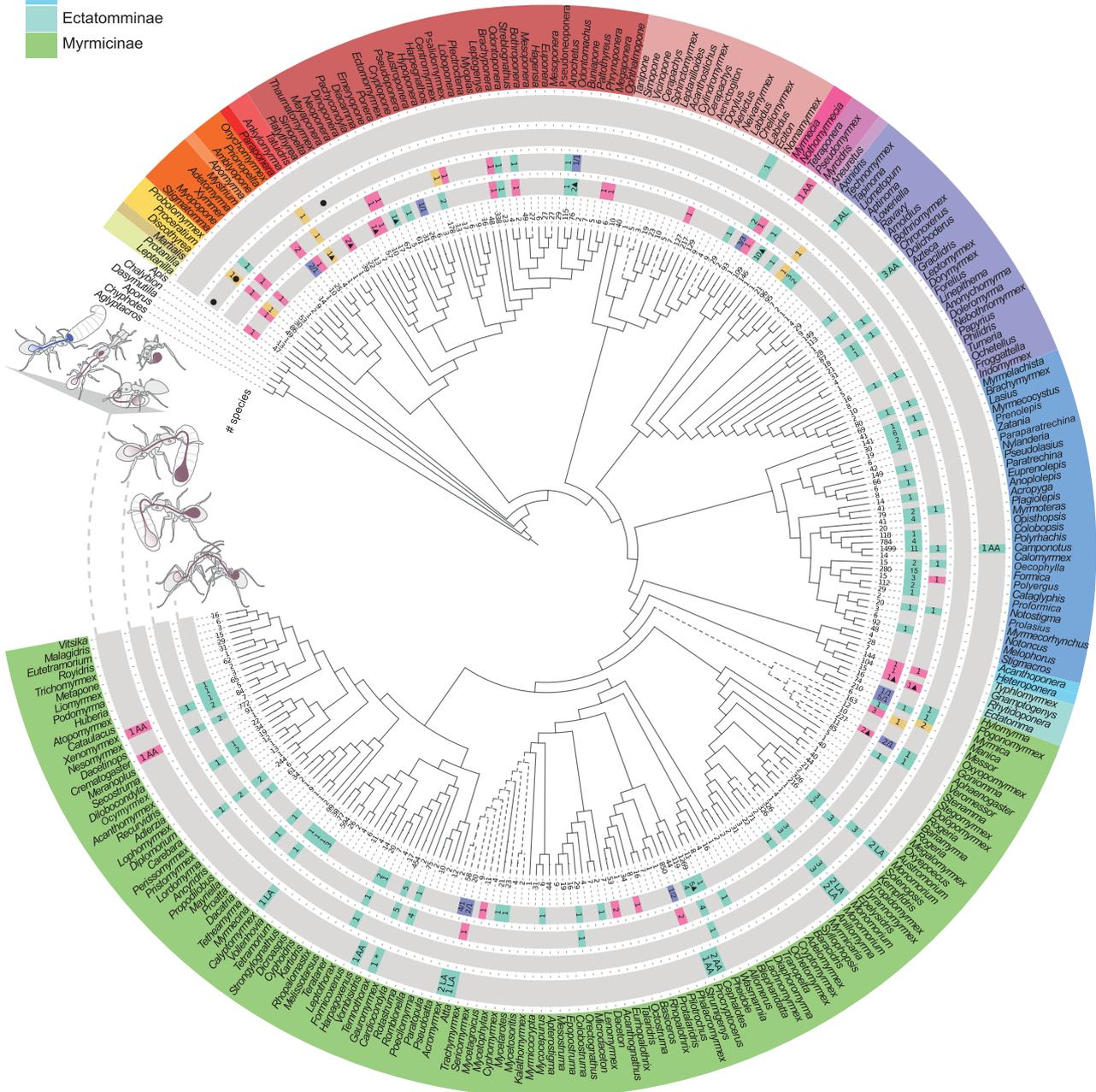
Fig. 3: Trophallaxis occurrence over the ant phylogeny. On this tree, we show the status (observed – green, not observed – pink, observed in some species of the genus but not in others – purple, debated or suspected – yellow, no report – grey) of trophallactic behaviors for each genus deduced from observations of species available in literature, listed in Appendix S1. We produced an ant phylogeny at the level of the genus where the 269 genera are grouped by subfamily (based on ECONOMO & al. 2018). Some genera such as *Chelaner* or *Ooceraea* are missing, as these were not included in the 2018 tree. Dashed tree branches represent non-monophyletic genera. This figure clearly displays the scarcity of literature on all forms of trophallaxis. The number of species for which we found reports in the literature is indicated for each trophallactic behavior. From the inner to the outer ring: the number of species recorded in the corresponding genus in August 2020 (ANTWIKI 2020); recorded stomodeal trophallaxis behavior between adults; recorded stomodeal trophallaxis behavior from adult to larva; recorded stomodeal trophallaxis behavior from larva to adult; recorded proctodeal trophallaxis behavior, either from adult to adult (AA), adult to larva (AL) or larva to adult (LA). ● indicates that at least one species of the genus shows larva-to-adult hemolymph feeding through a larval tubercle, a form of trophallaxis. ▲ indicates that at least one species of the genus uses pseudotrophallaxis. \* indicates that at least one species of the genus engages in adult-to-adult and / or adult-to-larva proctodeal trophallaxis. The figure was designed using iTOL (LETUNIC & BORK 2019).

### Ant subfamilies

- Leptanillinae
- Martialinae
- Proceratiinae
- Amblyoponinae
- Apomyrminae
- Paraponerinae
- Agroecomyrmecinae
- Ponerinae
- Dorylinae
- Myrmeciinae
- Pseudomyrmecinae
- Aneuretinae
- Dolichoderinae
- Formicinae
- Heteroponerinae
- Ectatomminae
- Myrmicinae

### Status of trophallaxis

- No report found in literature
- X Observed in X species
- X Never observed in X species
- X Debated or suspected in X species
- X/Y Observed in X species and never observed in Y species
- AL Adult → larva
- AA Adult → adult
- LA Larva → adult
- \* Larva → adult and adult → adult trophallaxis observed
- ▲ Adult → adult pseudotrophallaxis observed
- One or more species exhibits a larval hemolymph tap



negative data and to consider interactions amongst adults and larvae.

As trophallaxis occurs in many different ways and across many contexts, we have organized this review to describe this behavior across four sections that are tightly related to each other.

1. Participants and modes of trophallaxis (p. 6)
2. Mechanics of trophallaxis and contents of trophallactic fluid (p. 11)
3. Contexts and roles of trophallaxis (p. 14)
4. The evolution of trophallaxis (p. 19)

### Participants and modes of trophallaxis

The primary participants in ant trophallaxis are adult ants, ant larvae, and non-ant organisms (mutualists, parasites, trophobionts). These actors can be either donors or receivers of liquid through true trophallaxis or pseudotrophallaxis (Fig. 2). In this section, we detail forms of trophallaxis between these various actors and provide examples of such behaviors.

**Adult-to-adult trophallaxis:** Adult-to-adult stomodeal trophallaxis is the most well-known form of trophallaxis. It has become a *de facto* symbol of eusociality and of the altruistic behaviors that define social insects (SLEIGH 2002). Between adult ants, trophallaxis can be either stomodeal or proctodeal.

**Adult-to-adult stomodeal trophallaxis.** Adult-to-adult stomodeal trophallaxis is the primary form of trophallaxis behavior reported in ants, and it is often simply called trophallaxis. Based on observations reported in the literature for 267 species of ants (Appendix S1, as digital supplementary material to this article, at the journal's web pages) that we have extrapolated to the genus level, covering 104 genera (Fig. 3), we estimate that half of all ant species engage in adult-to-adult stomodeal trophallaxis.

This behavior is more prevalent in subfamilies whose members more often exhibit superorganismality, such as Formicinae, Myrmicinae, and Dolichoderinae (EISNER 1957, WHEELER & WHEELER 1979). We here define superorganismality as species where individuals belong to a permanent caste and workers cannot be mated (modified from BOOMSMA & GAWNE (2018)). Extrapolating from available observations (Fig. 3), all Formicinae and nearly all Dolichoderinae engage in stomodeal trophallaxis. Some species are over-represented in the literature, for example the highly studied invasive species *Solenopsis invicta* (Myrmicinae). There are large gaps in our knowledge, for example for Dorylinae. Informally, army ants are thought broadly not to engage in trophallaxis, yet the literature provides only one negative and one positive (rare) report for the entire species-rich subfamily. It remains unclear for nearly all ant species whether they are physiologically capable of regurgitation, whether they actually engage in stomodeal trophallaxis under natural conditions, and how much the behavior is necessary for a given species to thrive.

In many species that perform stomodeal trophallaxis routinely, this behavior occurs between all members of the

colony, between larvae and adults, foragers and nurses, sexuals and workers, and majors and minors, creating a network of fluid linking each individual (SORENSEN & al. 1985, BUFFIN & al. 2012, GREENWALD & al. 2018, LEBOEUF 2020). In formicine colonies (PROVECHO & JOSENS 2009, SCHWANDER & al. 2010, BUFFIN & al. 2011, GUO & al. 2013, GREENWALD & al. 2015), stomodeal trophallaxis events can last for a few seconds to several minutes, depending on known factors such as the sugar concentration of the ingested liquid food (SOLA & JOSENS 2016) and starvation conditions (JOSENS & MCCABE 2007), but also on factors that remain unclear. Some adult-to-adult stomodeal trophallaxis events can even be bi-directional (GREENWALD & al. 2015).

**Adult-to-adult proctodeal trophallaxis and autotrophallaxis.** Adult-to-adult proctodeal trophallaxis, also referred to as abdominal or anal trophallaxis, consists of an adult ant drinking secretions from the abdominal tip of another adult ant (Fig. 2). Proctodeal trophallaxis between nestmates is rarely studied relative to stomodeal trophallaxis (Fig. 3) and while it does involve ingestion of transmitted fluid, it does not appear to be a primary means to transfer nutrition. Simply from behavioral observation, one cannot distinguish the source of proctodeal trophallactic fluids, whether they originate in the digestive system or in abdominal glands, and this is likely to vary across species.

Proctodeal trophallaxis is suspected to happen in the contexts of microbiome transfer in *Cephalotes* (WILSON 1976, LANAN & al. 2016) and *Procryptocerus* (Myrmicinae) (WILSON 1976, WHEELER 1984) (see section Inoculation and maintenance of gut microbiota) and aggression as in slavemaking *Temnothorax* (Myrmicinae) species (STUART 1981) (see section Aggression).

Proctodeal trophallaxis may play a role in pheromonal communication or nourishment. Queens of *Camponotus senex* (Formicinae) produce anal secretions that workers collect (SANTOS & al. 2005) and either drink or transport to the material their nest is built with, suggestive of a pheromonal role. Queens sometimes also drink anal secretions from workers, for example, in *Dolichoderus quadripunctatus* (Dolichoderinae) (TOROSSIAN 1960). In the absence of brood, adults can stimulate other adults to produce proctodeal secretions by engaging in antennal contact. Whether these proctodeal trophallaxis events aim at sharing nutrients, communication cues or other molecules remains unclear.

Autotrophallaxis, auto-proctodeal trophallaxis, or acidopore grooming (TRAGUST & al. 2013, TRAGUST & al. 2020) consists of an adult drinking anal secretions from the tip of her own gaster. Whether autotrophallaxis falls under the definition of trophallaxis has been questionable, as autotrophallaxis does not involve two individuals and does not appear to have a nutritive role. According to our definition this is not a form of trophallaxis (See Table 1). However, it involves an excretion of fluids that in some contexts can be transferred to other individuals, for example, spread on brood, or added to trophallactic

Tab. 1: Examples of behavior that we do not consider trophallaxis.

