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论文题目：Multiple Behaviours of Advanced Intelligence: Exploring Social Division of Labour, Tool Use, and Play-like Behaviour in Ants

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Multiple Behaviours of Advanced Intelligence: Exploring Social Division of Labour, Tool Use, and Play-like Behaviour in Ants

Yangqinglan Zhang

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Abstract

Eusocial insects, notably formicids (ants), have evolved multifaceted social divisions of labour, facilitating both ant population densities and complex organizational structures; additionally, some taxa use tools to exploit previously inaccessible resources. Previous investigations have concluded that ant societies may be organized into three principle castes of labor: reproductives (queens), nurses (workers), and defenders (soldiers); within the worker class, further sub-specialization of labour has been observed: certain individuals of *Phiedol yeensis* workers devote more time and effort towards distinct tasks such as foraging, material translocation, or necrophoresis clearance. Laboratory trials under controlled circumstances were undertaken to test the tool-use behaviour of *Formica cunicularia* Latreille, 1798 when they encounter artificial adhesive substrates. We found *F. cunicularia* workers deposited significantly greater quantities of particles (sand) upon food-baited treated surfaces than upon unbaited treated control groups. We also found that ants repeatedly transported particles and exhibited a degree of division of labor.

Furthermore, we observed another interesting behavior: ants rolled spherical objects, displaying play-like behavior reminiscent of humans kicking a ball. Individuals propelled the object forward with their metathoracic (hind) leg and demonstrated a clear preference for spheres with lighter mass and greater movability. The metabolomic test indicated that play-like behaviour does indeed elevated dopamine levels, but due to testing constraints and random errors, the behaviour would appear to constitute a more complex phenomenon than simple playing.

Keywords

social divisions of labour; tool use; play-like behaviour; *Phiedol yeensis*; *Formica cunicularia*; *Odontomachus monticola*;

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Introduction

1.1 Long-lasting bias about ants

In discussions associated with intelligence and complicated social organizations, the term is often associated with mammals, in particular primates, with *Homo Sapiens* widely regarded as the most cognitively advanced and behaviorally intricate of all organisms. This long-lasting bias, however, may divert attention from less known taxa and obscure the fact that certain arthropod lineages likewise hold the ability of complex social repertories.

1.2 Ant intelligence and complex social organization behaviour

Formicids are known to exhibit three principal castes of labour—reproductives (queens), nurses (workers), and defenders (soldiers)—constituting the classic tripartite social structure. Among the most renowned groups is the hyperdiverse genus *Phiedol*, which displays a pronounced morphological and functional distinction between queens, workers, and soldiers. Divergent titres in juvenile hormone (JH) levels govern whether a larva differentiates into a worker or a soldier, and the imaginal disks that appear in soldier ants are essential for the formation of their disproportionately large heads and bodies (Rajakumar et al., 2018). Further task specialization within the worker caste of *Hymenoptera Formicidae* has been delineated into subcategories—mainly major, media, minor, and minim workers (Wilson, 1980); subsequent investigations have clarified the specific roles fulfilled by each subcaste in *Atta cephalotes*, making it clear that Minims are predominantly engaged in fungiculture and brood rearing, whereas Majors rarely participate in direct contact with the fungal gardens (Muratore et al., 2023).

Formicids have likewise evolved a noteworthy array of tool-using behaviors, utilizing environmentally provided objects such as rocks, dirt, and plant fragments. Among the best-documented instances is the utilization of particular materials to transport liquid nourishment. For instance, *Aphaenogaster subterranea* workers introduce small items (e.g., soil particles, sand grains, pine needles, leaf and grass fragments) into liquid food and subsequently convey the saturated materials back to their nest (Módra et al., 2020). *A. subterranea* further demonstrates discriminative material selection, favoring items that are either more portable or are more absorptive of fluids (Maák et al., 2017). In a similar vein, *Solenopsis invicta* workers exhibit tool use by depositing particles onto substrates that are otherwise arduous to traverse when food is present; foraging workers gather soil particles from remote locations and deposit them onto obstacle surfaces, thereby rendering the resource more readily accessible (Wen et al., 2020).

Play-like behaviour has been documented in bumble bees(*Bombus*), in which individual workers push wooden balls, they also exhibited age and sex difference in this behaviour, with yourger males playing more than any other individuals. But no play-like behaviour were documented among ants; however, in this study, *F. cunicularia* have indeed been observed in the

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following experiments to exhibit such behaviour when encountering polystyrene (Styrofoam) spheres, and no age differences were observed.

Materials and methods

1.1 Ant collection and maintenance

According to molecular identifications, *F. cunicularia* workers were identified. One colony of about 500 *F. cunicularia* workers was collected on July 24th, 2026, from Wangfu Road No. 7 (40.102379° N, 116.396032° E), Changping District, Beijing, China. Another colony of about 500 *F. cunicularia* workers was collected on July 25th, 2026, from Wangfu Road No.7 (40.106906° N, 116.401249° E). The nest provided is oval-shaped, about 60cm in shortest diameter and 100cm in longest diameter; workers were sucked into an insect aspirator after being dug up, larvae and pupae were all brushed into a centrifuge tube to be collected. The collected ants were then put into a 15cm high, 32cm in diameter cylindrical container with a 1cm thick plaster base, and a Fluon coating about 2 cm wide to prevent ants from escaping; the plaster base was made with modelling plaster powder, mixed with water in a 5:3 ratio; the nest in the middle of the container was imitated by a dragon stone.

One colony of *P. yeensis*, 20 queens and 3000 workers, was obtained via an online vendor on the Pin-duo-duo platform, with shipment originating from Guangxi province. The colony was housed in a 15cm high, 32cm in diameter cylindrical container with a 1.5cm thick plaster base and a Fluon coating about 2 cm wide to prevent ants from escaping; the plaster base was made with modelling plaster powder, mixed with water in a 5:3 ratio; although the initial design of the nesting chambers followed a hexagonal configuration, the ants have since modified the plaster surface through excavation, progressively constructing their own subterranean chambers at the base of the container.

Humidity levels in both containers are sustained daily through periodic water spraying; drinking water is provided in the form of droplets placed within a small container; nutritional provisions are supplied every 2 to 3 days and encompass a diverse array of substrates: honey water serves as an energy source while egg yolk supplies essential proteins; sanitation is maintained by removing food and honey residue that have accumulated over the preceding 2 days, and all deceased individuals are collected and discarded in a half-sealed bag.

