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Tool selection during foraging in two species of funnel ants

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A R T I C L E I N F O

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Keywords: ants Aphaenogaster food transport foraging tool use Tool use by nonhuman animals has received much research attention in the last couple of decades. Nevertheless, research has focused mostly on vertebrates, particularly primates and corvids, even though tool use has also been documented in insects. One of the best documented examples involves ants using debris (e.g. sand grains, mud, leaf fragments) to collect and transport liquid food to their nest. However, little is known about the factors that determine the selection of materials to be used as tools. We investigated tool selection in two species of *Aphaenogaster* ants by giving them the choice between different kinds of potential tools (natural and artificial objects). Ant workers showed a clear preference for certain materials to be used as tool objects. Tool selection was also shaped by familiarity with the material as ants developed a preference for artificial tools with a good soaking capacity that cannot be found in their natural environment. Our results indicate that ants of this genus have evolved unique foraging strategies and show plasticity in their behaviour.

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Once considered unique to humans, tool use is now known to be widespread in the animal kingdom (Bentley-Condit & Smith, 2010; Shumaker, Walkup, & Beck, 2011). Moreover, in the last two decades our general understanding of the mechanisms underlying flexible tool use has greatly increased (Sanz, Call, & Boesch, 2013). Some species can make tools to meet specific task demands (e.g. Auersperg, Szabo, von Bayern, & Kacelnik, 2012; Bird & Emery, 2009; Sanz, Call, & Morgan, 2009), use multiple tools in succession to fulfil the subgoals required to complete a task (Martin-Ordas, Schumacher, & Call, 2012; Mulcahy, Call, & Dunbar, 2005; Wimpenny, Weir, Clayton, Rutz, & Kacelnik, 2009) or select appropriate tools depending on their physical attributes or functional properties (Bird & Emery, 2009; Chappell & Kacelnik, 2002; Manrique, Gross, & Call, 2010; Visalberghi et al., 2009).

Although these findings are concerned with vertebrates, mostly primates (chimpanzees, *Pan troglodytes*, orang-utans, *Pongo pyg-maeus*, and capuchin monkeys, *Cebus apella*) and passerine birds

(New Caledonian crows, Corvus moneduloides, rooks, Corvus frugilegus, woodpecker finches, Camarhynchus pallidus) (Sanz et al., 2013), tool use also occurs in invertebrates. For instance, a recently compiled catalogue reports about 50 cases of tool use in insects, involving 30 different genera (Bentley-Condit & Smith, 2010). However, little is known about the occurrence of flexible tool use in invertebrates. For instance, weaver ants use the silk produced by their larvae in nest building but this is not considered 'true' tool use because ants use an animate object (Pierce, 1986). Antlions and wormlions throwing out sand to make small prey fall inside their conical pits does qualify as tool use. Although this behaviour is stereotyped, it is not completely fixed: antlions modify the characteristics of their trap in line with hunger level, prey availability, predation threat and other environmental conditions such as light and temperature; however, the influence of experience upon this flexibility is unclear (Scharf, Lubin, & Ovadia, 2011). Antlions and wormlions also prefer specific substrates for pit building or ambushing prey (Devetak & Arnett, 2015). The use of pebbles to close burrows containing eggs and prey in some apoid wasps (Ammophila, Sphex) is flexible because it requires the selection of suitable pebbles (Evans & Eberhard, 1970).

One of the best documented examples of tool use by insects involves using debris to transport food by some species of ants including the harvester ant *Pogonomyrmex badius* (Morrill, 1972),

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Solenopsis invicta (Barber, Ellgaard, Thien, & Stack, 1989) and several species of Aphaenogaster (Agbogba, 1985; Fellers & Fellers, 1976; McDonald, 1984; Tanaka & Ono, 1978), all belonging to the subfamily Myrmicinae. Many of the species from this subfamily are characterized by a very chitinous gaster (abdomen) and by the lack of a distensible crop, which prevents the transport of large amounts of liquid food inside their bodies, a feature very common in other subfamilies, such as Formicinae or Dolichoderinae (Davidson, Cook, & Snelling, 2004; Hölldobler & Wilson, 1990). Furthermore, some myrmicine genera, namely Aphaenogaster, do not perform trophallaxis (Delage & Jaisson, 1969; i.e. mouth-to-mouth exchange of liquid food, also common in other subfamilies) and so the foragers of these species cannot exchange liquids stored in their crops with in-nest workers performing other tasks, such as feeding the larvae. The characteristic anatomy of the digestive tract in myrmicine ants, in particular an absence of an expandable crop, may have favoured the evolution of tool-using behaviour, which allows for efficient gathering, transport and sharing of liquid food (Fellers & Fellers, 1976; Tanaka & Ono, 1978).

