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# Effect of Starvation on the Feeding Activity Toward Sugary Food in the Ant *Tapinoma nigerrimum* (Nylander, 1856)

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**Abstract**

In this study, we investigated the effect of starvation on the feeding behavior of the ant *Tapinoma nigerrimum*. In particular, we tested the response of ants that had experienced different levels of starvation, toward sucrose solutions of increasing concentration. As expected, starved ants promptly reacted to the sugary food sources with a higher rate of acceptance as compared to satiated ones. Acceptance increased both with sugar concentration and the length of the starvation period. However, a consistent fraction of the starved ants did not feed on the solutions, suggesting that starvation had different effects on different individuals, even though they all had food *ad libitum* before the beginning of the tests, had comparable body sizes, and were collected from the same trail. The different acceptance of sugary solutions may be, therefore, merely because ants fed on the experimental food at different times. Interestingly, in all the experimental groups, ants appeared to satiate quickly, irrespective of the solution tested and fasting duration. This would suggest that the rate of ingestion was independent of these factors, a result partially at odds with previous studies. This study is one of the few ones dealing with the behavioral response of an ant species to a famine event.

## Introduction

Finding enough food is a central challenge for all the organisms. In social insects, the amount of collected food must counterbalance both individuals and collective requirements, following the 'altruistic' rules dictated by the inclusive fitness (Abbot et al., 2011). Escaping from starvation is, therefore, a collective concern that may deeply affect the foraging behavior of ants, since food shortage may be a serious threat for the whole colony. When resource supply is low, ant colonies may put in place collective behaviors to counterbalance food shortage, as it happens in *Lasius niger*, in which the number of scouts that spontaneously leave the nest even in the absence of chemical or tactile signals increases in times of famine (Mailleux et al., 2010). Starvation may

negatively affect a colony's fitness in many ways, which range from subtle metabolic impairment to direct foragers death. In *Aphaenogaster picea*, for example, starvation reduces individual thermal tolerance (Nguyen et al.,2017), while in *Formica exsecta*, protracted food shortage affect the tissue-specific gene expression and impairs the efficiency of the tissues involved in the degradation of bacterial cell walls, melanization, and the encapsulation response (Bos et al., 2016). All these effects may, in turn, elicit cascade effects as decreased brood and nest hygiene (Dussutour et al., 2016), or unbalance interspecific interactions and facilitate the success of the species more resistant to the lack of food, as observed in *Linepithema humile* (McGrannachan & Lester, 2013). Although many details of the ecological and behavioral responses of ants to food shortage have been studied in the

past, such as the inter-caste interactions (Mailleux et al., 2011a), the spatial arrangement of the colony (Mailleux et al., 2011b), the interspecific aggressiveness (Liu et al., 2011), the exploration efficiency (Mailleux et al., 2010), and even the communication (Mailleux et al., 2006), the short-term behavioral responses to food resources received, surprisingly, little or no attention.

Tapinoma nigerrimum (Nylander, 1856) is a dominant, polydomous, usually polygynous, and even potentially invasive ant species widespread along the Mediterranean basin and a significant part of Europe (Blight et al., 2014; Dekoninck et al., 2015). This species is widespread in anthropic habitats, and its nest holes can be easily found in gardens, or along the crevices of sidewalks. It has been assumed that similarly to T. sessile (Buczkowski & Bennett, 2008) this species can limit the expansion of the invasive argentine ant (L. humile), because of its supposed strong ecological competitiveness (Blight et al., 2010, but see Frizzi et al., 2017). Moreover, this species is highly tolerant to high temperatures (Frizzi et al., 2017) but seems to be sensitive to food shortage as six days without food appear sufficient to cause the death of the majority of workers (unpublished observation). As in many other ant species, carbohydrates are a fundamental resource for the survival of T. nigerrimum colonies, and they are usually obtained from mealybugs honeydew (Cerdá et al., 1997; Mansour et al., 2012). Carbohydrate availability may affect individual behavior (e.g. aggressiveness, Grangier & Lester, 2014), colony size, and survival (Wittman et al., 2018), and their importance is supported by the strong preference for this resource shown by many dominant species (e.g. Grover et al., 2007; Abril et al., 2007; Frizzi et al., 2016).