1.2 Experiment 1: Social division of labour in *P. yeensis* workers and soldiers

The first experiment concerning the division of labour among worker ants was conducted within the colony enclosure. To ensure that the behaviors observed corresponded as closely as

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possible to those performed under natural conditions, the colony was initially left undisturbed for a period of one month to adapt to its surroundings. Subsequently, a 10 cm Petri dish containing water 3 mm in depth was introduced into the container. At the centre of the dish, an island of honey water was positioned, isolated within a small circular receptacle 3 cm in diameter. Blue sponges (about 2 × 2 × 2 mm^3) and white sand particles (about 0.3 × 0.3 × 0.3 mm^3) were provided to the ants and arranged around the perimeter of the Petri dish. Filming started at the instant the Petri dish was placed and continued for one hour.

The second experiment, examining the roles fulfilled by soldiers, was also conducted within the colony enclosure, one week following the first experiment. A single mealworm was introduced into the container in order to test the function of soldiers within the nest. Filming again started at the moment of introduction and continued for one hour.

The social divisions underlying necrophoretic clearance were first investigated; the trajectories travelled by three workers to deposit soldier cadavers were tracked by hand-drawn movement trajectories, supplemented with a heatmap and a behaviour point map. Next, the social divisions governing material translocation of sand grains were examined; the movements of three individuals—from picking up sand grains, to transporting and relocating them, and to returning to the original pick-up site—were tracked by hand-drawn movement trajectories, supplemented with a heatmap and a behaviour point map. Furthermore, the social divisions underlying foraging behaviour were investigated. These concerned the ants that constructed an ant raft to reach the honey water; the movements of three individuals—from building the raft, to returning to land, and ultimately to entering the nest—were tracked by means of hand-drawn movement trajectories, supplemented with a heatmap and a behaviour point map. Lastly, the social division of labour among soldiers was documented by hand-drawn movement trajectories indicating the movements of three individuals—from exiting the nest entrance, to joining the conflict, and ultimately to retreating into the nest—supplemented with a heatmap and a behaviour point map.

1.3 Experiment 2: Comparison of tool-use behaviour between three ant species *O. monticola*, *P. yeensis*, and *F. cunicularia*, with addition of the sand particle covering speed comparison of *F. cunicularia* workers on treated surface in three time intervals

The tool use behaviour, particularly the particle covering rate of *F. cunicularia* on viscous substrates, was investigated, with experiments conducted within the colony enclosure. To ensure that the behaviors observed corresponded as closely as possible to those performed under natural conditions, the colony was initially left undisturbed for a period of one month to acclimatize to its surroundings. A circular acrylic plate, 10 cm in diameter and 3 mm in thickness, treated with PET double-sided adhesive tape, was placed into the upper region of the colony container. Yellow sponges (about 3 × 3 × 3 mm^3), rocks (about 2 × 2 × 2 mm^3) and sand particles (about 0.3 × 0.3 × 0.3 mm^3) were provided to the ants and distributed evenly throughout the container. Two

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experimental conditions were established: one featuring a food-baited surface and one featuring an unbaited surface. Each condition consists of three trials, with each trial lasting 1.5 hours.

The start and end of each trial were analyzed using ImageJ; the amount of material and the extent of area covered were subsequently measured, and the changes in both material quantity and covered area were calculated and graphically represented.

1.4 Experiment 3: investigating the playing mechanism of *F. cunicularia* workers with three types of balls and comparing to *P. yeensis*

Animal play-like behaviour has a few criteria: no immediate survival function, was mentally rewarding, differed in form from functional behaviors, was repeated but not stereotyped, and occurred under non-stressful conditions. Behaviour of *F. cunicularia* toards spheres met the criteria for animal play-like behaviour.

Play-like behaviour in *F. cunicularia* was first observed upon the introduction of a Styrofoam sphere, prompting the design of a dedicated experiment. Owing to its execution immediately after the tool-use experiment, no further acclimatisation was required. Two movable Styrofoam spheres, two fixed Styrofoam spheres and four mud balls were presented to the ants. The mud balls proved too heavy for the ants to displace, and the fixed Styrofoam spheres were secured in place with double-sided adhesive tape. Filming continued for an hour, and 5 individuals were chosen to be documented with hand-drawn movement trajectories, supplemented with heatmaps and location point maps; the movement of all spheres was documented by means of hand-drawn movement trajectories, with heatmaps and location point maps.

Further metabolomic assays were conducted to examine the changes in brain secretion levels. 5 individuals that exhibited play-like behaviour towards either artificial Styrofoam spheres or mud balls were assigned to the play-like behaviour group sample, while 5 individuals that did not actively exhibit play-like behaviour towards provided spheres were assigned to the non-playing control group. Individuals from both groups were frozen at the instant of their action by immersion in or application of liquid nitrogen, in order to preserve cerebral secretions. They were subsequently dissected so that only the head remained, removing the body, neck, and antennae. The samples were then individually placed into 1mL centrifuge tubes and sent to E-ceshi for metabolimic group test in dry-ice-packed foam boxes.

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Results

1.1 Experiment 1: Social division of labour in *P. yeensis* workers and soldiers

Each labour caste exhibited pronounced disparities in the time devoted to its respective job; the trajectories undertaken by individuals further corroborated their social division of labour.

During the first experiment examining the social division of workers, individuals engaged in necrophoretic clearance allocated the majority of their time to corpse translocation and ancillary operations related to this role, including scouting trajectories and cadaver exchange (Fig. 1). They also spent a disproportionate amount of time in proximity to either the corpses pile or the nest where the cadavers were initially encountered. By contrast, those belonging to the social division of material translocation devoted most of their time conveying sand grains from the sand piles back to the nest (Fig. 2), expending greater effort in the transfer and retrieval of materials. Ants belonging to the material translocation labour caste were documented to exhibit a distinctive relaying behaviour: certain individuals transport materials (sand) solely from the sand pit to the nest entrance, while others continue the task from that point, collecting sand from the nest entrance and conveying it into the nest to be deposited as building material. Foraging ants were documented to build ant rafts to get to the food (Fig. 3), unlike previous reports of *S. invicta* preserving their nest from floods, and spent the majority of their time returning to the mainland, constructing and utilizing an ant raft, and subsequently walking directly back to the nest with their social stomach replete with honey water.

In the second experiment, upon exiting the nest, soldiers allocated the majority of their time tackling the mealworm and retreated only after the mealworm had ceased to constitute a threat to the colony (Fig. 4).

Subsequent investigations concluded that when the abundance of large workers declined so drastically that they became insufficient for the whole colony to function, and smaller workers proved incapable of fulfilling their roles, soldiers were able to assume workers' social division of labour and progressively take over all three labour castes.