Non-trophallaxis behavior	Explanation
Pseudotrophallaxis (any form)	The material has not been stored internally and thus endogenous components have not been added to it
Feeding of trophic eggs	Does not involve direct passage from donor to receiver; can be considered an alternative means of colony nutrient commerce to the trophallactic network
Drinking plant-wound sap	Non-consensual; the plant has not secreted this sap to / for the receiver
Larval hemolymph feeding by incision	Non-consensual; the larva has not secreted this hemolymph to / for the receiver
Feeding of infrabuccal pellets	Unclear. This material has not been ingested and regurgitated. Whether this behavior is or is not considered trophallaxis could depend on whether infrabuccal pellets contain endogenous components
Feeding of pre-masticated solids	Unclear. This material has not been ingested and regurgitated. Whether this behavior is or is not considered trophallaxis could depend on whether pre-masticated material contains endogenous components
Autotrophallaxis / autoproctodeal trophallaxis	Only involves one individual. No clear nutritive function.

fluid later fed to nestmates. Thus, this behavior is used in the context of defense against pathogens (see section Defense against pathogens) as an hygienic and prophylactic behavior. Autotrophallaxis is often observed in Formicinae, and we found no observation of autotrophallaxis in other subfamilies in literature (TRAGUST & al. 2020). However, venom proteins have been found in the trophallactic fluid of *Solenopsis invicta* (Myrmicinae) (LEBOEUF & al. 2016), suggesting that it might occur in Myrmicinae.

#### Adult-to-larva and larva-to-adult trophallaxis:

Adult and larval ants often interact because ant larvae are mostly unable to move on their own and rely on workers to feed them (LE MASNE 1953). Larvae can be provisioned with solid food, transported to a food source, as in *Gnaptogenys striatula* (Ectatomminae) (KAPTEIN & al. 2005) or *Lasius claviger* (Formicinae) (WHEELER & WHEELER 1953) and can be fed by workers through stomodeal trophallaxis. In species that rely mainly on trophallaxis to share nutrients, larvae are part of the trophallactic network. In species such as in *Acromyrmex subterraneus* (Myrmicinae), larvae are not only the food “sink” of the network, but are thought to be a digestive caste, providing processed food to workers (ERTHAL & al. 2007) as in *Vespa* (ISHAY & IKAN 1968) (see section Resources distribution). In this section, we detail examples of stomodeal and proctodeal trophallaxis behaviors that involve larvae playing roles of both donor and receiver.

Adult-to-larva and larva-to-adult stomodeal trophallaxis. Stomodeal trophallactic feeding of larvae occurs in many species of ants (Fig. 3), including members of Ponerinae (WHELDEN & HASKINS 1954), Myrmeciinae (WHELDEN & HASKINS 1954), Myr-

micinae (CASSILL & TSCHINKEL 1995, BROWN & TRANIELLO 1998), Dolichoderinae (LENOIR 1979) and Formicinae (WHEELER & WHEELER 1953). The fact that this behavior can be found across several subfamilies illustrates how widespread it is in ants. Cassill and Tschinkel studied what triggers adult workers of *Solenopsis invicta* (Myrmicinae) to feed larvae via stomodeal trophallaxis. They established that the bigger and hungrier a larva is, the more frequently that larva is fed, thus the more food the larva obtains (CASSILL & TSCHINKEL 1995). Larvae of many species including *Myrmica rubra* (Myrmicinae) (CREEMERS & al. 2003) and *Formica exsecta* (Formicinae) (PEIGNIER & al. 2019) can influence their food intake through trophallaxis by begging for food. Adult-to-larva stomodeal trophallaxis could also help build trans-generational immune priming (HARWOOD & al. 2019), thus playing a role in defense against pathogens (see section Defense against pathogens).

In some species of ants and wasps, larvae regurgitate fluid to workers (LE MASNE 1953, DELAGE 1968, ISHAY & IKAN 1968, DAVISON 1982, TSCHINKEL & KWAPICH 2016). In *Messor* (Myrmicinae) (DELAGE 1968), *Myrmica* (Myrmicinae) (OHLY-WÜST 1977), *Monomorium* (Myrmicinae) (OHLY-WÜST 1977, DAVISON 1982), *Solenopsis* (Myrmicinae) (SORENSEN & al. 1983), *Chelaner* (Myrmicinae) (DAVISON 1982), *Pheidole* (Myrmicinae) (CASSILL & al. 2005) and *Acromyrmex* (Myrmicinae) (ERTHAL & al. 2007) some studies suggest that larvae are a digestive caste.

Adult-to-larva and larva-to-adult proctodeal trophallaxis. Proctodeal trophallaxis from adults to larvae is more rarely reported than proctodeal trophallaxis from larvae to adults, which in turn is less common than stomodeal trophallaxis events between adults and larvae (Fig. 2).

Among the species engaging in larva-to-adult proctodeal trophallaxis, workers of leafcutter ants such as *Acromyrmex rugosus* (Myrmicinae), *Acromyrmex balzani* (Myrmicinae) (LOPES & al. 2005), and *Atta sexdens* (Myrmicinae) (SCHNEIDER 2000) are reported to ingest droplets of anal secretions produced by larvae. In other species such as *Monomorium pharaonis* (Myrmicinae), the queen has been observed to drink both stomodeal and proctodeal secretions directly from the larvae (BØRGESEN 1989).

More rarely, adults feed larvae with anal secretions, as in *Iridomyrmex humilis*, *Tapinoma erraticum* and *Dolichoderus quadripunctatus* (Dolichoderinae) (TOROSSIAN 1959, TOROSSIAN 1960, TOROSSIAN 1961). The abdominal droplet is collected by the adult in her mandibles and then fed to the larva. We estimate that this behavior falls under the proctodeal trophallaxis definition because a fluid is excreted from an adult's abdomen and fed to a larva, which is likely to be the only way an adult can feed anal trophalactic fluid to a larva. Additionally, this behavior involves a donor and a receiver and happens over a short amount of time, even though there is no direct contact between the adult's gaster tip and the larval mouthparts. In *Dolichoderus* (Dolichoderinae) (TOROSSIAN 1959), such trophallaxis events are suspected to have a control on larval growth and development (see section Manipulation of larval growth and development). Additionally, the fluid is sometimes stolen by adults indicating that it is appealing for adults and might have nutritive or signaling characteristics.

#### **Interspecies trophallaxis: friends and foes:**

Ants engage in trophallaxis with an incredible variety of partners, across kingdoms with plants, across orders with aphids and butterflies and clearly within Formicidae. Many ants feed on extrafloral nectar or honeydew, and some specialize on specific plants or insect species. The trophobionts – the food providers – typically get some return payment from this trophobiosis, such as protection, food or help in dispersal (BUCKLEY 1982). The food exchange from the insect trophobiont to the ant can be done through stomodeal or proctodeal trophallaxis. In less harmonious interspecies interactions, ants are often parasitized by other insects that are fed by the ants through stomodeal or proctodeal trophallaxis. In this section, we report examples of these mutualistic and parasitic interactions.

As Wheeler stated in 1918, the fact that ants drink plant liquid secretions can be considered trophallaxis, thus there are inter-kingdom interactions of fluid exchange. The most stunning symbol of ant-plant trophallaxis is that over 100 families of plants evolved extrafloral nectaries (MARAZZI & al. 2013) that secrete carbohydrate-rich fluid that primarily attracts ants in exchange for ant protection from herbivory. In one example, 15 species of Formicinae, Ectatomminae, Dolichoderinae, Myrmicinae and Pseudomyrmecinae were observed feeding from *Chamaecrista debilis* extrafloral nectaries (DO NASCIMENTO & DEL-CLARO 2010). The study also showed that the presence of ants is beneficial, as it reduces the number of herbivores

and allows plants to produce more fruit, improving the plant's reproduction. It is hypothesized that plants evolved extrafloral nectaries to bypass the honeydew-producers that wound plants with their piercing sucking mouthparts (BECERRA & VENABLE 1989, LANAN & BRONSTEIN 2013). With extrafloral nectaries, ants have direct access to carbohydrate-rich fluid and plants are not wounded by the honeydew-producers. Ants can also drink plant sap through wounds such as those made by herbivores. For example, *Oecophylla smaragdina* (Formicinae) drinks sap from wounds in *Cardwellia sublimis* and *Syzygium sayeri* (BLÜTHGEN & FIEDLER 2004), and *Pristomyrmex punctatus* (Myrmicinae) drinks sap from wounded leaves of *Quercus acutissima* (STAAB & al. 2017). A beautiful example of ant-plant mutualism involves *Pseudomyrmex* (Pseudomyrmecinae) and *Acacia*, where the plants evolved specialized nesting sites for ants, extrafloral nectaries and beltian bodies, and ants defend the plant from herbivores, even mammalian ones (BEATTIE 1985).

Honeydew is a liquid secreted by phloem-feeding plant pests, typically hemipterans (VÖLKL & al. 1999, STYRSKY & EUBANKS 2007, DETRAIN & al. 2010, ZHOU & al. 2014). Honeydew is generally rich in carbohydrates and comparatively poor in nitrogen, though some honeydew does indeed contain certain amino acids and even full proteins (SABRI & al. 2013). Because honeydew is often nitrogen-poor, many aphid-tending ants must complement their diet with protein-rich food-sources and / or take advantage of nitrogen-upgrading / -recycling endosymbionts (DAVIDSON 1997, COOK & DAVIDSON 2006). The composition of honeydew varies depending on the plant the trophobiont is feeding on, the trophobiont's needs and the trophobiont's symbiotic bacteria (BLÜTHGEN & al. 2004, DOUGLAS 2006). It is also suggested by DOUGLAS (2006) that whether a given species of Hemiptera is tended by ants could affect the honeydew composition. Ants generally drink honeydew via proctodeal trophallaxis. Some species can treat honeydew producers like cattle, protecting them against predators, shuffling them between pastures (OFFENBERG 2001, WIMP & WHITHAM 2001) and also preying on them (OFFENBERG 2001). Many ant species, especially Myrmicinae and Formicinae, feed on honeydew (LANAN 2014), for example *Lasius niger* (Formicinae) (VÖLKL & al. 1999), *Camponotus rufipes* (Formicinae) (PRINGLE 2020) and *Myrmecaria opaciventris* (Myrmicinae) (KENNE & DEJEAN 1999).

There are darker examples of trophallaxis between ants and other insects. Ants feed parasites by trophallaxis. Adult crickets *Myrmecophilus gracilipes* and *Myrmecophilus albicinctus* drink trophalactic fluid from their host ant *Anoplolepis gracilipes* (Formicinae). *Myrmecophilus albicinctus* receives all nutrition through trophalactic interactions with its host (PARMENTIER 2020). It was also shown that females of the aphid *Paralipsis enervis*, the beetle *Claviger testaceus* and the cricket *Myrmecophilus acervorum* are fed via stomodeal trophallaxis by respectively *Lasius niger* (Formicinae) (VÖLKL & al. 1996), *Lasius flavus* (Formicinae) (CAMMAERTS 1992) and *For-*

*mica rufa* (Formicinae) workers (PARMENTIER & al. 2014). Some myrmecophiles are exclusive to a given ant species, and some can be hosted by various ant species. There are also examples of beetles, like *Amphotis marginata*, staying near foraging trails of *Lasius fuliginosus* (Formicinae) and acting “as a highwayman,” stopping returning foragers and soliciting regurgitation of food (HÖLLDOBLER & KWAPICH 2017). Workers often feed myrmecophile larvae via stomodeal trophallaxis, as it is the case with some *Formica* sp. (Formicinae) that feed the rove beetle *Lomechusa emarginata* larvae during spring and summer (PARMENTIER 2020), or with *Formica polyctena* (Formicinae) feeding the larvae of the beetle *Atemeles pubicollis* (HÖLLDOBLER 1971). The parasitic blue butterfly caterpillar *Maculinea rebeli* produces anal secretions that are drunk by the workers of the host *Myrmica* species (Myrmicinae), and the same caterpillar is also fed by workers of at least six *Myrmica* (Myrmicinae) species (ELMES & al. 1991).

There are also parasitic interactions within Formicidae that involve trophallaxis, which are discussed in the sections on proctodeal trophallaxis and on aggression.

