Route improvement by tandem-running ants

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Abstract

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Protocol

Materials and method

Step 1.

We collected the 12 experimental colonies of the ant *T. albipennis* from the Dorset coast, England, UK on 14thMarch 2013. No specific permissions were required for sampling activities at this location because it is a disturbed quarry habitat and ants are not considered as endangered or protected species. The experiments commenced on 15th April 2013. In the laboratory the colonies were housed in standard nests consisting of a cardboard perimeter, sandwiched between two microscope slides (75 × 50 mm). The cardboard formed an inner nest cavity measuring (50 × 35 x 2 mm), with a 2-mm-wide entrance. The colonies had *ad libitum* access to water, honey solution and *Drosophila* flies, which were replenished weekly.

Materials and method

Step 2.

The experiments involved two treatments: a landmark-rich (L-R) and landmark-poor (L-P) environment. Under L-R, the ants were allowed to emigrate to a new nest and to navigate under the usual lab environment. The windows on the left side of the arena and the experimental arena itself were not covered so that the ants had access to celestial cues as well as all the prominent landmarks present around the arena such as computers behind the old nest and lab instruments on the right side of the arena. Under L-P, the ants were allowed to emigrate and navigate under manipulated lab conditions whereby the windows were covered with blinds and the arena surrounded with white muslin cloth thus barring access to celestial cues and prominent landmarks. Ants were not familiar with the landmarks around the arena because colonies were placed in a different area of the lab and were only brought to the experimental arena prior to the start of an experimental trial. The ant colonies were kept in square (100mm) petri dishes and cardboard was placed on top of colony nests to make them dark between experimental trials.

Experimental protocol

Step 3.

At the start of each trial for each of the L-P and L-R treatments, the old standard nest (ON) was destroyed to induce emigration. A new standard nest (NN) was placed 800 mm away from the front of the ON. When a tandem run began, the other remaining ants in the arena were removed and placed in a separate Petri dish. The ON was temporarily isolated by placing a Petri dish lid over it to prevent other ants making contact with the tandem pair. The tandem pair was tracked with a motorized gantry [29] until they reached the new nest (Fig 1, L-tr). We recorded the tandem run and either the returning leader or the returning follower alternately for each tandem run. During a tandem run, the gantry tracked the leader and follower ants simultaneously with the coordinates for each ant recorded in separate columns. Hence, we could determine which coordinates belonged to the leader and which belonged to the follower. We matched the return path for the leader or follower with the respective tandem-run path.

Experimental protocol

Step 4.

If the leader was tracked on the return trip, after the pair had reached the NN, we removed the follower when there was a gap between the two ants. This was done carefully so as not to disturb the leader. The leader ant was left to access the NN alone and was tracked on its return trip to the ON (Fig 1, L-rtn). Recording started from the NN entrance until the returning ant had reached the perimeter of the Petri dish that covered the ON. The tracked ant was then removed from the arena and kept in a separate Petri dish (as was the follower) until the end of the trial. The

Petri dish cover over the ON was lifted again to allow another tandem run to form.

Experimental protocol

Step 5.

If the follower was tracked on the return trip, the ants were tracked during tandem running from the ON until they reached the NN (Fig 1, F-tr). When the pair had reached the NN, the leader ant was removed and only the former follower ant was allowed to access the NN. After that, the former follower ant was tracked as it returned from the NN back to the ON (Fig 1, F-rtn). Then it was removed from the arena and kept in a separate Petri dish until the end of the trial.

Experimental protocol

Step 6.

The removal of the tracked ants from the arena and their isolation from the colony for the duration of an experimental trial avoided pseudoreplication. We tracked an ant (leader or follower) during her tandem run and return trip only once. On the scale of a 1000 x 1000 mm arena it is virtually impossible to follow individually marked ants that are 2-3 mm long and track them again in subsequent tandem and return trips. On this scale it is also difficult to keep track of more than one individual once they have reached and entered the ON.

Experimental protocol

Step 7.

After the end of each trial, the isolated ants were reunited with their colony and the arena floor was cleaned using alcohol and water to remove any traces of pheromone trails.