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necrophoretic clearance:				
		Start	End	Behaviour
	#1 (upper left)	00.14.000	00.16.766	carry corpse
		00.16.766	00.29.500	path exploring
		00.29.500	00.53.566	carry corpse
		00.53.566	01.06.633	path exploring
		01.06.633	01.53.966 (place in garbage)	carry corpse
		01.53.966	02.14.100	end
		Start	End	Behaviour
	#2 (upper right)	01.05.766	1.50.866	carry corpse
		1.50.866	1.52.099	switch carry position
		1.52.099	02.10.366	carry corpse
		02.10.366	02.20.233	rest
		02.20.233	02.50.533 (place in garbage)	carry corpse
		Start	End	Behaviour
	#3 (left middle)	00.00.000	00.19.133	carry corpse
		00.19.133	00.24.033	exchange corpse
		00.24.033	00.31.866	carry corpse
		00.31.866	00.34.133	exchange corpse
		00.34.133	00.49.799 (place corpse)	carry corpse
		00.49.799	00.55.833	carry corpse
		00.55.833	01.07.733 (place corpse)	carry corpse
		01.07.733	01.35.866	back to nest

Fig. 1 During the first experiment, 3 individuals of *P. yeensis* workers' necrophoretic clearance social division behaviour map

material translocation:				
		Start	End	Behaviour
	#4 (main nest)	00.00.000	00.06.433	wander around sand pit
		00.06.433	01.07.299	41 carrying behaviour
		01.07.299	01.09.166	finding another rout
		01.09.166 (new rout)	01.21.566	12 carrying behaviour
		01.21.566	01.32.766	back to nest
		Start	End	Behaviour
	#5 (3rd nest)	00.00.000	01.17.933	39 carrying behaviour
		01.17.933	01.21.700	back to nest
		Start	End	Behaviour
	#6 (main nest)	02.30.000	02.36.966	wandering
		02.36.966	03.23.866	23 carrying behaviour
		03.23.866	03.30.266	back to nest

Fig. 2 During the first experiment, 3 individuals of *P. yeensis* workers' material translocation of sand grains social division behaviour map

forager:				
		Strat	End	Behaviour
	#7 (1st to land)	03.15.033	03.45.800 (mainland)	leave feeding spot
		03.45.800	03.55.900	back to nest
		Start	End	Behaviour
	#8 (2nd to land)	03.16.400	04.31.033 (mainland)	leave feeding spot
		04.31.033	04.49.666	self cleaning
		04.49.666	05.02.066	back to nest
		Start	End	Behaviour
	#9 (3rd to land)	03.16.400	04.28.800 (mainland)	leave feeding spot
		04.28.800	04.33.699	back to nest

Fig. 3 During the first experiment, 3 individuals of *P. yeensis* workers' foraging behaviour social division behaviour map

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soldier :				
		Start	End	Behaviour
	#10 (1st to attack)	00.04.166	00.08.199	bite meal-worm
		00.08.199	00.10.466	let go
		00.10.466 (mis-harmed)	00.12.666	bite meal-worm
		00.12.666	01.42.633 (retreat)	bite meal-worm
		01.42.633	01.45.733	back to nest
		Start	End	Behaviour
	#11 (2nd to attack)	00.46.600	00.05.700	bite meal-worm
		00.05.700	00.52.899	got fling away
		00.52.899	01.04.533	5 attacks
		01.04.533	01.09.900	let go
		01.09.900	01.14.000	2 attacks - unsuccess
		01.14.166	01.43.833	4 attacks
		01.43.833	01.56.500	back to nest
		Start	End	Behaviour
	#12 (new soldier)	08.03.699	08.10.466	engage in fight
		08.10.466	08.17.733	bite meal-worm
		08.17.733	08.26.166	wandering
		08.26.166	08.30.966	bite meal-worm
		08.30.966	08.58.033	back to nest

Fig. 4 During the second experiment, 3 individuals of *P. yeensis* soldiers' fighting social division behaviour map

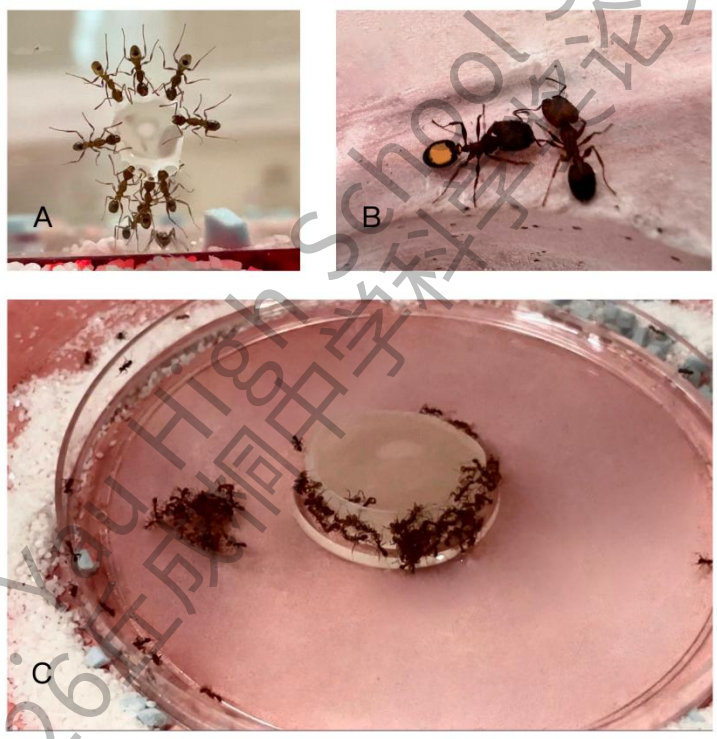


Fig. 5 *P. yeensis* exhibit feeding behaviour in presence of honey water (A); *P. yeensis* soldiers (B); *P. yeensis* exhibit ant raft building behaviour (C)

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1.2 Experiment 2: Comparison of tool-use behaviour between three ant species *O. monticola*, *P. yeensis*, and *F. cunicularia*, with addition of the sand particle covering speed comparison of *F. cunicularia* workers on treated surface in three time intervals

Odontomachus monticola, *P. yeensis* and *F. cunicularia* have all been observed to exhibit tool use behaviour in various circumstances and usage, *O. monticola* was observed to use quartz stones to manipulate the humidity levels in nursery room (Fig. 6); *F. cunicularia* was observed to exhibit tool use behaviour in order to get access to either water or honey water without the hazard of being drowned in 3cm wide 3mm deep round bottle cap (Fig. 7); P. yeensis have been observed to use sand particles to build dams in order to over the entrance of their nest (Fig. 8). According to further research about the difference in tool use behaviour regarding the presence of artificial viscous surface in *F. cunicularia*, *P. yeensis* and *O. monticola*, *F. cunicularia* and *P. yeensis* were observed to exhibit tool use to cross the hazardous substrate, while O. monticola proved to be ether unable to exhibit tool use behaviour in order to cross viscose substrates, or simply too large to feel hazards from the viscous surface.