**Trophic eggs:** Trophic eggs may replace trophallaxis as a means of sharing endogenous material across individuals for some species and in certain contexts. Trophic eggs, unfertilized eggs that have a solid envelope allowing easy storage and transport, are not within our definition of trophallaxis (Table 1). They can be shared over a colony and fed to workers, sexuals and larvae, and are eaten by ants of various subfamilies such as Formicinae (HÖLLDOBLER & WILSON 1983, BARONI URBANI 1991, LEE & al. 2017), Myrmicinae (BARONI URBANI 1991, WARDLAW & ELMES 1995, GOBIN & ITO 2000, DIJKSTRA & al. 2005, CHARBONNEAU & al. 2017), Ectatomminae (GOBIN & al. 1998, GOBIN & al. 1999, TAY & CROZIER 2000), Amblyoponinae (ITO & BILLEN 1998) and Ponerinae (DIETEMANN & PEETERS 2000). In *Technomyrmex albipes* (Dolichoderinae) trophic eggs are laid by all females and these eggs are eaten by both adults and larvae, perhaps to the exclusion of stomodeal trophallaxis (Appendix S1) (YAMAUCHI & al. 1991).

Trophic eggs are considered particularly important for fully claustral queens. Some claustral queens are suspected to recycle their limited internal energy storages (wing muscles, fat) into trophic eggs to feed their first larvae (HÖLLDOBLER & WILSON 1990, chap.3, BARONI URBANI 1991, SILVA & al. 2014). Claustral queens are also known to feed their larvae by stomodeal trophallaxis (HASKINS & HASKINS 1950). Observations of claustral queens feeding their larvae through stomodeal trophallaxis alone remain rare (HASKINS & HASKINS 1950) though whether this reflects biology or the lack of observations of larval feeding is difficult to disentangle. The development of a thoracic crop (see section The mechanics of trophallaxis in adults) in claustral queens is suggestive that stomodeal trophallaxis is a more frequent occurrence than has been reported.

Altogether, the fact that across different subfamilies queens or workers are able to lay trophic eggs suggests that it is a widespread form of food storage and dispersal within

colonies. As opposed to stomodeal trophallaxis, trophic eggs are a slow means to share nutrients as they take time to produce. However, they are solid and can be stored as units, which is an advantage compared to trophallaxis. Additionally, they could be a convenient way to recycle and externally store the claustral queen’s wing muscles and fat to raise her first brood. Trophic eggs are clearly rich in endogenous components while our understanding of trophallactic fluids has only recently revealed how rich this fluid is with endogenous components (LEBOEUF & al. 2016). Trophic eggs can be an alternative to stomodeal trophallaxis to share nutrients (YAMAUCHI & al. 1991) or a complement to it. The similarity and dissimilarity in nutrient content of trophic eggs and trophallactic fluid is worthy of study to understand how each is used in food sharing and storage processes. The interplay between the use of trophallaxis or trophic eggs for sharing material has, to our knowledge, never been studied and could improve our understanding of the dynamics of material sharing in ants.

**Other kinds of liquid sharing:** Many ant species have other ways of sharing liquids without regurgitation or excretion or that involve more extreme forms of excretion.

Mandibular pseudotrophallaxis, corporal pseudotrophallaxis and tool-mediated pseudotrophallaxis do not involve regurgitation or excretion, and do not fall within our definition of trophallaxis (Table 1, Fig. 2). They are thus unlikely to result in a social circulatory system because endogenous components are unlikely to be added in the fluid (Fig. 2). However, the efficiency of such liquid sharing networks to diffuse food over the colony remains to be studied.

Mandibular pseudotrophallaxis consists of an individual carrying liquid with surface tension to maintain a drop between her mandibles (HÖLLDOBLER & WILSON 1990, figs. 7-74, PEETERS 1997, HORA & al. 2009). Mandibular pseudotrophallaxis between adults tends to occur in species of ants with facultative worker sterility (PEETERS 1997), such as *Ectatomma ruidum* (Ectatomminae) (CORBARA & al. 2010), *Odontomachus chelifer* (Ponerinae) and *Odontomachus troglodytes* (Ponerinae) (DEJEAN & LACHAUD 1991, DEJEAN & LACHAUD 1992, ÁVILA NÚÑEZ & al. 2011), and *Neoponera apicalis* (Ponerinae) (FRESNEAU & DUPUY 1988). In *Ectatomma brunneum* (Ectatomminae) (LOCHER & al. 2009) and *Ectatomma permagnum* (Ectatomminae) (BRANDÃO & PAIVA 1989), even larvae have been observed to be fed by workers from infrabuccal drops, a form of pseudotrophallaxis.

Corporal pseudotrophallaxis is a form of pseudotrophallaxis that involves transporting fluid on the body and has been observed in *Pheidole megacephala* (Myrmicinae) soldiers (DEJEAN & al. 2005). When finding a food source on competitors’ territory, soldiers are recruited by foragers to maximally exploit the food source. Foragers and soldiers drink liquid food and store it in their crops. Additionally, soldiers dip their head and thorax in the food source until a drop remains under their head due to surface tension. Once back in the nest, nestmates will drink from this drop as they would in the context of

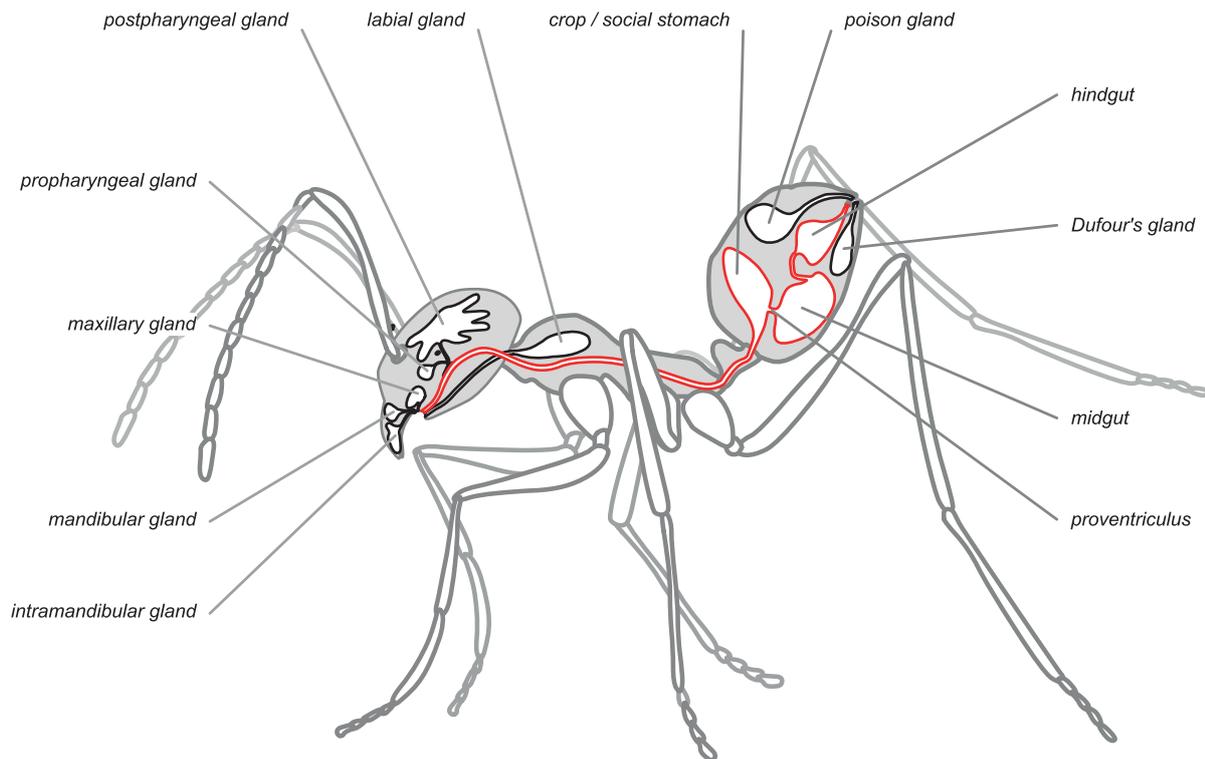


Fig. 4: Digestive tract and excretory glands of a formicine worker. This schematic is based on WENSELEERS & al. (1998) and represents a *Cataglyphis* (Formicinae) worker with digestive system (in red) and exocrine glands (in black). In the head, the ducts of the postpharyngeal gland, the propharyngeal gland, the maxillary gland, the mandibular gland and the intramandibular gland have an opening onto the alimentary canal and could contribute to the trophallactic fluid. The excreta of the labial gland, located in the ant thorax, can also be added in the trophallactic fluid. From the crop, material can be regurgitated, or pass the proventriculus to join the individual digestive system in the midgut and then in the hindgut. The fluid excreted during proctodeal trophallaxis can thus come from the digestive system or the abdominal glands.

mandibular pseudotrophallaxis. This technique allows multiple nestmates to drink from the droplet, increasing the rate of food distribution (DEJEAN & al. 2005). The transport of liquid food droplets under the head and thorax has also been observed in *Platythyrea conradti* (Ponerinae) workers (DEJEAN & SUZZONI 1997).

In tool-mediated pseudotrophallaxis, ant species use tools to share liquid with their nestmates, such as pine needles or pieces of dirt used as sponges to transport liquid (LÓRINCZI 2014, MAÁK & al. 2017, MÓDRA & al. 2020). To our knowledge, this tool-mediated pseudotrophallaxis has only been reported in *Aphaenogaster subterranea* (Myrmicinae) and *Aphaenogaster senilis* (Myrmicinae).

Larval hemolymph feeding consists of adults or queens drinking hemolymph directly from larvae, either by piercing the larval cuticle, or drinking from a specialized tubercle, called a larval hemolymph tap. In *Platythyrea schultzei* (Ponerinae) (VILLET & al. 1990), *Leptanilla japonica* (Leptanillinae) (MASUKO 1989, VILLET & al. 1990) and *Proceratium itoi* (Proceratiinae) (MASUKO 2019) larvae have a hemolymph tap. Because this fluid is excreted through the tap, we consider this behavior a form of trophallaxis. This secretion is thought to be used as food for workers when food is scarce, without killing and eating the brood, thus providing an interesting alternative to

brood cannibalism (SCHULTNER & al. 2017). It is unclear if this fluid is a secretion or simply hemolymph. Species such as *Amblyopone silvestrii* (Amblyoponinae) (MASUKO 1986) or *Myopopone castanea* (Amblyoponinae) (ITO 2010) cut the larval cuticle and drink larval hemolymph. As this appears to be non-consensual excretion of fluid from the larvae, this behavior is not considered trophallaxis (Table 1).

These alternatives and parallels to true trophallaxis are rarer than stomodeal trophallaxis. They are unlikely to bring about a social circulatory system for two reasons. With the exception of larval hemolymph feeding, the addition of endogenous material in the fluid is unlikely. Second, most of these behaviors occur rarely even in species who perform them, and consequently the rate of diffusion over this fluid network is likely insufficient for a colony to reliably transmit information. Pseudotrophallaxis could build a fluid network over the colony, though to our knowledge, this has not been investigated. Larval hemolymph feeding through a larval tubercle is an especially interesting trophallaxis behavior because it is observed sparsely over three different subfamilies. Whether this fluid differs from hemolymph or has commonalities with trophallactic fluid and whether it is similar across the three subfamilies are questions that will help us understand colony and larval physiology.

## The mechanics of trophallaxis, contents of trophallactic fluids, and proximal modulators

Given our definition (the direct ingestion by one individual of a fluid excreted, secreted or regurgitated by another), trophallaxis conveys material from one individual's body into the digestive system of another. In this section, we describe the physiology required for adult and larval ants to engage in trophallaxis, glands that could secrete molecules into trophallactic fluid, components that have been found in these fluids, and neurochemical modulators that may be involved in regulating this behavior.

**The mechanics of trophallaxis in adults:** The digestive tract of an adult ant (Fig. 4) starts with the mouthparts followed by three internal sections. The first is the foregut (pharynx, esophagus or alimentary canal, crop, and proventriculus). The proventriculus is a sphincter that separates the crop from the second section, the midgut. The hindgut is the last part of the digestive tract (pylorus, ileum and rectum) (CAETANO 1990, CHAPMAN 1998, chap.11, CASADEI-FERREIRA & al. 2020). This anatomy is common to all ant species, though the component parts have adapted to the particularities of each species' digestive needs.