With this preliminary laboratory study, we aimed to assess the short-term behavioral effect of starvation on T. nigerrimum, in terms of acceptance of sugary solutions of increasing concentration. In particular, we observed the behavior of groups of ants in the first few minutes after the end of starvation and we expected that the acceptance of a solution changes with its sugar concentration and starvation duration. To our knowledge, this is one of the few studies dealing with the early effects of the starvation on the foraging behavior in ants.

**Materials and methods**

The experiments were carried out from June to August 2014 within the Sesto Fiorentino University Campus, central Italy (43°49'05.05"N, 11°12'12.95"E). The sampling area is interspersed in an urban matrix, made up by University buildings surrounded by overgrown fields, public gardens with a few ornamental trees (Acer sp.), and traversed by low-traffic roads. Ants were collected from a paved lot (side 200m), where a total of 31 nest holes were counted. We randomly selected six nest holes, separated by at least 6 m from each other. As T. nigerrimum may form large super colonies (Dekoninck et

al., 2015), the likely non-independence of nest holes was taken into account in the statistical analyses. Since T. nigerrimum shows considerable polymorphism and body size can affect both individual survival (Kaspari & Vargo, 1995; Heinze et al., 2003) and the amount of food ingested, particularly carbohydrates (Josens et al., 2018), we took care to collect only major workers having a similar size. At the end of each experiment, ants were measured under a stereomicroscope, to exclude the presence of outliers (total body length = 3.28 ± 0.21SD mm).

In a single sampling event, we collected 300 ants from trails starting from a randomly selected nest hole. We repeated this procedure for each of the six nest holes. Trails were used by the ants to exploit the honeydew of mealybugs infesting the trees bordering the lot, so we took care to capture only unfed individuals, i.e. those walking in the direction of trees. Ants were housed in a plastic container (15 x 25 x 15 cm) with Fluon® coated walls and acclimated to laboratory conditions for three days with 25 g/L sucrose solution and water available ad libitum to empty the gut of the ants (Frizzi et al., 2017). After this acclimation period, we removed the sucrose solution only, and we collected five groups of 10 ants each (hereafter called SA, satiated ants), which were placed in a neutral arena (a 9 cm diameter Petri dish with Fluon® coated walls). After two minutes, a droplet of water or one of three different sucrose solutions (2, 4 and 16% weight/volume, expressed in g/L, following Frizzi et al., 2016) was released in the center of each dish (Fig 1). Droplets were sufficiently large so that all the tenants could drink at the same time. Two minutes later we started assessing the acceptance, measured as the proportion of drinking ants within a period of 15 seconds. We repeated this measure every two minutes for each group of ants, until a total time of 14 minutes and 15 seconds had elapsed. An ant touching the droplet with the mandibles was scored as drinking the solution only if contact lasted for more than 2 seconds. If the same ant drank more than one time during the 15 seconds of the observation, it was scored only once. To reduce the error probability, each trial was observed by at least two operators. At the end of each test, the ants were measured and released. The test was repeated after 48 and 96 hours (hereafter called SS, short starvation, and LS, long starvation, respectively). The length of the starvation period was chosen on the basis of previous observations, showing that T. nigerrimum ants well survived without access to food for at least 5 days. Water was always available to all ants for the entire duration of the experiments. The 14 minutes duration of the observations was decided on the basis of preliminary tests, showing that after this time both in starved and non-starved ants ceased to feed. To do this, we performed individual tests with four groups of fed (10 ants per group) and four groups of starved ants (four days of fasting). To each group we supplied a 16% sucrose solution and we counted every two minutes the number of ants feeding (over 15 seconds), for a total period of 60 minutes. After 14 minutes, only one fed ant and two starved ants drank the solution at most.
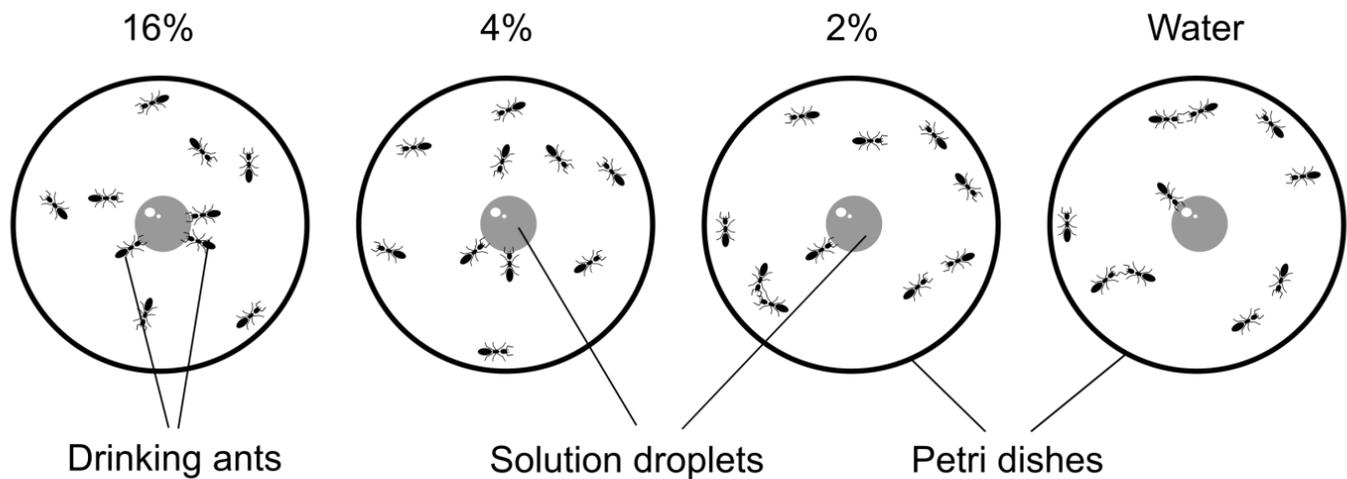
**Fig 1**. Experimental apparatus, which was used for all the three levels of starvation, satiated (SA), short starvation (SS), and long starvation (LS).