Fig. 6 *O. monticola* exhibit tool use behaviour in order to manipulate the humidity levels in nursery room



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Fig. 7 *F. cunicularia* exhibit tool use behaviour to keep from drowning when accessing water & honey water (A, B)

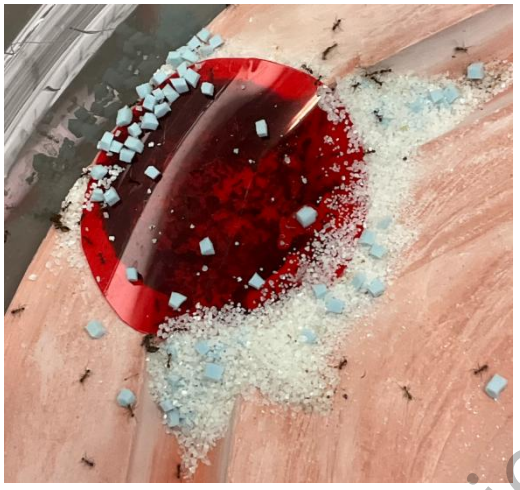


Fig. 8 *P. yeensis* exhibit tool use behaviour in order to cover the entrance of their nest

The first experiment regarding the tool use behaviour of *F. cunicularia* workers was tested using a 3cm in diameter and 3mm in height circular acrylic plate. Three testing circumstances were provided: food-baited with particles, unbaited with particles, food-baited without particles. (Fig. 9)

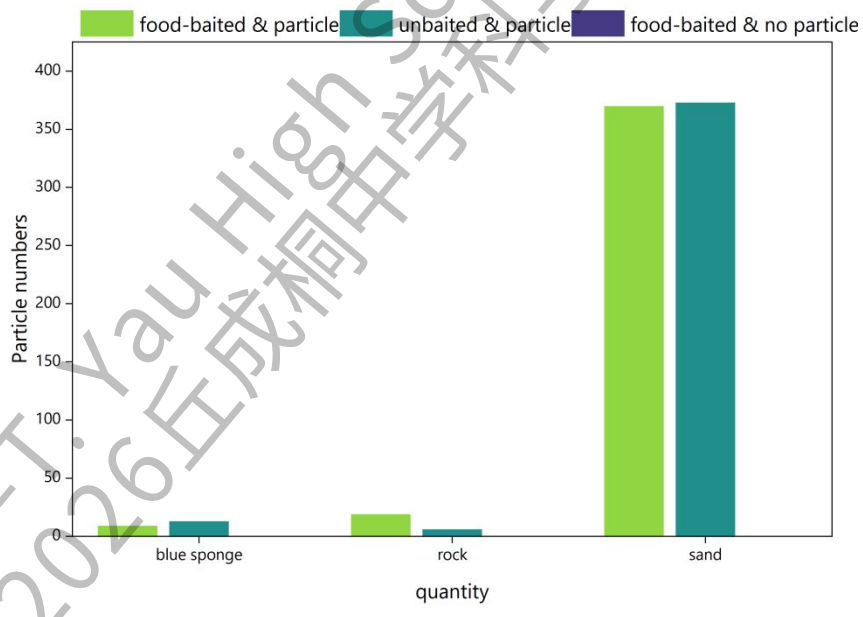


Fig. 9 *F. cunicularia* workers total deposit of 3 different particles onto viscous surface in three different circumstances

The presence of bait significantly affected the tool-use behaviour of *F. cunicularia* workers. The experiment yields a definitive conclusion: workers covered a greater extent of surface during every time interval when provided with food as a lure than the unbaited control groups. The food-baited surface without particles group displayed a striking behaviour: they employed plaster pieces broken from the edges of their nest as an instantaneous substitute for the previously provided particles. This suggests that ant tool use behaviour is not restricted to the environment

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and particles supplied to them, but that they also possess the ability to manipulate their surrounding environment and process tools for their own advantage. (Fig. 10)

Across the three 1.5-hour time periods, both the quantity of material translocated and the area covered on the 10cm circular acrylic plate were significantly greater in the food-baited group than in the unbaited group. (Fig. 11 & Fig. 12)

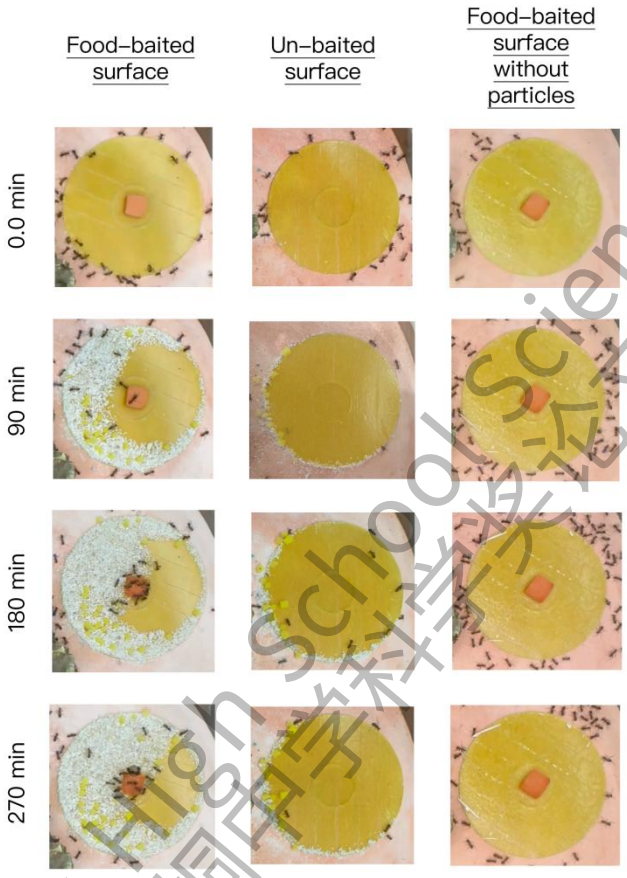


Fig. 10 *F. cunicularia* workers deposited significantly more material onto the baited viscous surface than the unbaited viscous surface

Scale:	1.0042 pixle/mm	Area:	314.1593 mm^2	Error(%):	0.038128137					
	Start	Inicial particle number	initial cover area (mm^2)	End	final particle number	final cover area (mm^2)	Particle increase	Particle increase error	Cover area increase (mm^2)	Cover area increase error (±mm^2)
#1	00.00.00.000	0	0	01.30.00.000	321	83.3501	321	16.05	83.3501	3.177983998
#2	01.30.00.000	578	85.1192	03.00.00.000	1052	91.3924	474	23.7	6.2732	0.239185426
#3	03.00.00.000	1052	91.3924	04.30.00.000	1372	109.4228	320	16	18.0304	0.687465554

Fig. 11 Food-baited group: *F. cunicularia* workers deposited a significant amount of material and covered more area on the baited viscous surface