The crop, often called the social stomach, is a critical organ in stomodeal trophallaxis, as it holds the liquid that can be regurgitated by the donor and stored by the receiver. As part of the foregut, the crop is lined with a cuticle layer and is surrounded by muscles (CHAPMAN 1998, chap.3.1.1, SOLIS & al. 2013). As an ant drinks, her empty wrinkled crop expands and takes on a round shape (SOLIS & al. 2013). The entire gaster of the ant has to allow for this expansion. For this reason, ants that frequently engage in trophallaxis have evolved an expandable gaster made of rigid segments separated by elastic membranes which allow the swelling of the highly elastic crop. The regurgitation of liquid from the crop to the oesophagus is thought to be enabled by the pharyngeal muscles (CAETANO & al. 1997).

Repletes are individuals with highly expandable gasters and large expandable crops found in Myrmicinae in *Carebara* sp., in Dolichoderinae in *Leptomyrmex* sp. and in Formicinae in *Camponotus* sp., *Myrmecocystus* sp., *Melophorus* sp., *Plagiolepis* sp., *Prenolepis* sp. and *Proformica* sp. (EISNER 1957, HÖLLDOBLER & WILSON 2009). Once their gaster is full of liquid, these repletes can hardly move, tend to stay in the nest and are used as a stock of food by their nestmates (WHEELER 1910, EISNER & HAPP 1962) sometimes for months (MORGAN 2007) or even a lifetime in the case of honeypot ants like *Myrmecocystus*. In most ant species, the crop is located in the abdomen (Fig. 4). However, workers in some species can also exhibit a dilation of the oesophagus in the thorax called a thoracic crop (CAETANO & LAGE FILHO 1982, CAETANO & al. 1986, CAETANO 1988, CAETANO & al. 1990, CAETANO & al. 1997). In *Pheidole aberrans* and *Pheidole deima*, major workers exhibit a thoracic crop (CASADEI-FERREIRA & al. 2020). It is suggested that in these species, the thoracic crop allows

majors to act as repletes while not being immobilized when full, and to thus remain efficient in defending the colony or processing food. In some species such as *Camponotus pennsylvanicus* (Formicinae), majors have a bigger abdomen than minors, and are used as “tankers” to transport large quantities of liquid food to their nestmates thus saving time and energy (ROSSI & FELDHAAR 2020), but without playing a long term food-storage role.

Crop-load capacity has been observed to vary over the ant phylogeny and with liquid foraging strategies (e.g., trophobionts vs. more solitary leaf foraging), where solitary leaf foraging Formicinae display the largest crop capacities (DAVIDSON & al. 2004). Early-branching subfamilies such as Ponerinae have rigid abdominal segments fused together, making the abdominal cuticle hard and solid, and preventing the crop's enlargement (PEETERS 1997). Nonetheless, some Ponerinae are known to engage in stomodeal trophallaxis (addressed later in this section). The crop is thus a critical organ in the stomodeal trophallaxis mechanics.

Similar to workers, in most species, the queen's crop is also located in the abdomen. However, in queens of various Myrmicinae and Formicinae, and in workers of some Dolichoderinae, Ectatomminae, Paraponerinae and Ponerinae, the oesophagus can also be dilated to become a thoracic crop (CAETANO & LAGE FILHO 1982, CAETANO & al. 1986, CAETANO 1988, CAETANO & al. 1990, CAETANO & al. 1997).

The crop is separated from the midgut by the proventriculus, a sphincter damming the passage of food from the crop to the midgut. With the crop evolving better storage abilities by elasticity and swelling, the proventriculus is thought to have evolved different shapes to enable a longer food storage (EISNER 1957, EISNER & BROWN 1958, DAVIDSON & al. 2004). These studies suggest that the proventriculus has two modes of action. It can be opened actively or passively, to allow the crop contents to pass to the midgut, from the social stomach to the more individual digestive tract.

The proventriculus' shapes across ants are widely varied and as EISNER & BROWN (1958) suggested may contribute to an ant's ability to store food in her crop. In his exploration of ants' proventriculus, EISNER (1957) compared the proventriculus shapes of 38 species from seven subfamilies and produced high-quality plates of the various proventriculi among ants, attempting to build an ant phylogeny based on the proventriculus structure. He suggested that Dolichoderinae and Formicinae have proventriculi best-suited for trophallaxis, but he misled the field on the topic of trophallaxis for Myrmicinae by focusing only on the most early-branching genera of this subfamily. Another surprise has been the report of stomodeal trophallaxis in early-branching ants such as some Ponerinae species (Fig. 3), contrary to EISNER (1957)'s predictions. Based on previous work (EISNER & WILSON 1952, EISNER 1957, EISNER & BROWN 1958), DAVIDSON & al. (2004) found a correlation between species having a highly modified passive proventriculus, fast liquid food intake,

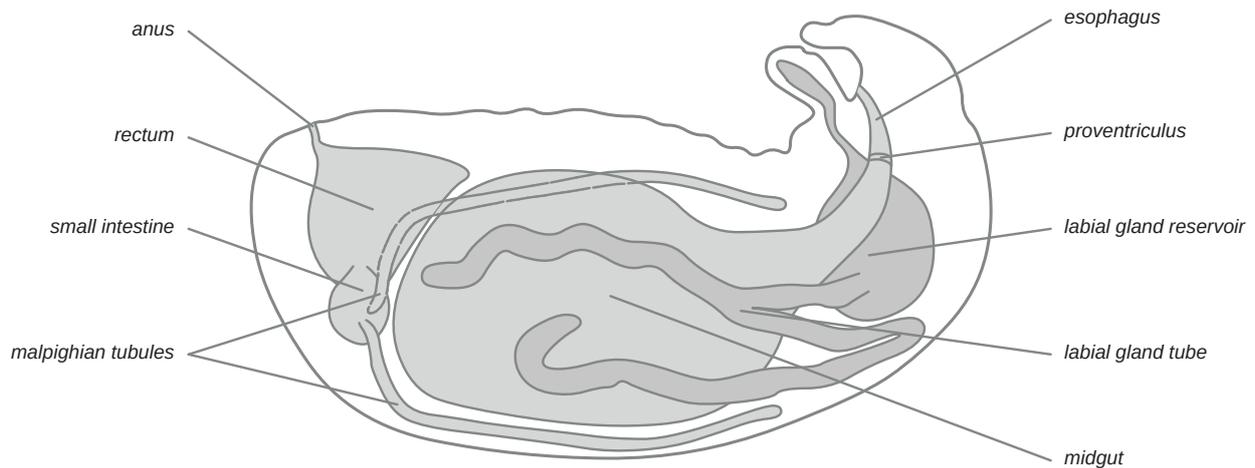


Fig. 5: Digestive tract and labial gland system of a myrmicine larva. This schematic is based on a *Solenopsis invicta* (Myrmicinae) larva (PETRALIA & VINSON 1980), and shows the digestive system (light grey) and labial gland system (dark grey). The food is ingested, and from the esophagus, it passes the proventriculus to join the blind midgut. The Malpighian tubules filter hemolymph, and excrete materials in the small intestine, and then to the rectum. The fluid involved during proctodeal trophallaxis possibly comes from the rectum, while the larval trophallactic fluid could either be regurgitated from the esophagus, or from the labial gland reservoir.

and high crop capacity, particularly in Formicinae and small Dolichoderinae such as *Forelius* and *Dorymyrmex*.

The proventriculus is also suspected to play a critical role in the maintenance of the microbiome, as in *Cephalotes rohweri* (Myrmicinae) (see section Inoculation and maintenance of gut microbiota), where newly hatched workers drink older workers' anal secretions that contain specific commensal bacteria. The highly specialized proventriculus acts as a filter such that only the correct bacteria may pass into the midgut (LANAN & al. 2016).

While not considered a classical component of the trophallaxis-associated apparatus, cuticle thickness may play a critical role in whether or not trophallaxis has evolved in a given lineage. Formicoid ants, especially dolichoderines and formicines, have a thinner and more flexible cuticle than do Poneroid ants (PEETERS & al. 2017). PEETERS & al. (2017) proposed that a thinner cuticle allows for "cheaper" workers and thus larger colony sizes, but it also could have enabled the expandable gaster necessary to effectively exploit liquid food sources. Further studies combining phylogeny, morphology and behavior will be needed to uncover causality.

**The mechanics of trophallaxis in larvae:** The liquid exchanges between adults and larvae, despite being quite common, are often described but rarely investigated, making it difficult to understand whether they consist of only nutrient transfer or if they are part of a more complex communication system.

Unlike adults, ant larvae do not clearly have a social stomach. Similar to adults, larvae also have foregut, midgut, and hindgut (Fig. 5) (LAPPANO 1958, PETRALIA & VINSON 1980, SOLIS & al. 2013). Unlike most insects, in Hymenopteran larvae the midgut is a blind sac, unconnected to the rectum until the very late in development when the meconium (waste collected over development)

is released (WEIR 1957, LAPPANO 1958). This general anatomy is similar for all ant larvae, though differences are observed between species, for example in the number of glands. Ant larvae typically have a slight indentation on their ventral side just beneath the mouthparts that is often seen to hold solids (PETRALIA & VINSON 1980). It is possible that some larvae engage in external digestion in this pocket but it has not yet been demonstrated.

Little research has been done on ant larval regurgitate. When ant larvae regurgitate, the liquid has been proposed to originate from the glandular reservoir of the labial glands (WHEELER 1918, LE MASNE 1953), though it remains feasible that it could come from the digestive tract. Labial glands are suspected to be involved in silk production (PETRALIA & VINSON 1980, PETRALIA & HAUT 1986, ERTHAL & al. 2007) and digestion (PETRALIA & VINSON 1980). Indeed, these glands were shown to produce enzymes implicated in the digestion of solid food in *Solenopsis invicta* (Myrmicinae) (PETRALIA & VINSON 1980). Labial glands are linked to a reservoir which can be highly developed for species that spin cocoons and can store large quantities of secretions. It was suggested from histological analysis of the labial glands that *Neoponera villosa* (Ponerinae) fourth-instar larvae go through two stages. First, their labial glands produce digestive enzymes, and in a second phase, they produce silk to spin a cocoon (ZARA & CAETANO 2002). Wheeler (1918) suspected that some species like *Carebara termitolestes* (Myrmicinae) use this labial gland reservoir to store food for adults. Wheeler hypothesized that it could be the case for other Myrmicinae that do not spin cocoons but have a well-developed labial gland system. Ant species are very diverse in their behaviors and physiologies, and even if some larvae regurgitate from the labial gland reservoirs, maybe it is not the case in all species.

The contents of rectal excretions of ant larvae drunk by workers have also not been extensively studied. Larvae of *Solenopsis invicta* (Myrmicinae) produce a white precipitate and a clear excretion at their rectum (PETRALIA & al. 1982). The clear excretion was found to be a solution of inorganic salts and was drunk by water-deprived adults. The authors concluded that the excreted liquid is drunk by adults because of the water it contains. Clear excretas were also observed by LE MASNE (1953) in Formicinae, Myrmicinae and Ponerinae species, and are considered to be rectal fluids originating from Malpighian tubules, as the midgut is closed on the rectal side (LE MASNE 1953). New analyses of the larval rectal secretions using recent -omics techniques could give additional information on why adult ants ingest these secretions.

**Trophallactic fluid contents and how they arrive in this fluid:** Trophallactic fluid contains exogenously and endogenously derived substances including a microbiome and endogenously produced proteins, nucleic acids, and small molecules. Exogenous substances arrive in trophallactic fluid by ingestion and both endogenous and exogenous proteins can be detected there by proteomic analysis (LEBOEUF & al. 2016). Endogenously produced proteins present in this fluid are likely to arrive in the foregut by secretion from glands in the head, by social transfer via trophallaxis, and by autotrophallaxis from the venom apparatus (LEBOEUF & al. 2016).