To quantify how acceptance changes over the testing time for satiated (SA) and starved ants (SS and LS), we fitted, for each of the sucrose solutions tested, five Generalized Linear Mixed Models (GLMM) of increasing complexity, with binomial distribution. These models were: the null model, two models including only one factor (level of starvation or testing time), a model including both factors, and finally a complete model including both factors and their interaction. We included the nest identity as a random factor to account for non-independence of nestmates. Models were ranked using the Akaike's Information Criterion corrected for small size samples (AICc). To assess the differences in the acceptance between the differently starved groups in pairs, we performed Tukey post-hoc multiple comparisons. To focus the acceptance change over time according to starvation, for all the observation times we compared the number of ants drinking among starvation levels using the Kruskal-Wallis test, followed by Dunn's non-parametric post-hoc test. Analyses were performed using the 'lme4' (Bates e tal., 2015), 'AICcmodavg' (Mazerolle, 2017), and 'multcomp' (Hothorn et al., 2008) R 3.5.1 (R Core Team 2018) packages.

## Results

The main result of the experiments is shown in Figure 2, which reports the temporal trends of acceptance of the four solutions tested, for the three groups of ants (SA, SS, and LS).

Results of model ranking and multiple comparisons are reported in Table 1 and 2, respectively. Water, was almost never accepted by ants, irrespective of starvation level, and no model performed better than the null one. When we analyzed the response to 2, 4 and 16% solutions, the best fitting model was always the full one, containing the length of starvation, time and their interaction as explanatory variables. In these cases, acceptance was always low or null for satiated (SA) ants, whereas for the 4 and 16% solutions both groups of starved ants showed a significantly higher acceptance. Acceptance was also higher at the beginning but rapidly declined as time goes by. For the 2% solution, LS ants showed higher acceptance than either SS and SA (SS = SA, Table 2). In Table 3, all mean values of drinking ants and the results of Kruskall-Wallis and Dunn's tests are summarized.