When foragers of these myrmicine species encounter liquid food sources (e.g. fruit pulp, body fluids of dead arthropods) they drop debris of various kinds (e.g. sand grains, soil particles, leaf fragments) into the food source and then transport the food-soaked debris back into the nest. Furthermore, some evidence suggests that these ants do not drop debris into nonfood substances (Agbogba, 1985; Banschbach, Brunelle, Bartlett, Grivetti, & Yeamans, 2006). Tool-assisted food transport has been observed in both field and laboratory experiments with artificial baits (Agbogba, 1985; Banschbach et al., 2006; Barber et al., 1989; Fellers & Fellers, 1976; Fowler, 1982; Lőrinczi, 2014; McDonald, 1984; Morrill, 1972; Tanaka & Ono, 1978). Ants use as tools different objects found near the food source including mud clods, leaf fragments, pine needles, sand grains or any particles of a suitable size (Banschbach et al., 2006; Fellers & Fellers, 1976; Lőrinczi, 2014; Morrill, 1972; Tanaka & Ono, 1978). So far, however, only one comprehensive study has been carried out on tool selectivity in these ants (Tanaka & Ono, 1978). Other studies have reported limited observations that might indicate selectivity in use of tools in Aphaenogaster species (e.g. Banschbach et al., 2006; Fellers & Fellers, 1976; Lőrinczi, 2014; Morrill, 1972).

This putative material selectivity is important because it may indicate that ants choose materials flexibly, something that has been mainly documented in vertebrates. However, little is known about the factors that determine ants' preference for various materials as suitable tools for liquid transport. The aim of this study was to comprehensively investigate material selectivity in liquid food transport in two Aphaenogaster ant species in the laboratory to assess their flexibility and establish a possible link with the literature on tool use in vertebrates. We adopted St. Amant and Horton's (2008, p. 1203) definition of tool use, which is followed also by Bentley-Condit and Smith's (2010): 'the exertion of control over a freely manipulable external object (the tool) with the goal of (1) altering the physical properties of another object, substance, surface or medium (the target, which may be the tool user or another organism) via a dynamic mechanical interaction, or (2) mediating the flow of information between the tool user and the environment or other organisms in the environment'. We chose this definition instead of Beck's (1980) or Pierce's (1986) because it provided a good balance between specificity and generality and, crucially, it fully captured the behaviour that we investigated here.

We confronted ants with a liquid food source away from the nest and a set of natural or artificial (novel) objects with different weight/soaking properties. One might expect that tools with more efficient soaking properties would be preferred over alternative choices. Additionally, we expected that ants would preferentially drop debris in nutritious baits. The presentation of natural objects allowed us to link this study with previous ones while the inclusion of the artificial objects allowed us to explore the ants' flexibility in learning to use the most efficient novel materials.

METHODS

Study Species and Housing

We studied two monogynous Mediterranean ant species belonging to the subfamily Myrmicinae, Aphaenogaster subterranea and Aphaenogaster senilis (Czechowski, Radchenko, Czechowska, & Vepsäläinen, 2012; Stukalyuk & Radchenko, 2011). Aphaenogaster subterranea is a highly thermophilous species distributed in southern and central Europe (Czechowski et al., 2012; Seifert, 2007). It lives in moderately wet and warm deciduous and pine forests, and builds nests under stones, in the soil, litter or occasionally in fallen branches. Colony size can vary from several hundred up to 2000 workers (Czechowski et al., 2012; Seifert, 2007; Stukalyuk & Radchenko, 2011). Aphaenogaster senilis inhabits open, sunny locations such as forest edges, lawns, fields and sand dunes. The nests are built into the soil, often sheltered by stones. Workers forage individually mostly at the ground level, but they can occur on shrubs and trees. Since these habitats have scarce food sources, workers can cover large areas with the help of their long legs. Colony size can vary between a few hundred to a few thousand workers (Boulay et al., 2007).