Adult trophallactic fluid contents have been analyzed to varying degrees and with varying tools in a handful of species, namely four species of *Camponotus* (Formicinae), two species of *Lasius* (Formicinae), five species of *Formica* (Formicinae), *Solenopsis invicta* (Myrmicinae) and two non-ant social insects (HAMILTON & al. 2011, TRAGUST & al. 2013, LEBOEUF & al. 2016, HE & al. 2018, TRAGUST & al. 2020). The protein content of trophallactic fluid has been analysed in *Camponotus floridanus* (Formicinae), *Camponotus fellah* (Formicinae), *Solenopsis invicta* (Myrmicinae), the honeybee *Apis mellifera*, and *Mastotermes darwiniensis*, a termite. In all species trophallactic fluid has been found to contain digestion-related proteins (e.g., amylases, maltases), immune or defense-related proteins (e.g., members of the prophenoloxidase cascade, DNases, RNases), and growth and development related proteins (e.g., juvenile hormone processing proteins, vitellogenins, major royal jelly proteins). Many consistently observed proteins fall across these boundaries, for example apolipoprotein, a nutrient storage protein implicated in development, and cathepsin D, a protease that has been implicated in digestion, immune defense and development (AHN & ZHU-SALZMAN 2009, SAIKHEDKAR & al. 2015). Beyond proteins, the trophallactic fluid of *Camponotus floridanus* (Formicinae) has been further studied, and has been found to contain hydrocarbons, cholesterol, microRNA, and the master developmental regulator juvenile hormone (LEBOEUF & al. 2016). Formicine ants have extremely acidic trophallactic fluid (pH 2-4) because they acidify their crop content by imbibing their formic acid (TRAGUST & al. 2020), detailed in the section on autotrophallaxis. Trophal-

lactic fluid also contains a variety of microorganisms making up a microbiome. The microbiome has been more thoroughly studied in social insects in the midgut where it is more stable, but even the crops of bees who engage in frequent trophallaxis appear to have somewhat consistent commensal bacterial populations (KWONG & MORAN 2016, BONILLA-ROSSO & ENGEL 2018).

The crop is not a porous structure, and thus endogenously produced materials in trophallactic fluid are likely to arrive in the crop through secretion by glands that open onto the alimentary canal. Indeed, the majority of the genes producing proteins found in trophallactic fluid carry secretion signals (LEBOEUF & al. 2016). BILLEN (2009) listed 75 glands present in ants. Amongst those in the head, many could excrete molecules into the trophallactic fluid through their openings on the digestive tract, such as the mandibular gland, the intramandibular gland, the maxillary gland, the infrabuccal gland, the basimandibular gland, the hypostomal silk gland, the postpharyngeal gland, the propharyngeal gland and sub-epithelial and tegumental glands (BILLEN 2009, BILLEN & al. 2015). The labial glands are located in the thorax, but their duct ends in the mouth so their excreta are also likely to be added to trophallactic fluid. There have been some studies on gland function, for example on the postpharyngeal gland (SOROKER & al. 1994, HEFETZ & al. 1996, SOROKER & al. 1998), propharyngeal gland (NICULITA & al. 2007), mandibular gland (BRAND & al. 1973, HOWSE 1990, HERNÁNDEZ & al. 1999, HUGHES & al. 2001), intramandibular gland (SCHOETERS & BILLEN 1994, CAETANO & DO AMARAL 2005, MARTINS & SERRÃO 2011) and labial gland (PAULSEN 1971, BILLEN & al. 2002), but so far, no work has directly shown that these glands impact the trophallactic fluid content. A proteomic study in honeybees (FUJITA & al. 2013) compared the protein content of royal jelly, a substance similar to honey bee trophallactic fluid (LEBOEUF & al. 2016), and three different glands in the head and thorax, the hypopharyngeal gland (analogous to the postpharyngeal gland), postcerebral gland, and thoracic gland. They demonstrated that these glands provide the proteins that make up royal jelly. Given that these same proteins have been found in honey bee trophallactic fluid strongly suggests that the analogous glands secrete the majority of proteins found in adult trophallactic fluid in ants.

The content of the fluid ingested during proctodeal trophallaxis and autotrophallaxis is little studied. In the context of proctodeal trophallaxis, the liquid excreted by termites (NALEPA 2015) and bees *Apis mellifera* (POWELL & al. 2014) comes from the hindgut and allows these creatures to share gut microbiota. In *Lasius neglectus* (Formicinae), it was shown to contain, in addition to the hindgut content, excreta from the Dufour's gland and poison gland (TRAGUST & al. 2013). In *Solenopsis invicta* (Myrmicinae), trophallactic fluid contains endogenous proteins found in venom (LEBOEUF & al. 2016), which is produced by the poison gland, even though it is not clear whether autotrophallaxis or proctodeal trophallaxis occur

in this species. COOK & DAVIDSON (2006) proposed that proctodeal trophallaxis may be a means for ants specializing on honeydew to incorporate essential amino acids from the endosymbionts that live in the gut. Abdominal glands could excrete proteins and molecules into this fluid, depending on the species and the context in which proctodeal trophallaxis occurs, but more research is needed.

**Proximal modulators:** It has been observed that workers isolated for a short time from the main colony, once reunited with nestmates, engage more in stomodeal trophallaxis than non-isolated workers (BOULAY & al. 2004). Trying to understand why, studies were conducted on neuromodulators such as octopamine, a biogenic amine whose homeostasis might depend on trophallaxis. Octopamine levels decrease in the brains of isolated workers of *Camponotus fellah* (Formicinae) (BOULAY & al. 2000), *Solenopsis invicta* (Myrmicinae) (VANDER MEER & al. 2008) and *Formica japonica* (Formicinae) (WADA-KATSUMATA & al. 2011). To understand the link between octopamine and increase of trophallaxis after isolation, isolated *Camponotus fellah* (Formicinae) workers were injected with octopamine and then reunited with a nestmate. Trophallaxis rates between injected ants and nestmates were similar to trophallaxis rates observed in non-isolated ants. Octopamine is generally linked to aggressive behaviors (YAKOVLEV 2018), but this study suggested that it might regulate the will of ants to engage in stomodeal trophallaxis. Thus, octopamine might be responsible for maintaining social interactions in ant colonies (BOULAY & al. 2000). Biogenic amines are likely to play a role in the neural regulation and response to trophallaxis and more research is necessary to understand these proximal mechanisms across contexts.

### The contexts and roles of trophallaxis

We have identified six contexts in which trophallaxis occurs: nourishment, decision making, defense against pathogens, social maintenance, aggression, and inoculation and maintenance of the gut microbiota. Depending on the context, trophallaxis can fulfill different roles in colony dynamics and physiology.

**Nourishment:** Nourishment is the primary context in which trophallaxis occurs. Division of labor necessitates a spatial compartmentalization of individuals inside and outside the nest (MERSCH & al. 2013, HEYMAN & al. 2017). The colony then requires nutrients to be shared among all individuals, including larvae, to meet communal nutritional needs (DUSSUTOUR & SIMPSON 2009, CSATA & DUSSUTOUR 2019). In species that rely heavily on trophallaxis for resource distribution, every individual in the colony is connected by this network of fluid exchange creating a social circulatory system for the colony or superorganism (BUFFIN & al. 2009, GREENWALD & al. 2015). Trophallaxis plays two roles in the context of nourishment: resource distribution and assessing the colony's satiety state.

**Resource distribution.** The primary role of stomodeal trophallaxis is likely to be resource distribution. In the context of famine relief, GREENWALD & al.

(2015) show the rapid dissemination of fluorescent food through colonies of *Camponotus* species (Formicinae), and previously BUFFIN & al. (2009) used radiolabeled food to a similar effect in *Formica* species (Formicinae). Beyond simply transmitting food from one individual to another, trophallaxis can also enable the colony to distribute specific nutrients to individuals who require them. In *Solenopsis* (Myrmicinae), stomodeal trophallaxis was found to allow nurses to receive proteinaceous food from foragers to feed larvae, while foragers consume more sugary nutrients (HOWARD & TSCHINKEL 1981, SORENSEN & al. 1981, SORENSEN & al. 1985, TSCHINKEL & CASSILL 1999, FELDHAAR 2014). A study on *Lasius niger* (Formicinae) also determined that ants can differentiate carbohydrate types such as sucrose and melezitose, the latter triggering an increase in trophallactic exchanges. This study also reports that the type of food impacts the frequency of trophallactic events but not the duration of trophallaxis events (BUFFIN & al. 2011). However, the structure of the trophallactic network in the context of famine relief was found to be robust and independent from the sucrose concentration of the food (BLES & al. 2018).

Repletes, as a food storage caste, represent this role of trophallaxis, in emblematic honeypot ants such as *Myrmecocystus mexicanus* (Formicinae) (RISSING 1984) and also in more generalist ants such as *Solenopsis invicta* (Myrmicinae) (GLANCEY & al. 1973). Honeypot replete castes are found in formicine genera *Myrmecocystus*, *Prenolepis*, *Camponotus*, *Cataglyphis*, and *Plagiolepis*, and Dolichodines *Leptomyrmex* (EISNER 1957, HÖLDOBLER & WILSON 2009). Repletes evolved to have an impressively expandable gaster, such that when their crop is full of liquid, they are not able to move. This is not the case for more flexible repletes in other species. In *Acromyrmex subterraneus* (Myrmicinae) and *Acromyrmex octospinosus* (Myrmicinae), empty foragers coming back to the nest engage much less in trophallaxis than replete foragers that had found food (RICHARD & ERRARD 2009). There was a low rate of trophallaxis between non-foragers in these two species, as measured by RICHARD & ERRARD (2009), suggesting that trophallaxis is involved mainly in food distribution for these ants. *Camponotus yamaokai* (Formicinae) tend to engage more in trophallaxis during starvation (SANADA & al. 1998). This could indicate that some individuals store nutrients under normal conditions and redistribute them over the social circulatory system of the colony when food is scarce.

Larvae can also be part of the trophallactic network. Several studies (DELAGE 1968, OHLY-WÜST 1977, DAVISON 1982, SORENSEN & al. 1983, ERTHAL & al. 2007) suggest that larvae of some species are not a food sink but should be considered a digestive caste, as they could be critically involved in the food processing and distribution network. DELAGE (1968) found that in *Messor capitatus* (Myrmicinae), stomodeal larval secretions contain lipases and proteases, but no carbohydrases, while the cephalic glands of workers produced carbohydrases but no proteases. This complementarity in enzymes production led

to the suggestion that larvae provide workers proteases and receive carbohydrases in return (DELAGÉ 1968). The enzyme content of the trophallactic fluid produced by larvae and adults remains to be analyzed to confirm such exchanges. Such an analysis was performed on the labial gland secretions of larvae from *Myrmica rubra* (Myrmicinae), *Monomorium pharaonis* (Myrmicinae) (OHLY-WÜST 1977) and *Solenopsis invicta* (Myrmicinae) (SORENSEN & al. 1983). It was suggested that larvae of these species use enzymes produced by their labial glands to predigest food and then share with workers through regurgitation (OHLY-WÜST 1977). DAVISON (1982) reported that in the seed harvester myrmicines *Monomorium rothsteini* and *Chelaner whitei*, adults unhusk and chew seeds before feeding them to larvae but do not eat seeds directly. DAVISON (1982) further observed that adults were only fed by larval regurgitation, highlighting the crucial role of larvae as a digestive caste. More recently, ERTHAL & al. (2007) studied the possibility of larvae to be a digestive caste in colonies of *Acromyrmex subterraneus* (Myrmicinae) and found that larvae show more diversity and activity of enzymes than workers. However, these results only suggest that larvae are adapted to degrade symbiotic fungus, and not to transfer digestion related proteins to adults. All other analyses were conducted much before the latest improvements in molecular biology. Now that more detailed proteomics analyses can be done, it is worth challenging dogmas established before the molecular era to improve our understanding of this behavior.

The previous examples we develop cover only stomodeal trophallaxis, because of its prevalence compared to other kinds of trophallaxis (Fig. 3). Though the nutritive role of proctodeal trophallaxis has never been investigated, in some species such as *Dolichoderus quadripunctatus* (Dolichoderinae), the anal droplet provided to larvae is sometimes stolen by workers (TOROSSIAN 1959) indicating that this fluid is appealing for adults and might have interesting nutritive or pheromonal characteristics.