**Table 2**. Results of multiple comparisons in the acceptance rate between differently starved groups in pairs. Percentages are weight/volume (g/L). SA = satiated ants; SS = short starved ants; LS = long starved ants. In bold significant p values.

| Contrast | 2% | | 4% | | 16% | |
|---|---|---|---|---|---|---|
| | z | p | z | p | z | p |
| SA - SS | 1.672 | 0.212 | 3.332 | **0.002** | 4.385 | **<0.001** |
| SA - LS | 4.448 | **<0.001** | 4.575 | **<0.001** | 4.821 | **<0.001** |
| SS - LS | 3.356 | **0.002** | 1.592 | 0.244 | 1.439 | 0.297 |

**Table 1**. AICc values of models ranked. In bold the significantly lowest AICc value. ΔAICc is the difference from the lowest value. Percentages are weight/volume (g/L). Starv = Starvation level (SA, SS, and LS). Time = time elapsed from the beginning of the test.

| Model | Water | | 2% | | 4% | | 16% | |
|---|---|---|---|---|---|---|---|---|
| | AICc | ΔAICc | AICc | ΔAICc | AICc | ΔAICc | AICc | ΔAICc |
| Null | 437.34 | 0 | 826.73 | 41.62 | 842.64 | 50.815 | 995.94 | 150.85 |
| Starv | 438.95 | 1.61 | 817.92 | 32.81 | 825.50 | 33.672 | 963.46 | 118.37 |
| Time | 438.92 | 1.58 | 798.24 | 13.13 | 813.32 | 21.496 | 891.49 | 46.40 |
| Starv + Time | 440.52 | 3.18 | 789.11 | 4.00 | 795.61 | 3.79 | 855.83 | 10.74 |
| Starv * Time | 439.23 | 1.89 | **785.11** | 0 | **791.82** | 0 | **845.09** | 0 |
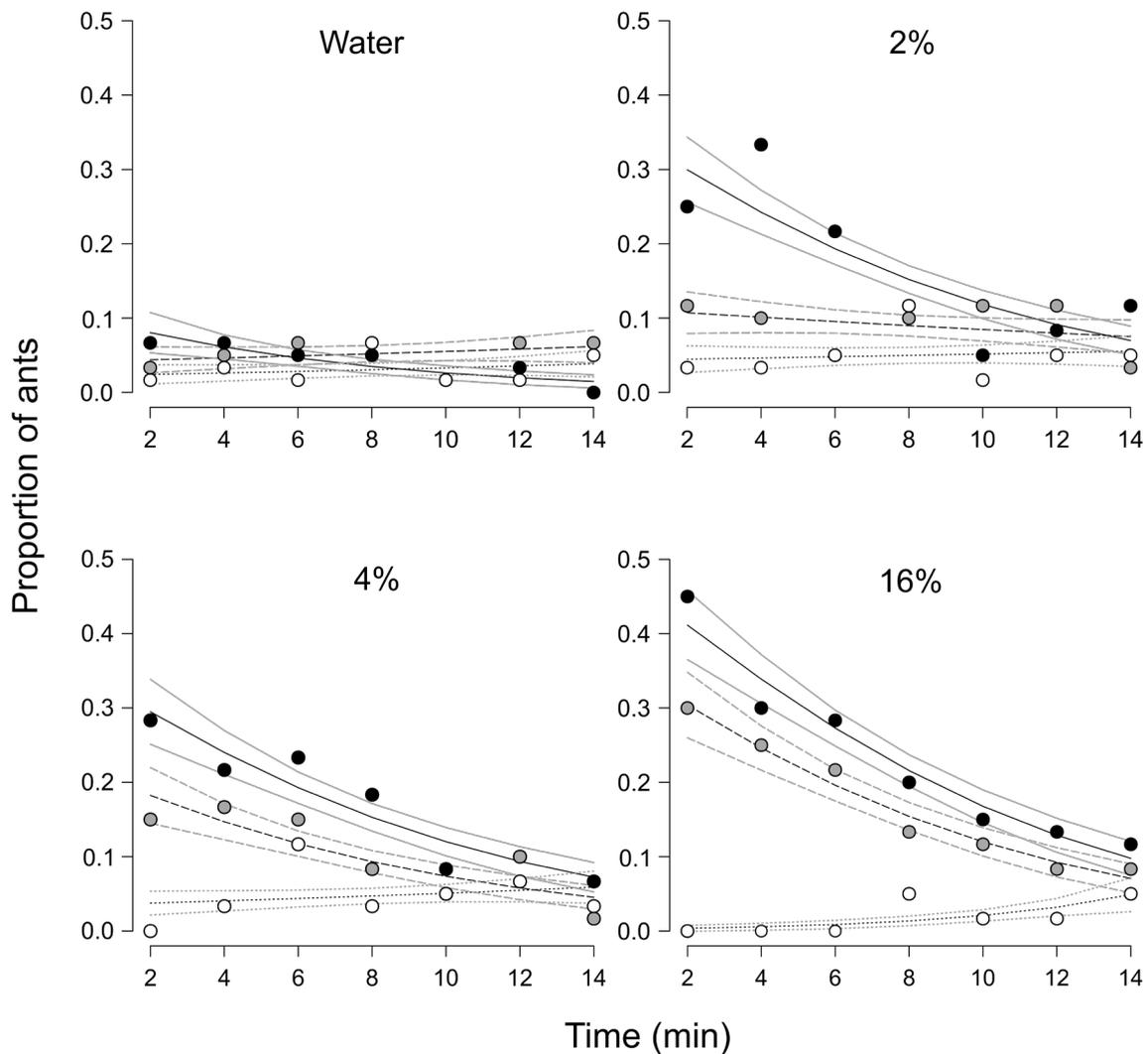
**Fig 2**. Proportion of drinking ants for water and all the three sucrose solutions tested over the experimental time, with their models fitted (black lines) and the standard errors of predicted values (grey lines). Percentages are weight/volume (g/L). Dotted lines, white circles = satiated ants (SA); dashed lines, grey circles = short starved ants (SS); Solid lines, black circles = long starved ants (LS). In Table 2 results of model comparisons are reported.

