CT Pre-Sampling Protocol

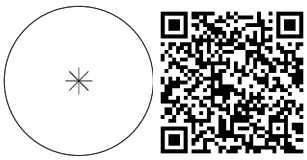
The goal is to determine if the ant species in your area perform or not cooperative transport (CT).

Preparation

Choose **3–5 ant species** from your area or laboratory colonies. For field species, **prioritize** those that have been identified at least to genus level; have been observed performing any type of food transport; are easy to find and have accessible nest entrances.

Assign each species to one of the following size categories:
→ **Small ant**: ~1–2 mm worker body length (e.g., *Wasmannia auropunctata*)
→ **Medium ant**: ~2–4 mm (e.g., *Linepithema humile*, *Solenopsis invicta*)
→ **Large ant**: >4 mm (e.g., *Camponotus sp.*, *Formica rufa or larger*)
If your ants fall near the boundary between two categories, choose the larger category.

For each species, print **5 copies** of the arena template matching its size category. Download the arena templates following the QR code.



Glue each arena template onto a rigid, smooth, unperforated cardboard surface matching the arena shape.

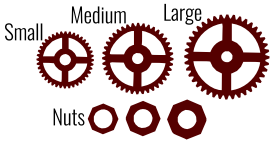
If you already know the preferred food of your target species, make sure you have 50–100 g available for the experiments.

If you do not know the preferred food of your target species, run this short food-preference test:
→ Place an arena next to a nest entrance.
→ Distribute on the arena small amounts of ham (1cm square), cheese (1cm square), cat food (1 unit), dry tuna (1 teaspoon), and egg (1 teaspoon). Distribute the food items evenly across the arena.
→ Wait 5 min for ants to recruit, then count the number of ants surrounding each food type.

Repeat the food-preference test **3 times**. Ideally, use a new nest entrance and arena for each trial, and rotate the position of the food items.
→**The preferred food** will be the food item surrounded with the highest number of ants across the trials. Use the preferred food in the following trials.

3D-printed cogwheels

Inside the CT KIT, you will find 3D-printed cogwheels matching the ant size categories.
→ **Small cogwheel**,
→ **Medium cogwheel**, and
→ **Large cogwheel**.



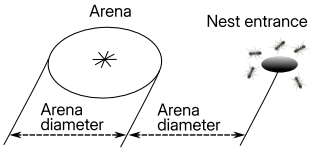
For each target species, use around **five** cogwheels matching the species size category.
You will also find **3-D printed nuts*** to adjust weight if necessary. Nuts are attachable to the cogwheel axis.

Cogwheel scenting

To make the 3-D printed materials attractive, place the cogs and nuts inside Ziploc bags in direct contact with the ant preferred food. Seal the bags and leave them for at least 2–5 h. For fresh food, keep the bags in the fridge.

CT behavior test

To examine CT behavior, place an arena approximately **one arena diameter** away from the nest entrance.



Fill in the metadata of the ***table sheet*** included in the CT Kit. Do not video record these tests.

Follow these two phases:
→ **Recruitment phase (5 min)**: pin a small piece (~1 cm square) of the preferred food on the arena "X" mark. Wait 5 min for ants to recruit.
→ **Transport phase (10 min)**: replace the food with the scented cogwheel and observe for 10 min. Follow the decision tree below.

Decision tree

If ants surround the cog wheel and successfully transport it outside the arena boundary:
→ Record a **successful CT trial** in the table sheet.
If ants cannot move or transport the cogwheel outside the arena boundary:
→ Record an **unsuccessful CT trial** in the table sheet.

Table sheets, a pencil, an eraser, pins, and tweezers are included in the CT Kit.

Run a total of 20 trials per species

Both successful and unsuccessful transport bouts count as trials.
Tip to increase sampling velocity: you should be able to run 2–3 of these cog wheel-testing trials in parallel using nearby nest entrances.

Reuse the cogwheels and arenas across trials. Just avoid using the same cogwheel and arena in consecutive trials.

If possible,
Sample across **4 or more nest entrances** and **3 or more days**.

Possible sources of confusion or error
→ Avoid using wet cogwheels. Let them dry for **5 min** after removal from the Ziploc bag.
→ *If one or two ants can easily move the cogwheel outside the arena, the item is probably not challenging enough to trigger CT. Add weight using the 3D-printed nuts and repeat the experiment.
→ If ants do not recruit to any cogwheel, improve scenting or try a different preferred food, then repeat the experiment.

Finalization

Once finished, take a photo of each completed ***table sheet*** and send them to andres.devegili@fu-berlin.de. For any issues, write to the same email.

Enjoy the sampling!