**Sensing the satiety state of the colony.** In colonies where hundreds or thousands of individuals must be fed, it is important that foragers monitor the satiety state of the colony and the colony's nutritional needs and adapt their foraging strategy accordingly. The monitoring of the satiety state of the colony can be done through stomodeal trophallaxis through the crop fullness of receiver ants as revealed by GREENWALD & al. (2018). They showed that the fullness of the crop of receiver ants controls food flow rates, while the fullness of the crop of donors, or foragers, regulates the frequency at which foragers will forage for food. Foragers typically don't unload their crop completely before going on a foraging trip, and receivers rarely get their crop filled in a single trophallactic event (GREENWALD & al. 2015, GREENWALD & al. 2018). While it has not been explicitly demonstrated, it is likely that a similar mechanism might be used to stigmergically monitor nutritional needs, an ability required for ants' incredible ability to collectively meet their colony's nutritional needs (DUSSUTOUR & SIMPSON 2009, CSATA & DUSSUTOUR 2019).

**Decision making:** For a colony to grow and reproduce, ants must make both short- and long-term collective decisions, such as choosing a food source or emigrating to a new nest-site for short-term decisions and when to shift from colony growth to dispersal for a long-term decision. At the scale of a colony, making such decisions requires consensus building either over minutes to hours or over months to even years. For short-term decisions, pheromones and tactile signals are the predominant modes of consensus building, while little is known about long-term consensus building. Ants can use the network of trophallactic fluid to build consensus using molecular components. In this section, we discuss how stomodeal trophallaxis is used by ants to build collective memory, learn information about food sources, and manipulate larval growth and development.

**Collective memory and learning.** The network of fluid created by stomodeal trophallaxis can transmit information about food quality, allowing individuals to adapt their behavior regarding the food source. SOLA & JOSENS (2016) investigated the ability of ants to make foraging choices depending on their own experience of a scented food source or the social experience of a scented food source. They discovered that in *Camponotus mus* (Formicinae) the social information shared by trophallaxis at the same time as the food (PROVECHO & JOSENS 2009), overcame the individual information, leading individuals to forage for food they would have otherwise avoided (SOLA & JOSENS 2016).

Trophallaxis was also shown to play a role in the ability of *Tetramorium tsushimae* (Myrmicinae) to learn which aphid species is a mutualist between *Aphis craccivora* and *Acyrtosiphon pisum* without having interacted with the aphids (HAYASHI & al. 2017). Ants that never tended aphids reduced their aggressiveness towards aphids after engaging in stomodeal trophallaxis with nestmates that had previously tended aphids. However, when mouth-to-mouth contacts were inhibited between individuals that did and did not tend aphids, individuals that never tended aphids were aggressive toward aphids. These results suggest that trophallaxis plays a role in sharing information about aphids, allowing inexperienced ants to learn which aphid species are worth tending.

These studies highlight that trophallaxis allows ants to build social memory and share knowledge about food, whether it consists of honeydew or other food sources, information that in turn impacts foraging behavior. Food information of a single forager is received by several nestmates, making stomodeal trophallaxis an efficient way to spread this information. It is worth noting that in the above example, social memory overcomes individual memory, strengthening sociality and facilitating effective decision making.

**Manipulation of larval growth and development.** Ant species vary in how they determine caste fate – whether a larva will develop into a worker or a queen, into a minor worker or a major worker. In some species it can be somewhat genetically determined, and

in most species, both abiotic and biotic environments (including indirect genetic effects) can impact caste determination (LINKSVAYER 2006, SCHWANDER & al. 2010, RAJAKUMAR & al. 2012, LIBBRECHT & al. 2013, NIJHOUT & al. 2014, ALVARADO & al. 2015, RAJAKUMAR & al. 2018). Deciding whether the colony should expand by producing more workers, or reproduce by raising sexuals is critical for the future of the colony yet the mechanism(s) by which colonies make this long-term decision have remained elusive. The social environment, in other words, the quantity and quality of brood care, are thought to determine which individuals follow which developmental trajectories (BRIAN 1979, FLETCHER & ROSS 1985, PASSERA 1985, WHEELER 1986, NIJHOUT & al. 2014). Thus, the term ‘environmental caste determination,’ in the case of ants, is often made up of indirect genetic effects (SCHWANDER & al. 2010). The social environment that contributes to developmental fate is not only licking and grooming but also quality and quantity of feeding typically performed by trophallaxis, which can be composed of both endogenous (of internal origin) and exogenous (of external origin) components.

Until recently, it was thought that only variation in externally sourced food could bring about the variation observed in developmental fate. This phenomenon is illustrated by the seed harvester *Pogonomyrmex badius* (Myrmicinae), where the larger the colony’s adults are, the more nitrogen is ingested relative to carbon, mostly found in prey insects and in seeds, respectively (SMITH & al. 2008, SMITH & SUAREZ 2010), though the direction of causality is unclear (LIBBRECHT & al. 2013, LILICO-OUACHOUR & ABOUHEIF 2017). Finally, workers even in species with genetic caste determination can control the content of the next generation simply by culling larvae through cannibalism as is observed in worker policing (SCHULTNER & PULLIAINEN 2020).

Clues in the content of trophallactic fluid fed by adults to larvae indicate that components passed by trophallaxis are a means by which adults can impact larval growth and development. There are proteins in the trophallactic fluid of each species studied (described in the section on trophallactic fluid contents) whose orthologs are involved in growth and development, for example, proteins involved in juvenile hormone processing, vitellogenins, hexamerins, and major royal jelly proteins (LEBOEUF & al. 2016). LEBOEUF & al. (2016) found endogenous microRNAs in trophallactic fluid, orthologous to those found in honey bee worker and royal jelly (GUO & al. 2013). Additionally, juvenile hormone III was also found in the trophallactic fluid of *Camponotus* (Formicinae), along with a group of juvenile hormone esterases that have sustained positive selection and repeated duplications since the split from *Formica* (Formicinae) (LEBOEUF & al. 2018). Finding growth-related proteins, juvenile hormone and microRNAs involved in developmental fate in trophallactic fluid suggests that trophallaxis is a means for ants to control larval fate and development, somewhat as royal/worker jelly does in bees (KAMAKURA 2011, LINKSVAYER & al.

2011, VILLALTA & al. 2016) but with finer potential for temporal and social control.

Presence of a given component in this exchanged fluid does not indicate that it is functional. Some work has been done to reveal functionality of trophallactic fluid components in influencing larval development. In *Camponotus floridanus* (Formicinae), when nursing workers’ trophallactic fluid is supplemented with either juvenile hormone itself (JH III, as found in the same species’ trophallactic fluid) or an inhibitor of the abundant juvenile hormone esterases found in *Camponotus floridanus* (Formicinae) trophallactic fluid, more larvae are reared to adulthood (LEBOEUF & al. 2016, LEBOEUF & al. 2018). Treatment with juvenile hormone alone mildly increased body size (LEBOEUF & al. 2016). These outcomes somewhat mirror previous research on juvenile hormone and development. Treating ant larvae topically with juvenile hormone or juvenile hormone analogs have been shown to influence the larval fate and development (NIJHOUT & WHEELER 1982, CNAANI & al. 1997, BLOCH & al. 2000, MUTTI & al. 2011). While the precise mechanisms by which topical and trophallactic fluid manipulations influence development remain unclear, they suggest that trophallactic fluid components are likely to function in regulating larval development.

Transcriptome analyses also support a role of endogenously produced trophallactic proteins in directing larval development. WALSH & al. (2018) looked at differentially expressed genes in the head and abdomen of *Monomorium pharaonis* (Myrmicinae) nurses specialized in tending either first- or last-instar larvae (WALSH & al. 2018). In nurses tending first-instar larvae, vitellogenin and a major royal jelly protein were more expressed than in nurses tending last-instar larvae. These proteins are involved in larval fate determination in bees (AMDAM & al. 2003), where caste determination occurs before the end of the first instar (WARNER & al. 2018), and both vitellogenin and major royal jelly proteins have been found in the trophallactic fluid of other ants (LEBOEUF & al. 2016).

Proctodeal trophallaxis is also suspected to play a role in larval growth and development in *Dolichoderus quadripunctatus* (Dolichoderinae). In this species, male larvae are fed with proctodeal fluids produced by workers, which could suggest that proctodeal trophallaxis is a means of providing these larvae with specific molecules (TOROSSIAN 1959). This study, however, did not test the content of the fluid and the impact of proctodeal trophallaxis on male larvae growth and development remains largely unknown.

**Defense against pathogens:** Because food is often shared through a colony by trophallaxis, poisons (HOOPER-BUI & RUST 2000, RUST & al. 2004) and pathogens (NAUG & CAMAZINE 2002, HAMILTON & al. 2011) can also be spread throughout the colony this way. In the case of pathogens, members of the colony can be informed that an infection has occurred, and their immune systems triggered (CREMER & al. 2018). In this section, we discuss the role of trophallaxis in prophylaxis, in the transmission of the information about infection or contamination and in building social immunity.

Autotrophallaxis or acidopore grooming (see section on autotrophallaxis) is thought to play a role in immune defense, at least in Formicinae. TRAGUST & al. (2020) observed that *Camponotus floridanus* (Formicinae) drink secretions from their highly acidic poison glands after ingestion of food, increasing their crop acidity. By infecting *Camponotus floridanus* (Formicinae) with *Serratia marcescens*, TRAGUST & al. (2020) observed increased survival rate in ants allowed to perform autotrophallaxis compared to infected ants prevented from ingesting their acidopore secretions. Stomodaeal trophallaxis combined with acidopore grooming in the infected donor improved survival of receivers compared to when acidopore grooming is prevented in the infected donor. Autotrophallaxis is thus thought to be a barrier to food borne bacterial pathogens and to be prophylactic (TRAGUST & al. 2020). The same study reveals that crop acidification allows the colonization of gut microbes *Acetobacteraceae*. Adult *Lasius neglectus* (Formicinae) were also observed by TRAGUST & al. (2013) to groom their acidopore and apply the collected fluid on the pupae in order to remove pathogenic fungus conidiospores. However, it is unclear if in this behavior the poison is drunk and regurgitated or just kept temporarily in the oral cavity (TRAGUST & al. 2013).

When infected by pathogens, the contact network of *Lasius niger* (Formicinae) changes (STROEYMEYER & al. 2018), likely in order to protect the queen and young. One would expect trophallactic exchanges to decrease, in order to avoid the transmission of pathogens through the colony. However, experiments done by HAMILTON & al. (2011) revealed that *Camponotus pennsylvanicus* (Formicinae) workers that were immune-challenged engaged more in trophallaxis than non-immune-challenged ants. The same increase in trophallactic rates has been observed with immune-challenged *Camponotus fellah* (Formicinae) (DE SOUZA & al. 2008) and *Solenopsis invicta* (Myrmicinae) (QIU & al. 2016). An increase in trophallactic rates could be due to the fact that trophallactic fluid contains defense-related proteins, such as cathepsins, prophenoloxidases, serine proteases, RNAses, DNAses, serpins and lectins (HAMILTON & al. 2011, LEBOEUF & al. 2016). Cathepsin D has been discovered to be present in higher quantities in the trophallactic fluid of immune-challenged *Camponotus pennsylvanicus* (Formicinae) workers compared to control ants (HAMILTON & al. 2011). The same study showed that engaging in trophallaxis with immunized nestmates allowed ants to improve their survival rate to a bacterial infection. However, other studies highlight a decrease in trophallaxis rates with immune-challenged ants in *Formica polyctena* (Formicinae) (AUBERT & RICHARD 2008). Finally, *Lasius neglectus* (Formicinae) individuals, when exposed to a pathogenic fungus, do not increase or decrease trophallactic exchanges (KONRAD & al. 2012). It then seems that when ants are immune-challenged, trophallactic exchange rates either increase, decrease, or remain constant depending on species, which could illustrate different defense strategies when facing infection, and may reflect trophallactic fluid content.