Area:	314.1593 mm^2	Error(%):	0.023464242							
Scale:		Start	Inicial particle number	initial cover area (mm^2)	End	final particle number	final cover area (mm^2)	Particle increase	Particle increase error	Cover area increase (mm^2)	Cover area increase error (±mm^2)
6.0006 pixle/mm	#1	00.00.00.000	0	0	01.30.00.000	223	14.5907	223	11.15	14.5907	0.342359716
6.4382 pixle/mm	#2	01.30.00.000	126	14.5907	03.00.00.000	267	16.822	141	7.05	2.2313	0.052355763
5.8444 pixle/mm	#3	03.00.00.000	267	16.822	04.30.00.000	355	19.5155	88	4.4	2.6935	0.063200936

Fig. 12 Unbaited group: *F. cunicularia* workers deposited significantly less material and covered less area on the unbaited viscous surface

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Fig. 13 Examples of adhesive tape: *F. cunicularia* 10cm baited and particle group (A, D); *F. cunicularia* 10cm unbaited and particle group (B, E); *F. cunicularia* 10cm baited and no particle group (C, F); *F. cunicularia* 3cm baited and particle group (G); *F. cunicularia* 3cm unbaited and particle group (H); *F. cunicularia* 3cm baited and no particle group (I); *P. yeensis* 3cm baited and particle group (J); *P. yeensis* 3cm unbaited and particle group (K); *P. yeensis* 3cm baited and no particle group (L)

For the baited group, the rate of area expansion declined dramatically following the first 1.5-hour trial, which nonetheless represents an increase of 83.3501 ± 3.1780 mm^2. The second 1.5-hour trial exhibited a significant reduction relative to the first interval, with an increase of only 6.2732 ± 0.2392 mm^2. The third 1.5-hour trial remained lower than the first trial, but displayed a moderate increase of 18.0304 ± 0.6875 mm^2. (Fig. 14)

The number of material transferred onto the viscous surface (PET double adhesive tape) remained comparatively stable across all three time intervals: 321 ± 16.05 grains of sand during the first interval, 474 ± 23.7 grains of sand during the second interval, and 320 ± 16 grains of sand during the third interval. This indicates that the act of paving roads in response to the pressure of a hazardous surface—thereby enhancing their prospects of reaching food across the artificial viscous substrate—is not a random act; rather, it is a stable, unambiguous, and repeatable instance of highly specialized tool use behaviour. (Fig. 15)

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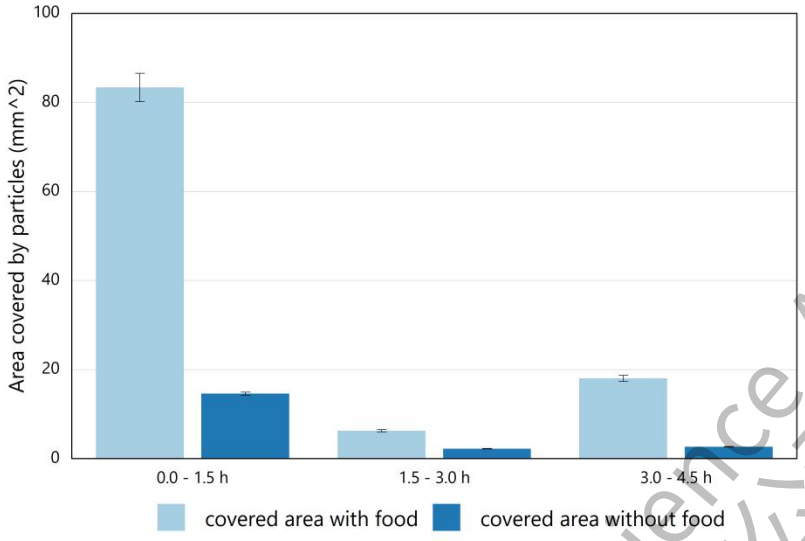


Fig. 14 *F. cunicularia* workers' rate of area increase over three 1.5-hour trials on an artificial viscous surface, including both food-baited and unbaited groups

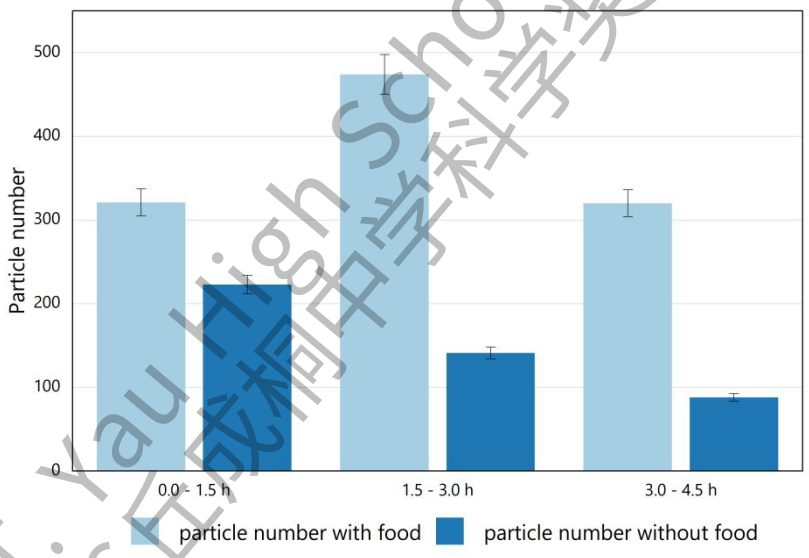


Fig. 15 *F. cunicularia* workers' change in the quantity of translocated sand grains in three 1.5-hour trials on an artificial viscous surface, including both food-baited and unbaited groups

In the food-baited group, given that the capacity of the ants to translocate materials remains essentially constant, the pronounced fluctuations in area covered are likely attributable to the changes in worker strategy across the successive periods. During the first period, the ants appeared to be more inclined to cover area and reach the target, inevitably leaving interstices between sand grains and thereby exposing proportions of the viscous surface that remained hazardous to traverse. Subsequently, during the second period, the ants endeavoured to reinforce the pavement to sustain their pursuit of food, resulting in a marked reduction in area covered. Finally, the ants paved the vicinity surrounding the food, culminating in an increase in area covered.

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1.3 Experiment 3: investigating the playing mechanism of *F. cunicularia* workers with three types of balls and comparing to *P. yeensis*

Experiments regarding the play-like behaviour of *P. yeensis* and *F. cunicularia* were investigated. *P. yeensis* does not exhibit any play-like behaviour towards the presence of artificial spheres without moving them. *F. cunicularia* exhibited play-like behaviour towards artificial spheres, exhibiting high numbers of play-like behaviour and can even determine which kind of sphere they like best(Fig. 17 & Fig. 18 & Fig. 19). Play-like behaviour towards balls turned out to be a community project, there were no significant differences between older or younger workers, all individual ant participated in this activity whenever they encountered a sphere.

