

Live-in if you must: density-dependent nest-sharing between two competitive ant species

Linepithema humile (Mayr, 1868), commonly known as the Argentine ant, qualifies as one of the '100 of the world's worst invasive alien species'¹. *Tapinoma nigerrimum* (Nylander, 1856), commonly known as the odorous ant, is native to the Mediterranean coast and has parapatric distribution with the invading *L. humile*². To study the behaviour of incipient colonies of an invasive species in the presence of a native competitor, we carried out density-dependent competition experiments. While numerous examples of nest-sharing between ants, as well as ants and other invertebrates exist³, an aggressive global invader like *L. humile* has not been reported to display nest-sharing with other species. This note describes unusual and novel behaviour between *L. humile* and *T. nigerrimum*, and reports the extent of behavioural flexibility in *L. humile* under adverse conditions, especially when it is numerically inferior. Several colonies of *L. humile* and *T. nigerrimum* were collected near Marseille, France, and were transported to a laboratory facility in Université Paris-Sud, Orsay, France. All experiments were carried out between April and September 2013 and typically lasted for 60 days or till the colonies of both species perished, whichever occurred first. Each experiment was carried out with either 30 or 300 workers, and three queens of the two species. The colonies were provided a synthetic food source⁴. Each experimental set-up consisted of two nesting arenas and two foraging arenas, one for each species, separated by a common interaction arena in the middle (Figure 1a). We kept the interaction arena closed for the first five days and then opened it so that the two species could interact. Queen and worker survival, brood production and behaviour of both species were recorded daily between day 1 and day 10, and every fifth day thereafter, till the end of the experiment. In total, 18 experiments of the 4 different colony size-based categories (*L. humile* workers – *T. nigerrimum* workers, 300-300: $n = 5$, 300-30: $n = 3$, 30-300: $n = 5$ and 30-30: $n = 5$) were carried out.

In 15 out of 18 experiments, one species quickly emerged as a clear winner and had killed all workers, queens and

brood of the opponent and had monopolized the complete experimental set-up, including its foraging and nesting arenas. The identity of the species that monopolized the arena depended on its absolute and relative group sizes. Quite unusually, however, in three out of five replicates of 300-300, although both species showed aggressive interactions, neither succeeded in complete monopolization. Both species survived, and stayed in their own arenas, with gradually decreasing

aggressive interactions between them, and the workers of the two species showed steady presence in the nesting arenas of each other. After a few days, *L. humile* queens had moved to the *T. nigerrimum* nesting arena. This was met with no aggression or resistance from *T. nigerrimum* queens or workers. Queens and workers of both species interacted, but these now consisted of non-aggressive behaviours such as antennation, without display of escape or evasive

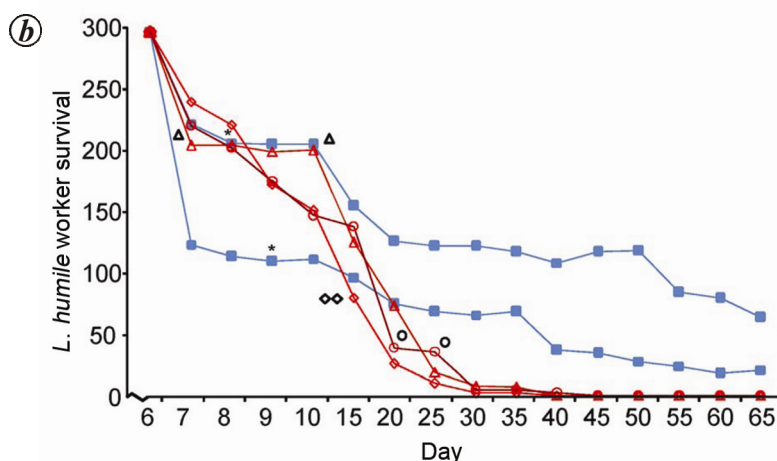
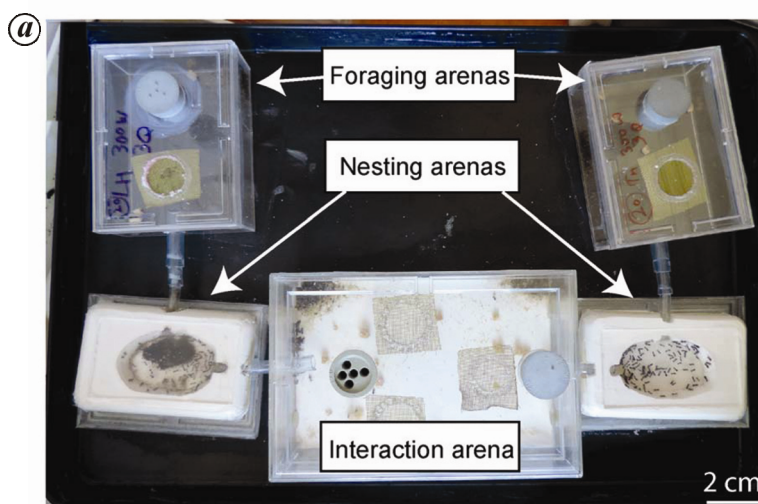