It is also worth noting that arthropods lack a protein-based adaptive immune system, but use RNA interference to fight viruses (MARQUES & IMLER 2016). This defence system involves the action of small RNAs (TASSETTO & al. 2017, WHITFIELD & al. 2017). Small RNA have been observed in trophallactic fluid of *Camponotus floridanus* (Formicinae) (LEBOEUF & al. 2016). While more analyses are clearly required to link trophallactic fluid RNA to insects' adaptive immune system, it is plausible that immune RNA would be shared through stomodaeal trophallaxis to improve the adaptive immunity of the colony. In bees, vitellogenin is metabolized to produce royal jelly, which when fed to the larvae, is suspected to participate in trans-generational immune priming (HARWOOD & al. 2019). Similarly, because vitellogenin has been found in the trophallactic fluid of ants (LEBOEUF & al. 2016), trophallaxis could contribute to trans-generational trans-individual immune priming.

Stomodaeal trophallaxis also dilutes and may neutralize toxic compounds. When bringing liquid food to the nest, foragers will unload only a portion of their crop load to several nestmates. Nestmates will drink from several foragers, and thus mix their crop content (GREENWALD & al. 2019). This mixing allows dilution of a potential poison such that the ant's crop contains fewer toxins, which might reduce the death rate. This is challenging for pest control, as poisonous baits must not repel foragers and or kill at high concentrations (HOOPER-BUI & RUST 2000, RUST & al. 2004). In *Temnothorax albipennis*, the presence of repletes might help in testing the toxicity of the collected food before it is distributed to nestmates (SENDOVA-FRANKS & al. 2010).

In sum, stomodaeal trophallaxis is important for immune defense, by diluting potentially harmful molecules, spreading defense molecules through the colony and helping to build social immunity. Additionally, formic acid, drunk via autotrophallaxis by adults and transmitted by stomodaeal trophallaxis plays an important role in protecting formicine ants against pathogens. However, it remains unclear what molecules spread through stomodaeal trophallaxis are involved in building both individual and social immunity.

**Social maintenance:** Interactions between ants of different colonies and different species typically involve aggressive behavior, while ants from the same colony recognize one another through a characteristic colony odor. We discuss the role of stomodaeal trophallaxis in the homogenization of the colony odor and in the maintenance of social interaction in colonies.

**Homogenization of colony odor:** Ants can recognize nestmates from non-nestmates. Nestmate recognition is based on the recognition of cuticular hydrocarbons (CHCs) (LAHAV & al. 1999, OZAKI 2005, VAN ZWEDEN & D'ETTORRE 2010, BOS & al. 2011, SHARMA & al. 2015, SPRENGER & MENZEL 2020). These hydrocarbons serve an ancient role in preventing desiccation, and a secondary role in communication and nestmate recognition. Within a species, the CHCs are generally the same but vary in quan-

tity between individuals and colonies (MARTIN & DRIJFHOUT 2009), while different species show different sets of CHCs (KATHER & MARTIN 2012). In *Lasius niger* (Formicinae), CHC profiles were shown to differ between castes in the same colony, and on different parts of the body of an individual and can vary simply with humidity and temperature (LENOIR & al. 2009, SPRENGER & MENZEL 2020).

Both trophallaxis and allogrooming are behaviors that can homogenize CHCs across the colony (CROZIER & DIX 1979, Bos & al. 2011), creating a dynamic and complex mix of hydrocarbons on the cuticle (BOULAY & al. 2004). This complex mixture is often referred to as the colony odor or colony signature, allowing individuals to recognize their nestmates (SOROKER & al. 1995, SLEIGH 2002). However, the role of trophallaxis in colony odor homogenization is often difficult to separate from that of allogrooming. CHCs are produced by oenocytes in and around the fat body (KOTO & al. 2019), and they are sequestered in the postpharyngeal gland, located at the back of the head and linked to the mouthparts (SOROKER & al. 1994, OLDDHAM & al. 1999). The hydrocarbon profile of the postpharyngeal gland is similar to that of the cuticle (BAGNÈRES & MORGAN 1991). Additionally, SOROKER & al. (1994) discovered that the postpharyngeal gland receives newly synthesized CHCs by grooming and through hemolymph. The authors suspected CHCs to be transmitted by trophallaxis between nestmates of *Cataglyphis niger* (Formicinae), as labeled CHCs from donors were found in the postpharyngeal gland and on the cuticle of receivers. However, receivers with blocked mouthparts couldn't engage in trophallaxis, and still received labeled CHCs from donors with blocked mouthparts, though admittedly less than when their mouthparts are not blocked. This result suggests that CHC homogenization can be performed through trophallaxis but that other methods are also used to homogenize colony odor (SOROKER & al. 1994).

Trophallaxis events may also help renew this combination of molecules after isolation (SOROKER & al. 1995, DAHBI & al. 1999). BOULAY & al. (2004) showed that 20-day isolated workers were strongly aggressed by nestmates, illustrating that isolated workers were not recognized as nestmates. The same study found that ants' CHC profile differs from the colony's after 20-days of isolation. This suggests that there is a turnover in the CHC production, and that the colony CHC signature can differ from an individual CHC signature. However, if the isolated workers were allowed to engage in trophallaxis and grooming with five young nestmates for five days before reintroduction to their colony, aggression was greatly reduced. This suggests that grooming and / or trophallaxis helped the isolated workers to adjust their CHC signature to that of the colony (BOULAY & al. 2004). After 20 days of isolation, workers from different colonies of the same species showed less aggression and engaged in trophallaxis, while workers from different species showed no decrease in aggression rates (BOULAY & LENOIR 2001). After five days of isolation, workers engage in more trophallaxis events than non-isolated workers, and that fed workers engage less

in trophallaxis than starved workers (LENOIR & BOULAY 1999). In the polygyne and supercolonial *Formica paralugubris* (Formicinae), CHAPUISAT & al. (2005) monitored the stomodeal trophallaxis events between ants from two distinct populations. They observed that stomodeal trophallaxis was more frequent between non-nestmates than between nestmates, they thus hypothesize that the higher rates of trophallaxis between non-nestmates could play a role in colony odor homogenization.

There are a number of reasons that trophallaxis is likely not the only means by which CHCs are homogenized. While CHCs were found to be present in trophallactic fluid of *Camponotus floridanus* (Formicinae) workers, the trophallactic hydrocarbons presented a different profile from the hydrocarbons on the body (LEBOEUF & al. 2016). Bos & al. (2011) showed that in *Camponotus aethiops* (Formicinae), CHCs present in the nest material play a critical role in nestmate recognition, thus making CHCs exchanges by trophallaxis less important, later supported by HEYMAN & al. (2019). However, soil CHC analyses in *Lasius niger* (Formicinae) nests showed that CHCs profiles were not colony specific (LENOIR & al. 2009). Finally, ants that were never observed to engage in trophallaxis (TAY & CROZIER 2000) such as *Rhytidoponera* sp.12 (Ectatomminae) manage to recognize nestmates and show aggression toward non-nestmates (PEETERS 1988, LENOIR & al. 2009). These studies suggest that even if trophallaxis is involved in colony odor homogenization in some species, it is not the only mechanism and it is not the method used for all ant species.

**Aggression:** In some species, ants live in social communities where individuals need to express dominance. Here, we detail how stomodeal and proctodeal trophallaxis can be used by ants in the context of aggression and dominance within a colony and between colonies, either of the same or of different ant species.

**Aggression within species.** Stomodeal trophallaxis is a form of appeasement behavior, wherein an oppressed individual will regurgitate trophallactic liquid to appease her aggressor. LIEBIG & al. (1997) observed that in *Ponera coarctata* (Ponerinae) colonies, the stomodeal trophallaxis rate increases after queen removal compared to queenright colonies. The same behavior is observed in the slave-making ant *Temnothorax muellerianus* (Myrmicinae). After the queen is removed, some more dominant workers will bite subordinate slave-makers, which results in oppressed ants regurgitating trophallactic liquid (HEINZE 2010). Another example deals with interactions between individuals from two populations of polygyne and uniclonial *Formica paralugubris* (Formicinae). Workers were more likely to engage in trophallaxis with non-nestmates than with nestmates, possibly in the context of a dominance-submission interaction (CHAPUISAT & al. 2005). From these examples, we see that trophallaxis can be important in building hierarchy and unifying over distant nests.

**Aggression between species.** These aggression behaviors also happen between colonies of different

species. Stomodeal trophallaxis was reported to occur when *Solenopsis invicta* attacked *Solenopsis geminata* and *Pheidole dentata* workers. The latter two species were fed with radiolabeled sucrose solution that was later detected in *Solenopsis invicta* workers (BHATKAR & KLOFT 1977). More radioactivity was recorded in major workers than minors of *Solenopsis invicta* (BHATKAR 1979), suggesting that they received more food from submissive ants than minor workers. However, radioactivity could have also been transmitted by *Solenopsis invicta* eating workers of the other species.

The slavemaker *Temnothorax americanus* (Myrmicinae) workers have been observed to provide anal droplets to hosts *Temnothorax ambiguus* (Myrmicinae) and *Temnothorax longispinosus* (Myrmicinae) through proctodeal trophallaxis. The slavemaker may dominate the host through components transmitted via proctodeal trophallaxis. Indeed, it could explain why this parasite would provide potentially valuable nutrients to its host (STUART 1981).

Although these behaviors could aim at offering food to appease the aggressor, it is unlikely the case when slavemaker ants provide fluid to the host. An alternative possibility is that the donor intentionally shares molecules with the receiver through either stomodeal or proctodeal trophallaxis, though this has yet to be investigated.

Inoculation and maintenance of gut microbiota. If trophallaxis allows pathogens to spread over an ant colony (NAUG & CAMAZINE 2002, HAMILTON & al. 2011) and even between ants and their parasites (DEDEINE & al. 2005), it can also diffuse symbiotic bacteria through ant colonies. In termites and bees, there is evidence that trophallaxis plays a role in sharing gut microbiota (MARTINSON & al. 2012, MCFREDERICK & al. 2012, ENGEL & MORAN 2013, BRUNE & DIETRICH 2015, NALEPA 2015). Gut microbiota plays a crucial role in digestion (ENGEL & MORAN 2013), and helps the host degrade food into nutrients. Though gut microbial populations have been studied in ants (VAN BORM & al. 2002, RUSSELL & al. 2009, ANDERSON & al. 2012, SANDERS & al. 2014, LIBERTI & al. 2015), comparatively little work has been done on horizontal transfer of gut microbiota between ants. To our knowledge, the only example of gut microbiota inoculation is recorded in *Cephalotes rohweri* (Myrmicinae) (LANAN & al. 2016). Larvae and adults of this species have stable gut microbiomes, but these commensals are lost during pupation. Turtle ants feed on a primarily plant-based diet requiring a specialized microbiome. LANAN & al. (2016) found that these ants have a highly specialized proventricular filter that blocks bacteria from passing from the crop into the midgut. Shortly after emergence and before the development of this filter, callow workers engaged in proctodeal trophallaxis with older workers to populate their gut microbiota. The same behavior is observed in *Procrystocerus scabriusculus* (Myrmicinae) (WILSON 1976, WHEELER 1984), presumably for the same reason, even though it has not been investigated in the context of the microbiome. Both stomodeal and proctodeal trophal-

laxis could play this role in other species. Studying the microbiome transmitted in trophallactic fluid would be a first step into investigating this hypothesis.

### The evolution of trophallaxis

The phylogenetic distribution of insects that engage in stomodeal trophallaxis, from social insects through gregarious insects (e.g., earwigs, cockroaches) to even solitary insects (e.g., burying beetles) raises the question of how this behavior has evolved.

Given our analysis of stomodeal trophallaxis prevalence over the ant phylogeny, what can be concluded about the evolution of trophallaxis behaviors?

The often sparse representation of trophallaxis across several orders of insects (Hymenoptera, Blattodea, Dermaptera (FALK & al. 2014), Coleoptera (CAPODEANU-NÄGLER 2018)) implies that all insects might be physiologically capable of regurgitation or that regurgitation machinery is sufficiently labile to effect this behavior if it sufficiently improves fitness. Indeed, regurgitation occurs in insects even outside the context of social interaction (GRANT 2006).