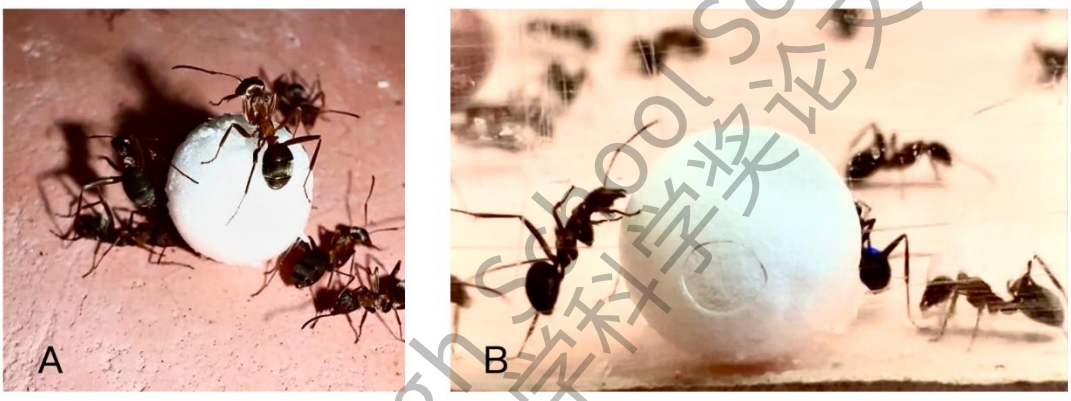


Fig. 16 *F. cunicularia* exhibit play-like behaviour towards spheres(A, B)

The mud balls proved to be too heavy, and the fixed Styrofoam spheres too difficult for the ants to displace without becoming detached from the substrate; consequently, their speed and displacement could not be reliably quantified. However, the speed and distance displaced by the movable Styrofoam ball proved instrumental in ascertaining the certainty of the *F. cunicularia* play-like behaviour, since the spheres should not move in the absence of the ants and should shift only marginally if the ants encounter them incidentally due to chance without displaying play-like behaviour towards them. (Fig. 17 & Fig. 18)

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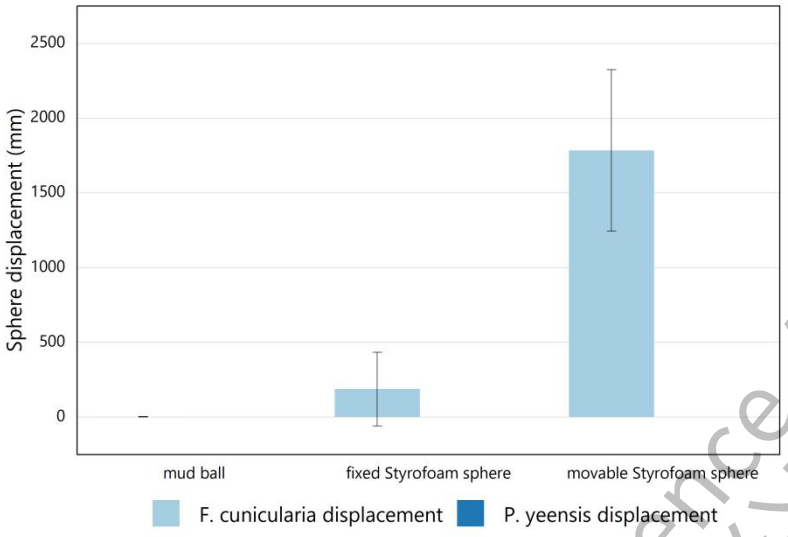


Fig. 17 The average distance displaced by the spheres in the presence of *F. cunicularia* workers

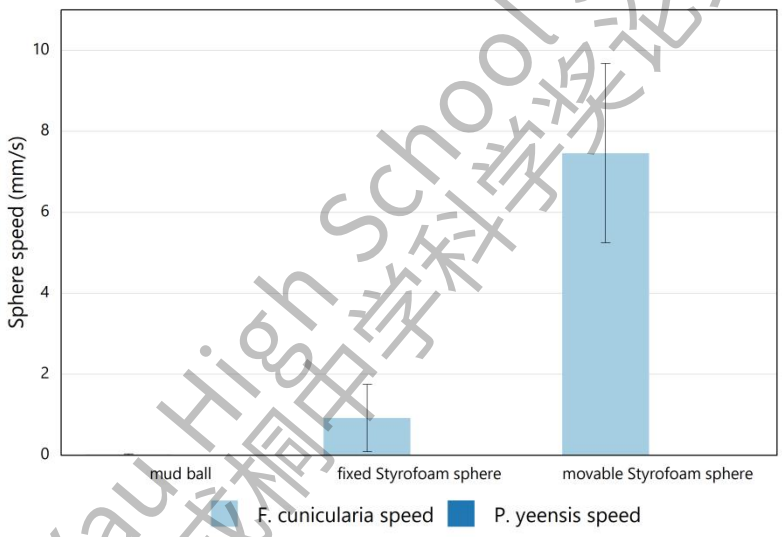


Fig. 18 The average speed of the balls in the presence of *F. cunicularia* workers

The mean number of interactions the ants exhibit towards each sphere type may serve as a practical metric for comparing their preferences. Fixed Styrofoam spheres emerged as the clear top preference, whereas mud balls and movable Styrofoam balls were less preferred, with no significant differences between the instances of playing directed towards them (Fig. 19).

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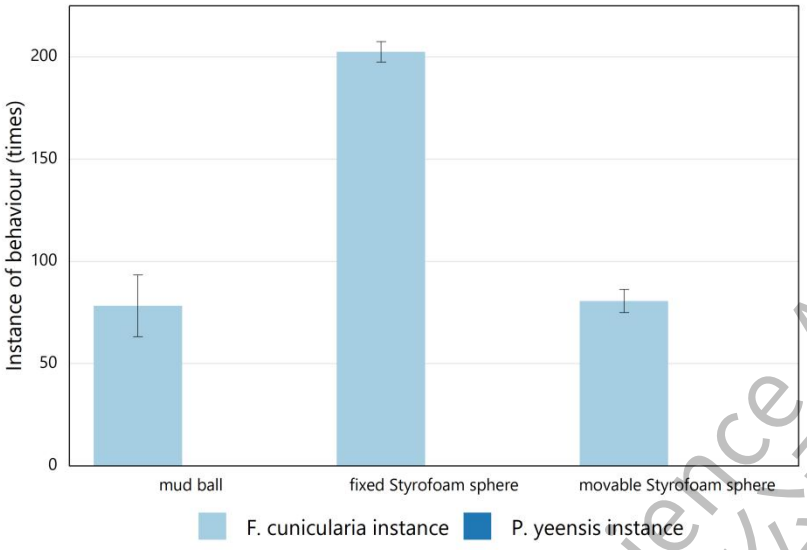


Fig. 19 The average number of interactions of *F. cunicularia* workers with three kinds of spheres

