### Discovery of new social behaviour in ant colonies

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are eusocial insects belonging to nts Formicidae (Hymenoptera: the family Formicoidea). They are ubiquitous and so abundant that the biomass of all ants is more than the combined biomass of wild birds and mammals, and equals 20% of the human biomass (Schultheiss et al., 2022). They live in colonies of thousands and millions of highly coordinated individuals, that's why they are also known as 'Super Organisms'. However, many individual interactions in social insect societies remain poorly understood or incompletely known, therefore requiring systematic studies on these interactions. Recently, a new social behaviour of ants was discovered based on the experiments of Snir et al., 2022, where they found immobile pupae stage extrudes a nutrient-rich social fluid or moulting fluid which was rapidly consumed by adult ants and young larvae. This nutrient-rich social fluid or moulting fluid of pupal ants is analogous to mammalian milk and it elicits parental care behaviour. This behaviour is crucial for the pupal survival and development of young larvae. Moreover, they found that this derived social function of the pupal social fluid or moulting fluid is conserved across studied ants.

#### Is pupal social fluid essential for young larvae?

The experiments of Snir et al., 2022 found that the beginning of pupal secretions and larval hatching are tightly synchronized events in the studied clonal raider ant, *Ooceraea biroi* (subfamily: Dorylinae) colonies and proved that pupal fluid serves as a 'milk-like' substance for newly hatched larvae. Larvae with pupae in their colonies have increased larval growth and survival during the first days after hatching, compared to larvae without pupae in their colonies. Based on observations, it was found that *O. biroi* adults will readily place young larvae on pupae, where they can feed on pupal fluid. Overall, their findings suggest that the pupal moulting fluid has a crucial, yet previously unknown, role in the social structure and colony fitness.

# How pupal social fluid hinders the survival of the pupa itself?

Based on the experimental studies on *O. biroi* pupae, it was found that a substantial amount of pupal social fluid was secreted toward the end of the pupal stage *i.e.*, secretion begins six days before eclosion and secreted volume increased over time till eclosion. Further, they found that if the social fluid is not removed from the pupae in isolation under a clean environment, they drown in their own secretions. Moreover, ant nest environments are not clean and when the pupae are placed in vacant used nest boxes, the fluid droplets become contaminated with fungi, and these infections spread and ultimately killed all pupae. In contrast, when fluid was removed daily manually, a high rate of pupal survival and eclosion was reported (Snir et al., 2022).

#### Molecular Composition of the pupal social fluid

Based on proteomic and metabolomic profiling it was found that the pupal social fluid contains a variety of micro and macronutrients viz., all essential amino acids, multiple carbohydrates including N-acetyl-d-glucosamine (GlcNAc), nucleic acids, and vitamins. Proteomic Gene Ontology enrichment analysis identified the processes of protein and chitin degradation, which are respectively represented by activities of peptidase and chitinase. The protein and chitin degradation activities are related to the degradation of the old cuticle, which is one of three major molecular pathways characteristic of insect moulting fluids. Many free amino acids and GlcNAc was found to be increasing as pupae approached eclosion. Further, it was demonstrated that the pupal social fluid possesses the molecular and physiological traits of insect moulting fluids and is rich in a range of proteins and metabolites (Snir et al., 2022).

# Is the role of pupal secretions conserved across the ants?

Although Snir et al. (2022) mainly worked on *O. biroi* (Dorylinae), they also explored the same social behaviour in four other species to cover the five major ant subfamilies: *Solenopsis invicta* (subfamily: Myrmicinae), *Nylanderia flavipes* (Formicinae), *Tapinoma sessile* (Dolichoderinae) and

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Fig. 1. Ant pupae of different species with secretion droplets at their abdominal tip accumulated over 24 h in social isolation. In species *P. pennsylvanica*, the cocoon has been removed. Source: Snir et al. (2022)

*Ponera pennsylvanica* (Ponerinae). They observed that melanized pupae of all four species emit liquid droplets from the abdominal tip. The composition of these secretions is similar to that of *O. biroi*, according to metabolomic profiling. This indicated that across the different taxon of ants, pupae secrete a liquid derived from the moulting fluid. Moreover, in species whose pupae are enclosed in cocoons, for those species adults consume the social fluid through the permeable silken fabric.

### Conclusions

The ant pupal social fluid or moulting fluid has acquired novel social functions within an ant colony, which was previously unknown. It plays an important interdependence link between pupae, larvae, and adults. It has been demonstrated that pupal fluid is detrimental to ant pupae if not removed and that it is an important food source for early larvae. Moreover, this behaviour is widely conserved in different ant taxon. This milk-like pupal secretion is perhaps having additional far-reaching effects on larvae and other colony members. The evolutionary aspects of pupal secretions across the Hymenoptera and ants will be interesting to see in future investigations.

### References

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