Eight medium-sized colonies of A. subterranea (two queenright and six queenless, between 500 and 1500 workers) were collected in a black pine forest near the village of Litér (Hungary) and kept under standard conditions (temperature 24 ± 4 °C; relative humidity 42–43%; 12:12 h light:dark cycle) in the laboratory. Together with some material coming from the original habitat (soil, dried pine needles and leaf fragments), the colonies were kept in plastic boxes (44×31 cm and 23 cm high) with their cover cut in a circular shape (diameter 15 cm) and covered with a fine-wired metal mesh for ventilation and easy moistening of the nest. Every box containing a colony was connected with a 10 cm long plastic tube to an arena (60×30 cm and 15 cm high). They were watered daily, and fed every second day with a commonly used artificial diet (Bhatkar & Whitcomb, 1970) in a distant location of the foraging arena. During the experimental period the colonies were not fed, to increase motivation for food found on the baits. Water was always provided.

Three queenright colonies of *A. senilis* (colony size 500–1500 workers) were collected at Banyuls-sur-Mer (France) in a sandy area and kept in the laboratory under standard conditions (temperature 24 ± 4 °C; relative humidity 50–60%; 12:12 h light:dark cycle). They were housed in artificial nests, each consisting of a cylindrical plastic box (diameter 12.5 cm) with regularly moistened plaster floor, and a hole giving access to the foraging area, which was represented by the space left in a larger plastic box (18 × 25.5 cm and 7.7 cm high) containing the circular nest. The standard diet for each colony consisted of five dead crickets, *Acheta domestica*, and about 5 g of apple/honey mix twice a week. During the experimental period, to increase motivation for food, colonies were fed with only three crickets and 2 g of apple/honey mix (twice a week); water was always provided ad libitum.

Experimental Set-up

Ant colonies were given food baits and different types of potential tools in the foraging arena (Figs. 1 and 2). The methodology used and described below is slightly different for *A. subterranea* and *A. senilis* because this study is the combined output of two initially





separate studies conducted independently in two different laboratories.

Aphaenogaster subterranea

The experiments lasted 3 h, which was enough time to observe the transport of tools into the nests. Each observation period lasted 1 min, repeated every 4 min until the end of the experiment. During this time, we noted the number of tools transported to the bait and from the bait into the nests. Tool preference was tested for tools found and used in nature: small soil grains (diameter 1 mm), large soil grains (diameter 2–3 mm), and fragments of pine needles and leaves. Additionally, we also used a fifth tool type of anthropogenic origin with good soaking/weight ratio: small pieces of sponges (diameter 5 mm; Fig. 1 and Appendix Fig. A1). Tools were placed 12 cm from the baits in a random order. To estimate the soaking properties, we weighed 10 tools of each type with a precision analytical balance (10 mg accuracy) before and after soaking them in the different types of baits (Table 1). The objects were placed on the surface of the baits and left there for 10 min. Three different baits were tested: water, honey diluted in water (1:3 ratio; further honey-water) and honey (condensed with sugar powder; further



Figure 2. Experimental set-up and tool types offered to Aphaenogaster senilis.

Table 1

Soaking properties of	f the different	tools used by Ap	haenogaster subterraned
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Tool type	Imbibed/initial weight (weight ratio)		
	Honey-water	Honey	
Sponges	7.45	1.29	
Leaf fragments	1.94	11.9	
Pine needles	1.33	1.85	
Small soil grains	1.1	1.48	
Large soil grains	0.7	0.64	

honey) placed in plastic plates (diameter 4 cm, Figs. 1, A1). The baits were given to the colonies in a random order.

Aphaenogaster senilis

The experiments lasted 3 h, during which the activity of the colonies was videotaped. The tapes were analysed as for *A. subterranea*. The experiments with the same set-up were repeated 10 times per colony.

Tool use preference was tested for a total of six different, novel potential tools, such as pieces of paper, sponges, artificial foam, twigs, string and parafilm (Fig. 2). Groups of 10 tools of each type (average weight for each tool: 2.5 mg) were placed 12 cm from the food source (diluted honey on a piece of aluminium foil). The soaking properties were estimated by weighing 10 tools of each type with a precision analytical balance (10 mg accuracy) before and after soaking them in diluted honey (Table 2).

Ethical Note

Ant colonies were collected with care in the field and maintained in nearly natural conditions in the laboratory. Ants were provided with suitable nesting sites, food and water thus minimizing any adverse impact on their welfare. Our experimental designs include only behavioural observations, no insect was harmed or stressed during the experimental procedure and all ant colonies were healthy at the end of the experiments.