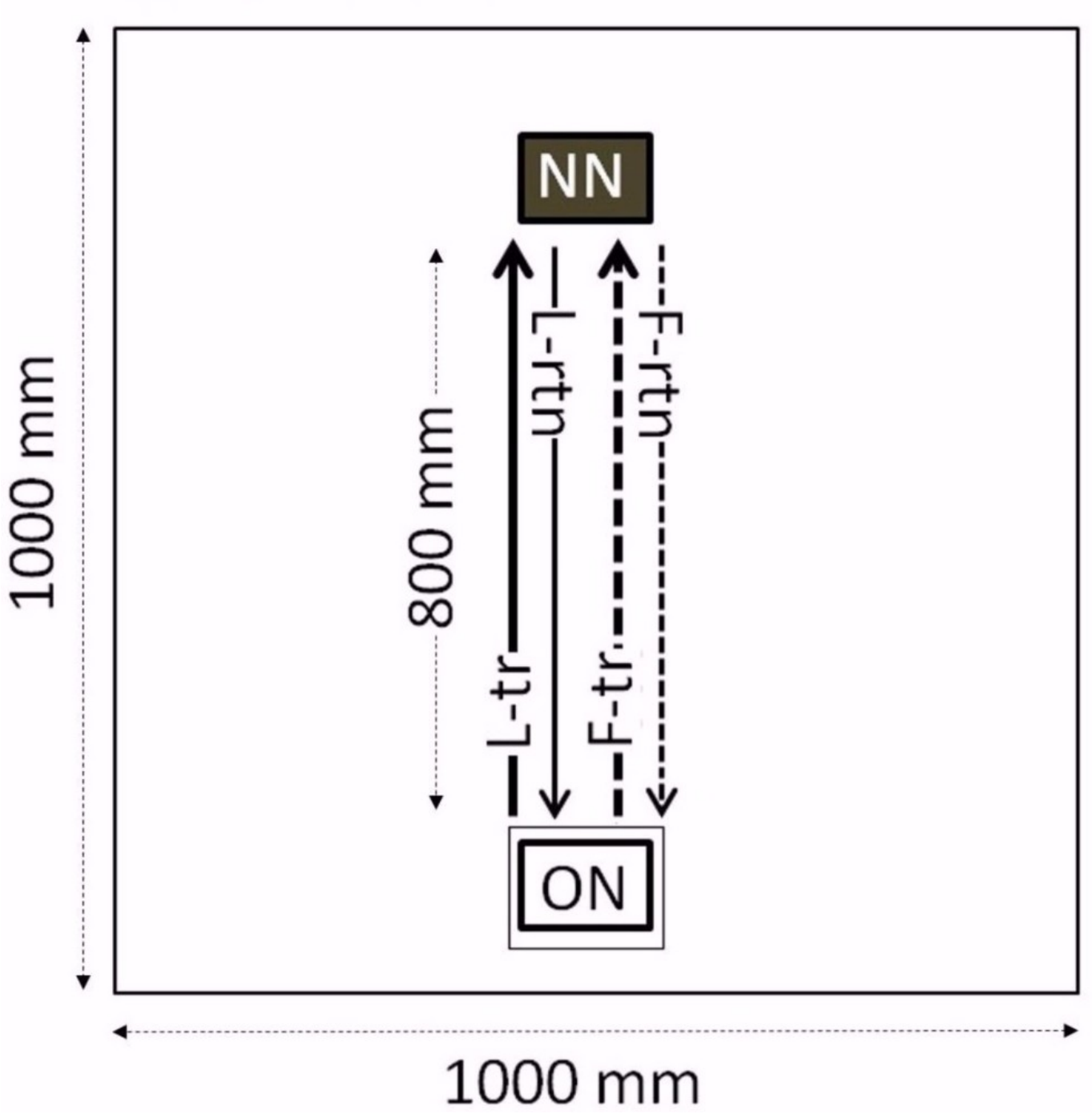
Experimental protocol

Step 8.

The L-R treatment was performed first on all 12 colonies and a day after it was completed the L-P treatment was performed on the same colonies. This was done to keep the environmental set-up as similar as possible for all trials under the L-R treatment. The 12 colonies were used alternately throughout a treatment with at least a 6-day gap [40] before the same colony was used again for another trial. Each colony was used twice, at most, throughout the experiment. We recorded tandem run data from each of the 12 colonies under at least one of the two treatments but not under both treatments because in some trials colonies either did not emigrate to the new nest or the tandem runs broke off before they reached the new nest. Overall, we analysed data from 10 trials with 10 colonies under the L-R treatment and 11 trials with eight colonies under the L-P treatment. In total, 64 pairs of tandem-run and return paths were recorded: 15 for leaders and 17 for followers under the L-R treatment and 15 for leaders and 17 for followers under the L-P treatment.

Experimental protocol

Step 9.



Experimental protocol

Step 10.

Fig 1. Experimental set-up.

The set-up was the same for the landmark-rich (L-R) and the landmark-poor (L-P) treatments. Experiments were conducted in a 1000 × 1000 mm arena. The new nest (NN) was placed 800 mm away from the front of the old nest (ON). The rectangular box around the ON depicts the Petri dish used to cover the ON during the tracking of each tandem run. L-tr: leader's tandem run path; L-rtn: leader's return path; F-tr: follower's tandem run path; F-rtn: follower's return path. The return paths of former leaders and followers were tracked with a motorized gantry in alternate tandem runs. During the L-P treatment, the arena was surrounded with a white muslin cloth to bar access to celestial cues and external landmarks.