Since workers manipulated Styrofoam spheres, which are considerably lighter than mud balls (Fig. 20), more frequently than mud balls, this suggests a predilection for lighter objects as play stimuli. Furthermore, they interacted with the movable Styrofoam sphere more than with any other sphere type, indicating a preference for spheres that are fixed in place rather than those possessing mobility. (Fig. 21)

	Number	Mass (g)	Average mass
Mud ball	3	2.233	0.744333333
Styrofoam ball	3	0.004	0.001333333

Fig. 20 The measured mass of Styrofoam spheres and Mud balls shown

Scale:	1.4878 pixles/mm					
	Average speed	STDEV	Average displacement	STDEV	Average instance	STDEV
Mun ball	0.018583333	0.012004243	2.65425	2.317434523	78.25	15.12754417
Fixed Styrofoam sphere	0.918333333	0.829200552	187.7828333	246.8078438	202.5	4.949747468
Movable Styrofoam sphere	7.460833333	2.217722568	1783.502167	540.3240974	80.66666667	5.656854249

Fig. 21 The movement distance, speed, and encounters of *F. cunicularia* workers have when exhibiting play-like behaviour against spheres of different kinds shown

5 individual ants were documented with hand-drawn movement trajectories indicating the path undertaken by each individual, with heatmaps and point maps recording the percentage of time each individual spent in each location. They devoted about 50% of their time to play-like behaviour triggered by spheres, while the remaining 50% was allocated to cleaning (both self-grooming and allogrooming), communicating with conspecifics, and resting. (Fig. 22 & Fig. 23)

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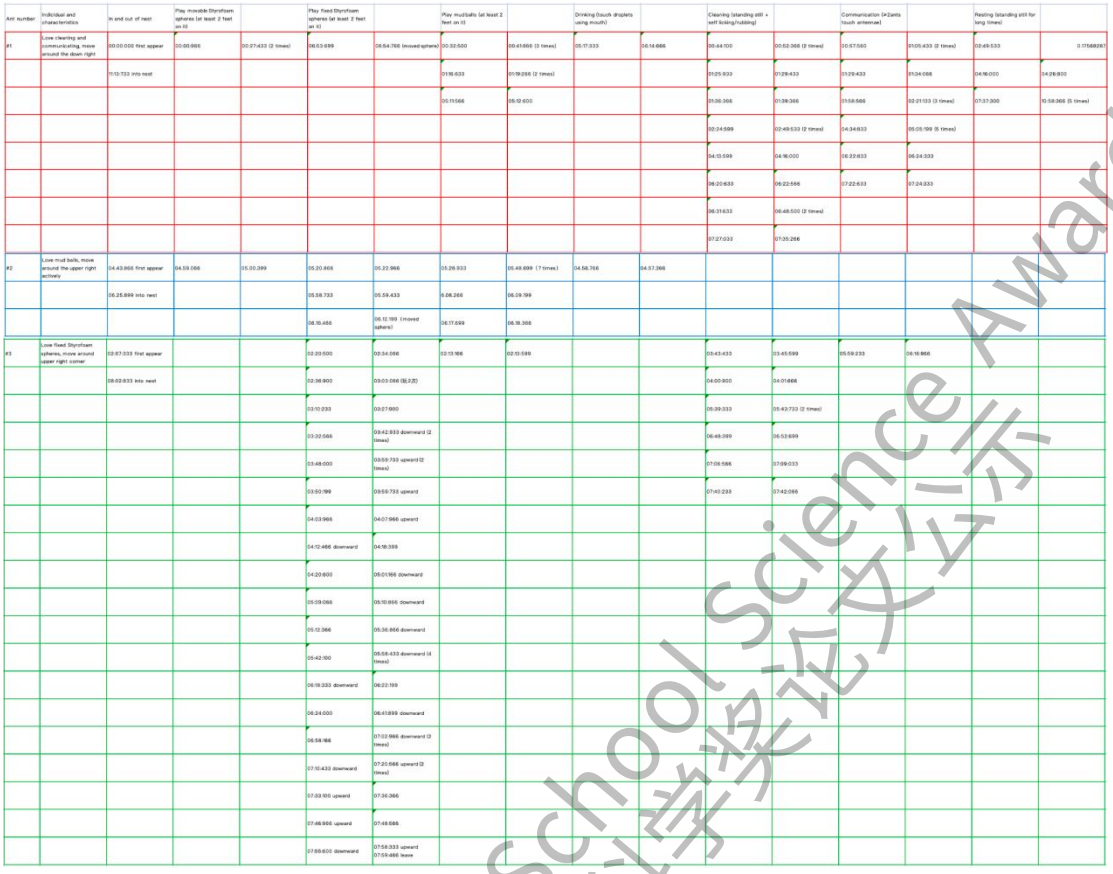