## Discussion

As expected, the starved groups reacted quickly and started feeding, with concentration-dependent acceptance rates, while fed ants showed no interest for any of the test solutions. However, the results also showed unexpected behaviors, which are worth examining. First, the acceptance rate by starved ants was not as high as we expected, as they did not exceed 0.5 even in long starved ants on the most concentrated solutions. It is quite surprising if compared to a previous field study on the acceptance of different food sources for the same sucrose solutions concerning the Mediterranean ant *Crematogaster scutellaris*, which showed a level of acceptance ranging from 0.6 to 0.8 (Frizzi et al., 2016). Although this species belongs to a genus evolutionarily quite far from *Tapinoma* and it likely has different nutritional needs, in absence of other previous studies regarding *Tapinoma* these values can be taken as a general reference, because both species can be usually found in mild/

hot climates and urban contexts, thus climate conditions and, partially, feeding opportunities in sites they inhabit are similar. However, the possibility that the difference in the low level of acceptance of *T. nigerrimum* compared to *C. scutellaris* was completely due to the different biological features between these two species cannot be ruled out. Nonetheless, other environmental factors may have a role. For example, the effect of water shortage and dehydration, which were effective in Frizzi et al. (2016) study, but not here. The fact that not all ants promptly started consuming the sucrose solutions needs some further investigations, as its determinants are not entirely clear. This pattern could, in principle, be explained hypothesizing that not all ants in a group (and hence subjected to the same fasting period), shared the same level of hunger. In an ant colony, the inter-individual response to food shortage may be determined by several endogenous factors, such as the difference in morphology, the different allocation of fat bodies among nestmates, the physiological status, or age

**Table 3**. Mean number of ants drinking at solution droplets (± SE), for each concentration and at each time (in minutes), and the results of Kruskall-Wallis and Dunn's tests. KW is the significance of the result of the Kruskall-Wallis global test. Contrasts concern the Dunn's test. SA = satiated ants; SS = short starved ants; LS = long starved ants. Significance levels: 'ns' not significant, '*' < 0.05, '**' <0.01, '***' <0.001.

| Time | Water SA | | | SS | | | LS | | | KW | Contrasts SA-SS | SA-LS | SS-LS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.167 | ± | 0.167 | 0.333 | ± | 0.211 | 1.167 | ± | 0.477 | ns | - | - | - |
| 4 | 0.333 | ± | 0.211 | 0.500 | ± | 0.224 | 0.667 | ± | 0.333 | ns | - | - | - |
| 6 | 0.167 | ± | 0.167 | 0.667 | ± | 0.211 | 0.500 | ± | 0.227 | ns | - | - | - |
| 8 | 0.667 | ± | 0.494 | 0.667 | ± | 0.211 | 0.500 | ± | 0.227 | ns | - | - | - |
| 10 | 0.167 | ± | 0.167 | 0.167 | ± | 0.167 | 0.167 | ± | 0.167 | ns | - | - | - |
| 12 | 0.167 | ± | 0.167 | 0.667 | ± | 0.333 | 0.333 | ± | 0.211 | ns | - | - | - |
| 14 | 0.500 | ± | 0.224 | 0.667 | ± | 0.333 | 0 | | | ns | - | - | - |