Figure 1. a, Experimental set-up. b, *Linepithema humile* worker survival for the entire experimental duration. The lines with filled squares represent replicates where *L. humile* exterminated *Tapinoma nigerrimum*. Asterisks represent the days on which *T. nigerrimum* colonies were decimated in the two replicates. The three replicates where nest-sharing was observed are represented by lines with hollow markers. The number and position of the hollow symbols alongside the lines depict the number of queens (one symbol per queen) and the day on which they moved into *T. nigerrimum* nests. Note that only six out of nine queens relocated in the three replicates because one queen per replicate was already dead by then.

behaviour from either species. While all its workers died over the next few weeks, the queens of *L. humile* survived for more than 100 days. Figure 1b provides details on worker survival and movement of *L. humile* queens to *T. nigerrimum* nests. Though the figure shows data only for 65 days, we maintained the replicates that showed nest-sharing. In these replicates we found *L. humile* queens surviving for more than 100 days in *T. nigerrimum* nests, even without a single *L. humile* worker.

To the best of our knowledge, there are no previous reports on the non-aggressive, non-evasive, conflict-free coexistence and co-nesting of *L. humile* inside the nest of another ant species. This is in contrast with the characteristic highly aggressive behaviour of *L. humile* workers, which usually leads to them emerging competitively superior against other species². This coexistence is also different from the escape and evasive behaviour shown by *L. humile*, when in small numbers, against other ant species⁵. Results seem to suggest a survival mechanism in an extreme condition when the worker population of *L. humile* had dwindled to a small number. *L. humile* queens that had moved to the *T. nigerrimum* nests survived for several weeks even without a single worker. This is unusual since *L. humile* queens suffer 75–100% mortality without their workers⁶. Co-nesting and coexistence as seen here, therefore, could be a desperate and risky survival strategy on part of *L. humile* queens. Absence of *L. humile* workers indicates that either *L. humile* queens did not reproduce in *T. nigerrimum* nests, or the eggs laid by them did not survive. This also indicates a survival and reproductive dead-end for *L. humile* queens, unless co-habitation was used as a temporary strategy.

The simplest explanation might be that *T. nigerrimum* could not recognize and differentiate between their own and *L. humile* individuals owing to factors such as laboratory conditions and the same diet, which could have led to similar cuticular hydrocarbon profiles. However, lack of nestmate recognition seems a less plausible reason because high aggression was still observed during the first few days in these three replicates. Moreover, in many simultaneously running replicates, species recognition was observed.

Though co-habitation between other ants, as well as ants and invertebrates has been reported before (for example, mixed ant nests, parasitic ants, lycaenid butterflies in ant nests, myrmecomorphic spiders and other myrmecophilous organisms in ant nests)³, to the best of our knowledge, coexistence of a global invader such as *L. humile* with other ants has not been reported either in the laboratory or in the field. Detailed investigations into scenarios under which such rare behaviours are displayed should be conducted. More broadly, such studies may give important insights into the novel mechanisms employed by an invasive species to survive under unfavourable conditions.

Our experiments comply with the Association for the Study of Animal Behaviour/Animal Behaviour Society Guidelines for the Use of Animals in Research, and with regulations of animal care in France.

The authors declare no conflict of interest.

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Nine million-year-old ape-like fossils found at Haritalyangar, India

The Middle Siwalik sediments exposed at Haritalyangar (31°32'N, 76°38'E), India are best known for the diversity of fossil primates that no longer inhabit the Sub-Himalaya, including the late surviving¹ large hominoids *Sivapithecus* and *Indopithecus*¹ as well as primitive lemuriform primate, such as *Indraloris* and *Sivaladapis*. However, one poorly known

species of primate from Haritalyangar is represented by an isolated and heavily worn upper third molar found in the 1970s. The specimen was initially identified as *Pliopithecus krishnaii*², but was later transferred to a new genus *Krishnapithecus*^{3,4}. No additional finds of this enigmatic primate have been made in the intervening four decades, and as a result

its place in primate evolution has been difficult to determine. Recently, two lower molars, consistent in size and morphology to *Krishnapithecus*, have been discovered at Haritalyangar. If they prove to belong to the same species, they will offer important clues to clarify its taxonomic status and understand its evolutionary relationships. Preliminary