Fig. 22 Ant #1, #2, and #3 behaviour chart

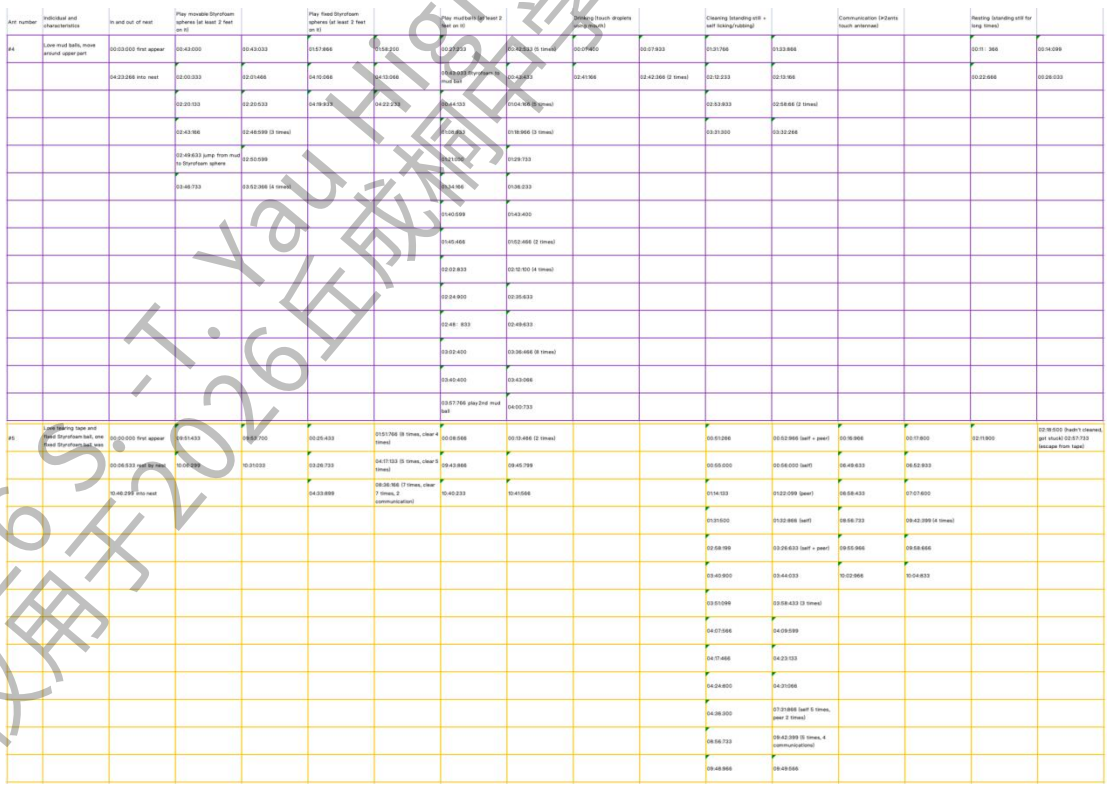


Fig. 23 Ant #4 and #5 behaviour chart

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Under the conditions of this metabolomics test, ant head samples were submitted to the E-ceshi center, where the metabolic difference between the playing group and the non-playing control group was investigated. The playing ant samples were frozen in liquid nitrogen only after 10-20 seconds of playing, which may lead to uncertainty that such brief stimuli are insufficient to elevate dopamine secretion in the ant brain to detectable levels. This assay concluded that there were no statistically significant differences in dopamine secretion between the playing group and the non-playing control group. The finding suggests that play-like behaviour in *F. cunicularia* does not originate from the single activation of dopamine secretion; individual sample variations, stress induced by instantaneous freezing and dissection, or an insufficient stimulation period could all influence and obscure the results of neuron activity and secretion.

An exploratory metabolomic comparison detected differences in dopamine-related measurements between the two groups, with the playing behaviour group's dopamine levels are 0.8-2.2 ng while the dopamine levels of the non-playing control group is 0.2-0.6 ng, but the data is highly scattered, and did not enquire significant scientific difference. Given the samples were limited in number and the playing group experienced only short periods of stimulus, this data may not have been sufficient to reveal the neuronal activity underlying play-like behaviour in response to spheres; therefore, this result should not be interpreted as direct evidence to prove that dopaminergic signaling is the main cause or effect of play-like behavior towards spheres. Further methodological improvements include: lengthening the period during which ants respond to the stimulus of play-like behaviour, ensuring adequate time prior instantaneous freezing and dissection; increasing the number of biological replicates; and repeating the experiment to elucidate deeper neuron regulatory mechanism underlying play-like behaviour. (Fig. 24)

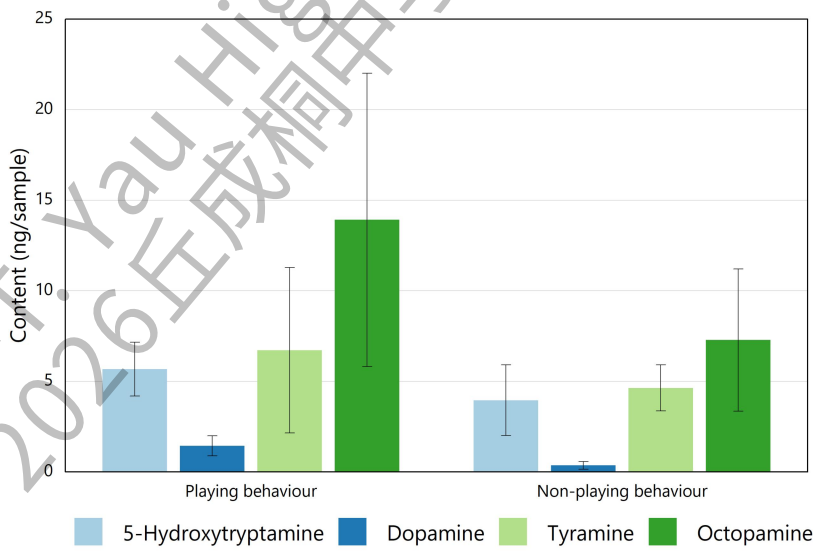


Fig. 24 Metabolomics test result of *F. cunicularia*, with playing behaviour group and non-playing control group

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Discussion

1.1 Summary of findings from three experimental tests

The study on division of labour demonstrated that: (i) under experimental conditions, *P. yeensis* workers exhibited complex divisions of labour, encompassing necrophoretic clearance, material translocation, and foraging behaviour; (ii) the ants exhibited a relaying tool translocation behaviour to increase efficiency; (iii) the ant raft building behaviour employed to attain food is elicited solely by the presence of honey water.

The study on the tool use behaviour demonstrated that: (i) under experimental conditions, *F. cunicularia* and *P. yeensis* workers were documented to exhibit tool use behaviour against the pressure of an artificial viscous surface, and are capable of manipulating the physical conditions of their surroundings to their own advantage; (ii) the ants exhibited a change in their objectives across different intervals of the translocation of sand particles.

The study on the play-like behaviour demonstrated that: (i) under experimental conditions, *F. cunicularia* workers showed play-like behaviour towards movable Styrofoam spheres; (ii) they exhibited a clear preference for lighter mass over heavier mass, and non-movability over movability; (iii) there was significant difference in dopamine-related measurements between the playing and non-playing groups, but there were high uncertainty and too less samples, therefor, this result should not be interpreted as evidence that dopaminergic signaling is the main cause or effect of the play-like behavior towards spheres.

The ant raft building behaviour of *P. yeensis* constitutes the first documented instance among all ant species of this behaviour occurring for a purpose other than the need of nest protection against floods. The play-like behaviour of *F. cunicularia* likewise represents the first documented instance among all ant species to exhibit play-like behaviour.

1.2 Applications and future outlook

This study documented a range of advanced behaviors in ants. Both *F. cunicularia* and *P. yeensis* exhibited objective tool-use behaviour, modification of substrates to obtain materials for their own needs, relaying material translocation, collective self-rescue by using an ant raft, and clearly differentiated social division of labour. Most notably, *F. cunicularia* engaged in play-like behaviour towards spheres under no survival pressure. As a form of non-utilitarian exploratory behavior not previously recorded, this offers a new case for understanding higher cognition in insects, specifically formicids.

These collective behaviors can serve as naturally occurring reference models for swarm robotics and bio-inspired multi-agent systems. Ants manage complex tasks such as path construction, raft building, and relaying translocation of materials without any central control, relying only on simple interactions among individuals, also known as swarm intelligence. This carries clear bio-mimicry implications for the design of distributed cooperative systems.

The play-like behaviour being documented also extends our understanding of the cognitive boundaries of insects. Given the constraints of our experimental setup, this study has limitations.

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Future work could include the comparision of behaviour between field and experimental conditional experiment to further examine the regulatory mechanisms behind these advanced behaviors.

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