Statistical Analysis

In *A. subterranea*, we did not observe any transport of tools into the nests in the case of water baits; furthermore, the transport rate towards the water baits was much lower than towards the other baits, so we focused on the analysis of tool use in honey-water and honey baits. The effect of the bait type on the total number of tools dropped into the baits was analysed using a GLMM model (Poisson errors, maximum likelihood fit, log link). In the full model, bait type was included as a factor and colony ID as a random factor (N = 12) to account for within-colony similarities. Differences in the number of tools of different types dropped into baits by different colonies were analysed with GLMM models (Poisson errors, maximum

Table 2	
Soaking properties of the different tools used by Aphaenogaster sen	ilis

Tool type	Imbibed/initial weight (weight ratio)	
	Honey-water	
Paper	28.37	
Sponges	23.31	
Artificial foam	6.37	
Twigs	3.06	
String	2.00	
Parafilm	1.47	

likelihood fit, log link). In the full model, tool type was included as a fixed factor and colony ID as a random factor ($N_{A,su} = 30, N_{A,se} = 18$).

The transport rate of tools towards the baits for the colonies of both species was analysed with the help of a log rank test with Monte Carlo (100 000) simulations ($N_{A.su} = 60$ corpses, $N_{A.se} = 30$). The removal rate was tested until at most the 10th tool was removed. The different tool types were included as dummy variables, while colony ID was included as a random factor. The transport rate of the different tools into the nests was analysed with the same model construction in both species.

In A. subterranea, the transport of tools to the different baits was analysed in separate models, and because two colonies did not transport anything to the baits, only six colonies were used for the analysis. In A. senilis, the same model constructions were used for the analysis of the first, fifth and 10th trials. The 3 h experimental period was enough in the case of both species to observe the transport of tools into the nests. In A. subterranea, three colonies in the case of honey-water and six in the case of honey baits actively transported tools into their nests, so only these colonies were used in the analysis with the same model constructions as described before ($N_{\text{honey-water}} = 15$, $N_{\text{honey}} = 30$). All statistical analyses were carried out in R (version 3.0.2, The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). GLMMs were performed using the glmer function in the lme4 package (Bates, Maechler, Bolker, & Walker, 2013). In the analyses, all tools used were included. A log rank test was carried out with the surv test function in the coin package (Hothorn, Hornik, van de Wiel, & Zeileis, 2008). Pairwise comparisons were performed with separate log rank tests and the Relevel function was used to carry out post hoc sequential comparisons among factor levels when performing GLMM analyses. We applied table-wide sequential Bonferroni-Holm corrections to reveal the exact significance levels in these cases.

RESULTS

Aphaenogaster subterranea: Transport to the Baits

Workers of *A. subterranea* dropped significantly more tools into honey-water (GLMM: z = 12.5, N = 24, P < 0.001) and honey (z = 11.97, P < 0.001) than into water, but no difference was observed between the honey-water and honey (z = -0.96, NS). We

therefore concentrate on honey-water and honey baits in the following analyses.

Overall, small soil grains were the most frequently transported tool to the honey-water, significantly more than sponges (z = -3.33, P < 0.001) and leaf fragments (Fig. 3a), with the latter being the least frequently dropped tools compared to every other tool type (z < -3.22, P < 0.01). Furthermore, leaf fragments were transported at the lowest rate (log rank test: $13 < \chi^2 < 29.76$, P < 0.001) while sponges were transported at a slower rate than small soil grains ($\chi^2 = 29.76$, P < 0.01; Appendix Fig. A2a).

In the case of honey baits, the preferred tools were the small soil grains and pine needles, which were transported in significantly higher numbers than leaf fragments (z < -3.07, P < 0.001) and large soil grains (z < -2.65, P < 0.05); sponges were also used more frequently than leaf fragments (z = -2.83, P = 0.03; Fig. 3b). Every tool type was transported at a faster rate than leaf fragments ($11.5 < \chi^2 < 40.29$, P < 0.003; Fig. A2b).

Aphaenogaster subterranea: Transport into the Nests

In the case of honey-water baits, small soil grains were transported into the nest in higher numbers than pine needles (z = 3.43, N = 24, P < 0.001), leaf fragments (z = 3.56, P < 0.001) and sponges (z = 2.91, P < 0.001; Fig. 4a). Small soil grains were also transported at a faster rate than every other tool type (11.43 < χ^2 < 29.26, P < 0.003; Fig. A3a).