| Time | 2% SA | | | SS | | | LS | | | KW | Contrasts SA-SS | SA-LS | SS-LS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.333 | ± | 0.211 | 1.167 | ± | 0.477 | 2.500 | ± | 0.671 | * | ns | ** | ns |
| 4 | 0.333 | ± | 0.333 | 1.000 | ± | 0.365 | 3.333 | ± | 0.614 | *** | ns | *** | * |
| 6 | 0.500 | ± | 0.341 | 0.500 | ± | 0.341 | 2.167 | ± | 0.601 | * | ns | * | * |
| 8 | 1.167 | ± | 0.401 | 1.000 | ± | 0.365 | 1.167 | ± | 0.477 | ns | - | - | - |
| 10 | 0.167 | ± | 0.167 | 0.167 | ± | 0.307 | 0.500 | ± | 0.341 | ns | - | - | - |
| 12 | 0.500 | ± | 0.224 | 1.167 | ± | 0.401 | 0.833 | ± | 0.307 | ns | - | - | - |
| 14 | 0.500 | ± | 0.224 | 0.333 | ± | 0.333 | 0.167 | ± | 0.307 | ns | - | - | - |

| Time | 4% SA | | | SS | | | LS | | | KW | Contrasts SA-SS | SA-LS | SS-LS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0 | | | 1.500 | ± | 0.563 | 2.833 | ± | 0.872 | ** | * | ** | ns |
| 4 | 0.333 | ± | 0.333 | 1.667 | ± | 0.558 | 2.167 | ± | 0.601 | ns | - | - | - |
| 6 | 1.167 | ± | 0.308 | 1.500 | ± | 0.562 | 2.333 | ± | 0.803 | ns | - | - | - |
| 8 | 0.333 | ± | 0.333 | 0.833 | ± | 0.654 | 1.833 | ± | 0.307 | * | ns | ** | * |
| 10 | 0.500 | ± | 0.341 | 0.500 | ± | 0.223 | 0.833 | ± | 0.307 | ns | - | - | - |
| 12 | 0.667 | ± | 0.422 | 1.000 | ± | 0.516 | 1.000 | ± | 0.258 | ns | - | - | - |
| 14 | 0.333 | ± | 0.333 | 0.167 | ± | 0.167 | 0.667 | ± | 0.333 | ns | - | - | - |

| Time | 16% SA | | | SS | | | LS | | | KW | Contrasts SA-SS | SA-LS | SS-LS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0 | | | 3.000 | ± | 0.931 | 4.500 | ± | 0.341 | *** | ** | *** | ns |
| 4 | 0 | | | 2.500 | ± | 0.428 | 3.000 | ± | 0.894 | ** | ** | ** | ns |
| 6 | 0 | | | 2.167 | ± | 0.601 | 2.833 | ± | 0.601 | ** | ** | *** | ns |
| 8 | 0.500 | ± | 0.341 | 1.333 | ± | 0.494 | 2.000 | ± | 0.817 | ns | - | - | - |
| 10 | 0.167 | ± | 0.167 | 1.167 | ± | 0.654 | 1.500 | ± | 0.341 | ns | - | - | - |
| 12 | 0.167 | ± | 0.167 | 0.833 | ± | 0.401 | 1.333 | ± | 0.421 | ns | - | - | - |
| 14 | 0.500 | ± | 0.341 | 0.833 | ± | 0.401 | 1.167 | ± | 0.401 | ns | - | - | - |

(Dussutour et al., 2016). In our experiments, we used only equally-sized ants collected from the same trail, which should imply similar morphological traits and size of fat bodies, and hence physiological status (Couvillon et al., 2011). As for the effect of health state, when we measured the size of ants under the microscope, we also checked for the presence of ectoparasites or fungus infestations, and none of them was found positive. On the other hand, the effect of age cannot be excluded, although the majority of the ants carrying out the same task- foraging in this case - should in principle have similar age (Tripet & Nonacs, 2004). Overall, therefore, the contribution of all the quoted factors to the response variability should be rather low. The simplest explanation of these uneven food use is probably that during the three days when resources were fully available, ants ate different amounts of food and, therefore, some of them started the

satiation period with greater energy reserves. Additionally, it is even possible that captured ants practiced an unbalanced trophallaxis among them (Cassill & Tschinkel, 1999), but, since we did not observe their behavior during the housing in the laboratory, we could not investigate the influence of this possible behavior.