The common ancestor of ants, bees and wasps had a wasp-waist, a sting and lived a solitary parasitic lifestyle that involved stinging prey, laying eggs in it, and likely no further brood care (PETERS & al. 2017). While trophallaxis is part of the behavioral repertoire of most if not all social wasps (HERMANN 1979), early-branching wasps do not engage in trophallaxis. Some solitary wasps (e.g., Eumeninae) progressively provision their larvae with pre-masticated food and others do not (HUNT 1982, TURILLAZZI 1989); Stenogastrinae are facultatively eusocial wasps that engage in more fluid transfer between adults and larvae though these transfers appear indirect, for example, “adults regurgitate drops of liquid food into the middle of the coiled larvae” (TURILLAZZI 1989). Given this progression over wasp phylogeny, trophallaxis appears to have come about in wasps through the path of parental care.

Despite the presence of stomodeal trophallaxis in other Hymenopterans, some Formicidae engage in stomodeal trophallaxis and some do not (Fig. 3). This suggests that stomodeal trophallaxis in ants either evolved several times or was lost several times (WHEELER & WHEELER 1979, BRADY & al. 2006). From the fossil record, it has been deduced that the most recent common ancestor of all ants was likely social, nesting, predatory and had similar sized winged-queen and wingless-worker castes (BARDEN & GRIMALDI 2016). This collection of traits is still present in many early-branching ants. There is no evidence to suggest that the ancestor of all ants engaged in trophallaxis. To the contrary, the idea that the common ancestor of ants did not engage in trophallaxis is consistent with the observation that trophallaxis is not a routine behavior, if performed at all, in most early-branching ants (Fig. 3). Adult→ adult stomodeal trophallaxis has been reported in nine Ponerinae (LE MASNE 1948, HASHIMOTO & al. 1995, LIEBIG & al. 1997, FUJIOKA & OKADA 2019), two Myrmecinae (WHELDEN & HASKINS 1954), suspected in two additional

Ponerinae species (HASKINS 1931, HASKINS & HASKINS 1950) (Fig. 3, Appendix S1) and has been observed in one Dorylinae (RETTENMEYER 1963). There are a handful of genera scattered over the phylogeny that show adult→ larva or larva→ adult stomodeal trophallaxis behaviors but not adult→ adult stomodeal trophallaxis. The observation of stomodeal trophallaxis events in early-diverging species remains rare, even in those species, and is likely not a prevalent way of sharing food. Nonetheless the distribution of species throughout the ant phylogeny that do perform trophallaxis (Fig. 3) suggests that trophallaxis has evolved multiple times (BRADY & al. 2006).

What circumstances might promote the adoption and fixation of trophallaxis as a fundamental component of a species' social life? Across ants, bees and wasps, trophallaxis behaviors often occur in the contexts of nutrient sharing in cooperative brood care or dominance interactions and tend to correlate with both superorganismality and the reliance on liquid food. We here discuss the ecological niches that have been hypothesized to have resulted in trophallaxis.

William Morton Wheeler suggested that the characteristic stomodeal trophallaxis behavior observed in many ant species evolved from mothers feeding their young (WHEELER 1918) as is likely the case in wasps. Given that all ants descended from a social common ancestor, they all engage in parental or alloparental care (excepting some socially parasitic species). However, many early-branching ants progressively provision their young, typically by offering pieces of insect prey to larvae. This has been sufficient in a majority of early-branching species for ~100 million years without resulting in trophallaxis behavior.

Stomodeal trophallaxis in many ants, bees and wasps occurs in the context of dominance where it is performed as an appeasement behavior to resolve conflicts (LIEBIG & al. 1997, WCISLO & GONZALEZ 2006, HEINZE 2010). This behavioral context could have led to the fixation of trophallaxis behaviors. In ants, trophallaxis-as-appeasement has been described both within and between colonies, and between different species (see section Aggression). Despite the fact that all ants have aggressive interactions within and across species, trophallaxis has not evolved in all aggressive ants (e.g., *Dorylus*), suggesting that the need for social conflict resolution is not a sufficient motivator alone to evolve trophallaxis.

Trophallaxis behavior is loosely correlated with superorganismality and at least in some cases may have become fixed out of the need for greater communication, cooperation and consensus building that came with this major evolutionary transition. We consider ant species with workers physiologically capable of being mated (GOTOH & al. 2016) as non-superorganismal, due to this increased space for competition within the otherwise cooperative colony. The roles played by stomodeal trophallaxis in maintaining social interactions, such as collective learning, cooperation, decision making and social immunity make this behavior tightly entangled with the committed eusociality seen in superorganismal species. Trophallaxis

has been hypothesized to have evolved from allogrooming, as a more efficient means to share recognition cues (SOROKER & al. 1998, LENOIR & al. 2001, see section Social maintenance). Stomodeal trophallaxis occurs more frequently in species with obligate worker sterility. Many Ponerinae workers maintain a functional spermatheca (GOTOH & al. 2016), and to our knowledge, have only nine species that engage in stomodeal trophallaxis (Fig. 3, Appendix S1), while Formicinae all have obligately sterile workers and to our knowledge all engage in stomodeal trophallaxis. Nonetheless, while there are correlative links between superorganismality and trophallaxis, there are superorganismal genera (e.g., *Dorylus*, *Sericomyrmex*) (WHEELER 1925, WEISSFLOG & al. 2000) that do not engage in trophallaxis, so far as we are aware.

The most parsimonious explanation for the distribution of trophallaxis behavior is that the trophallactic habit has evolved out of the need for ants to transport liquid food back to the nest, which itself was brought about by a new resource to be exploited in the environment, plant-derived carbohydrates. The most robust predictor of trophallactic behavior across the ant phylogeny appears to be whether the species' diet includes liquid food such as extrafloral nectar or honeydew, though quantitative and phylogenetic comparisons are merited. Internal storage of liquid food in the context of a colony necessitates the use of stomodeal trophallaxis (DAVIDSON 1998, DAVIDSON & al. 2004, COOK & DAVIDSON 2006). Further, foraging on liquid food (e.g., honeydew, extrafloral nectar) instead of insect prey could be at the origin of the evolution of repletes or semi-replete major workers. This hypothesis is supported by an increased diversification rate in ants in the late Cretaceous when there was a rise of angiosperms (MOREAU & al. 2006, MOREAU & BELL 2013). With the rise of Angiosperms, insects had access to various liquid foods such as nectar, honeydew, and later extrafloral nectar which could only have been exploited thanks to the evolution of trophallaxis behavior. However, more phylogenetic work needs to be done to understand the link between the rise of angiosperms, the diversification of liquid food sources and the diversification of ants that led to the evolution of trophallaxis (NELSEN & al. 2018, KAUR & al. 2019, KAUR & al. 2019). Behavior can be both a cause and consequence of evolution (DUCKWORTH 2009). At least in the case of extrafloral nectaries, the food-for-protection mutualism between ants and plants is likely to have evolved before structures to secrete extrafloral nectar (NELSEN & al. 2018).

This specialization on liquid food, be it direct through extrafloral nectaries or indirect through honeydew-secreting insects, represents a food-for-protection mutualism that allowed ants to massively increase their ecological dominance, likely outcompeting many more ancient ants (BARDEN & GRIMALDI 2016). With the exception of army ants, the species that dominate ecosystems with colonies of tens to hundreds of thousands of individuals tend to be species that utilize plant-derived carbohydrates.

Mandibular pseudotrophallaxis may have been an evolutionary precursor of stomodeal trophallaxis (HÖLL-

DOBLER & WILSON 1990, p.293), though it does not provide the added benefit of a social circulatory system. Many Ponerinae use mandibular pseudotrophallaxis instead of stomodeal trophallaxis, while later-branching ant species, from Formicinae and Myrmicinae engage in stomodeal trophallaxis (Fig. 3). Other ways of transporting liquid, such as pseudotrophallaxis have drawbacks. The tool or droplet can be easily lost, contaminated or stolen (LAPIERRE & al. 2007) on the path back to the nest, and these risks are reduced in the case of true trophallaxis, where the food is kept safe inside the ant's gut. It is possible that stomodeal trophallaxis replaced pseudotrophallaxis as ants diversified (HÖLLDOBLER & WILSON 1990, p.293). Additionally, *Ectatomma* (Ectatomminae), thought to have given rise to Myrmicinae, engage in mandibular pseudotrophallaxis but were never observed to engage in stomodeal trophallaxis (Fig. 3).

In light of the fact that nearly all Formicinae and Dolichoderinae engage in trophallaxis and tend honeydew producers and / or extrafloral nectaries, the behavior may have been part of the behavioral repertoire of the ancestral Formicinae and the ancestral Dolichoderinae. Indeed, the only living species of the subfamily Aneuretinae (sister group to Dolichoderinae), *Aneuretus simoni*, engages in frequent trophallaxis and has repletes (BILLEN 2017). However, the nearest-neighbor subfamilies Myrmicinae and Ectatomminae clearly do not all engage in trophallaxis (Fig 3). There is a compelling but incomplete parallel here with the presence and absence of the sting: Formicinae and Dolichoderinae have lost the sting, while in most cases, it has been retained in Myrmicinae and Ectatomminae (TOUCHARD & al. 2016). However, Aneuretinae both sting and engage in trophallaxis. There may also be links with the evolution of these traits and cuticle thickness. To understand the relationships between sting presence, cuticle thickness and use of trophallaxis, more quantitative models will need to be done over the ant phylogeny. Trophallaxis behavior and liquid food appear fundamental to the behavioral ecology of Formicinae and Dolichoderinae. Consequently, it is in these subfamilies that we can most expect to find the most highly developed adaptations that build upon the resultant social circulatory system.

Behavior has often been considered a pacemaker of evolution, either driving it with rapid adaptation to new niches or slowing it by avoiding the full force of natural selection due to environmental change (DUCKWORTH 2009). Here, trophallaxis behavior has enabled at least some lineages of ants to successfully exploit novel resources and inhabit new ecological niches. The evolution of stomodeal trophallaxis, as a requisite component in a species' behavioral repertoire, was likely provoked by ants eating, sharing and storing liquid foods such as honeydew and extrafloral nectar, though in some lineages this behavior may have come about through other routes. In the cases of Formicinae and Dolichoderinae this behavior is likely to have become fixed before the radiations of these subfamilies, most likely because of the importance of liquid food for these ants and the consequent physiological

adaptations that have followed the commitment to this behavior. This behavior has likely been instrumental in these subfamilies' ecological success.

## Conclusion

Trophallaxis is a complex behavior that is a crucial aspect of many ant species' behavioral ecology. Perhaps because it occurs in so many contexts, the importance of trophallaxis, its modes of action at the molecular level and its evolution remain surprisingly unstudied.

We hope to have clarified our collective knowledge of this captivating and complex behavior in order to give researchers tools to report trophallaxis in a more accurate way. A challenge lies in the fact that when trophallaxis is not observed, it is rarely mentioned. As a result, for many species such as army ants or early-branching species, even though it is unlikely that these species engage in trophallaxis, it is difficult to prove a negative. In the same vein, likely because of the difficulty of observing this intimate behavior, the way larvae are fed is rarely known for any given species.

Because of the unequal information in literature over the ant phylogeny, it is important to have a clear strategy to report which ants do engage in trophallaxis, based on more than proventricular shape, or the genus or subfamily to which a given species belongs. Indeed, the diversity and variability of behavior within the same subfamily or even genus does not allow us to extend results across species of ants. The example of Ponerinae, previously thought not to undergo trophallaxis while some species clearly do, illustrates the need we have to assess the capability of individual species to undergo trophallaxis.

Within a colony, for a given species undergoing trophallaxis, this behavior is a powerful tool linking all individuals in an efficient way. The evolutionary origins of this social network can be debated, but the most parsimonious explanation is that adult→ adult stomodeal trophallaxis arose from the need to transport and share liquid food. It seems clear that adult→ adult stomodeal trophallaxis evolved multiple times in ants, and for this reason, it is interesting to have a closer look at how trophallaxis occurs between individuals and within the colony across these different species, as there may be variation. Such research could also shed light on the distinct roles this suite of behaviors plays over the ant phylogeny. In addition, the fact that the trophallactic network within a colony represents the circulatory system of this superorganism makes trophallaxis a critical behavior to study in order to understand the organization of ant societies. In sum, we see that what began as food-for-protection mutualism between ants, plants and honeydew-producing insects has evolved into a deeply social behavior instrumental in ants' ecological dominance.

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