The second interesting result is that, despite starvation, ants continued to select the resources actively and consumed the richer solutions at a higher rate than the poorer ones. Even though being selective is not surprising in well-fed animals, it seems unexpected for starved ones, particularly for those who underwent a long period of starvation that leads them close to death. This finding would imply that workers continue to evaluate, rank and accept the resources, even when starved. What we might have expected is that all the starved ants, particularly the ones which have experienced the most extended period of food shortage (four days), have fed on all the sucrose solutions, irrespective of the concentration, to avoid death from starvation. The effect of fasting is, therefore, not that to just switch on/off the acceptance of a solution, but that of increase or decrease the acceptance threshold. Such behavior may arise because some of the ants, although they fasted for four days, were not enough starved to stop searching more nutritive resources, instead of filling their gut with a low energetic food, following the general principles of the contingency model (Stephens & Krebs, 1986). However, this point deserves further investigations.

The last evidence concerns the variation of the acceptance rate with time, which decreased rapidly after a few minutes from the beginning of the experiments. For example, for the four-days starved ants, the acceptance rate rapidly declined over the six-eight minutes from the start, until the mean number of feeding ants become similar to that characterizing the less starved ones. This point is also underlined by the fact that significant differences in the acceptance among differently starved ants occurred particularly during the first minutes. Interestingly, all ants seemed to be satiated approximately at the same time, irrespective of the fasting period, suggesting that the intake rate of feeding ants is similar in all the experimental conditions. This result seems to be partially in contrast with findings of previous studies, such as in *Camponotus mus*, where the intake rate of the sugary food was found to be dependent on both the concentration (Paul & Roces, 2003) and the level of starvation (Falibene et al., 2009). It should be noted that the concentrations of the solutions used in the tests did not significantly affect the viscosity of the liquid and hence a possible difference in the ingestion flow due to anatomic constraints should be excluded (Josens et al., 1998). Nonetheless, this point would suggest that when the feeding behavior is triggered, foragers eat until their gut is full, with no regulation due to the energetic value of the resource. This point should be surveyed more in-depth in future studies. Finally, although carbohydrates are central resources for the ants, since the need for sugary sources

may affect their behavior, from the interspecific interaction to the collective decision-making (e.g. Grover et al., 2007; Arganda et al., 2014; Sola & Josens, 2016; Frizzi et al., 2018; Wittman et al., 2018), an interesting improvement of the study would be to repeat the experiment with foods rich in fats or proteins, maybe including some different indicators in the analysis, such as hormone secretion, frequencies of trophallaxis and recruitment behavior. Moreover, we selected these experimental solutions on the basis of previous studies, which showed a clear scalar response of workers. However, given that the acceptance was not full, it should be also useful to test more concentrated solutions in future. As a final issue, it must be stressed that *T. nigerrimum* can build large super colonies (Seifert et al., 2017), and it is possible that most if not all the nests employed in the study belonged to the same large colony, which may be subjected to the same local environmental features. Hence, the effect of the colony as analytical factor cannot be considered as fully investigated. Elucidating this effect would require samples to be taken from distant and clearly independent nests. Nonetheless, given the largeness of colonies and the relatively low number of individuals involved in the experiments, we believe that all trials can be considered as independent, and the experiment reliable in general.

In conclusion, in this study, we assessed the behavioral responses of workers of *T. nigerrimum* subject to different levels of starvation toward different sugary solutions. We found a rapid reaction of the most starved ants, although the satiation appeared to occur at the same time, irrespective of the concentration supplied and level of starvation. Moreover, it seems that the level of starvation tested was not sufficient to make the foragers entirely cease to evaluate and select the resources. To our knowledge, this is one of the few studies dealing with the effect of the starvation on the feeding behavior of the ants.

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