In the case of honey baits, sponges were transported into the nest in the highest numbers, which differed significantly from every other tool type (z < -4.02, P < 0.001). The second most preferred tools were small soil grains, which were transported significantly more often than leaf fragments (z = -2.79, P = 0.03; Fig. 4b). Sponges were transported at a faster rate than every other tool type ($8.56 < \chi^2 < 37.39$, P < 0.01). Leaf fragments were transported at the slowest rate, which differed significantly from the small ($\chi^2 = 22.31$, P < 0.001) and large soil grains ($\chi^2 = 7.07$, P < 0.05; Fig. A3b).

Aphaenogaster senilis: Transport to the Baits

Overall, the number of tools used by *A. senilis* workers changed significantly across trials (from the first to the fifth to the 10th trials: GLMM: z = 3.09, N = 36, P < 0.01), and there was no significant difference between the fifth and the 10th trial (z = 0.95, NS);



Figure 3. Number of different tools transported to (a) honey-water and (b) honey baits by *Aphaenogaster subterranea* workers. Box plots show the median (internal line), quartiles (box edges), the range of values within the interquartile range (whiskers) and values outside this range (grey symbols). Different letters above boxes represent groups that differ significantly from each other.



Figure 4. Number of different tools transported into the nest from the (a) honey-water and (b) honey baits by *Aphaenogaster subterranea* workers. Box plots show the median (internal line), quartiles (box edges), the range of values within the interquartile range (whiskers) and values outside this range (grey symbols). Different letters above boxes represent groups that differ significantly from each other.

therefore the use of potential tools was analysed separately for each trail (Fig. A4).

In the first and fifth trials, there was no significant difference in the number of different tools transported to the bait (between every tool type: first: z > 0.2, N = 18, NS; fifth: z > -2.2, N = 18, NS; Fig. 5), although in the first trial paper was transported at a significantly faster rate than the artificial foam (log rank test: $\chi^2 = 12.72$, P < 0.01; Fig. A5a). In the fifth trial, the sponges (log rank test: $\chi^2 = -4.33$, P < 0.05; Fig. A5c) and the pieces of paper (log rank test: $\chi^2 = -3.82$, P < 0.05) were transported significantly faster than every other tool types, but there was no significant difference between these two tool types (log rank test: $\chi^2 = -0.28$, NS), and paper did not differ significantly from string (log rank test: $\chi^2 = -2.33$, NS; Fig. A5c).

In the 10th trial, the preferred tools were paper and sponges ($\chi^2 = 0.89$, NS), which were used more frequently than artificial foam (z > 3.24, N = 18, P < 0.01; Fig. 5) and transported at a significantly faster rate than parafilm and twigs (respectively:

 χ^2 > 8.66, *P* < 0.05 and χ^2 > 8.22, *P* < 0.05; Fig. A5e). Artificial foam was transported at a significantly slower rate than every other tool type (χ^2 = 9.3, *P* < 0.05; Fig. A5e).

Aphaenogaster senilis: Transport into the Nest

Overall, the number of tools transported into the nest by *A. senilis* workers changed significantly across trials (GLMM: z = 2.79, N = 36, P < 0.01), and there was no significant difference between trial 5 and 10 (z = 1.01, NS); therefore, the use of artificial tools was analysed separately for the first, fifth and 10th trials (Fig. A4).

In the first trial, there was no significant difference in the number (GLMM: 1.82 > z > 0.001, NS) and transport speed (log rank test: $0 > \chi^2 > 1$, NS) of different tools brought into the nest (Figs. 6, A5b). In contrast, in the fifth trial significantly more pieces of paper and sponge were transported than pieces of foam (z > -3.16, P < 0.05) and parafilm (z > -3.38, P < 0.01; Fig. 6). Moreover, paper



Figure 5. Number of different tools transported to the baits in the first, fifth and 10th trials by *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and range (whiskers).



Soaking power

Figure 6. Number of different tools transported into the nest in the first, fifth and 10th trials by *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and range (whiskers).

and sponge (log rank test: $\chi^2 = 0.29$, NS) were transported at a higher rate than every other tool type ($\chi^2 > 2.85$, P < 0.05; Fig. A5d). Strings and twigs ($\chi^2 = -0.91$, NS) were transported at a higher rate than foam ($\chi^2 > -6.24$, P < 0.01) and parafilm ($\chi^2 > 2.48$, P < 0.05; Fig. A5d).

In the 10th trial, paper was the most frequently transported tool to the nest, differing significantly from the number of artificial foam (*z* = 2.73, *P* < 0.05) and parafilm tools (*z* = 2.73, *P* < 0.05; Fig. 6). The fastest transport rate was observed for paper tools, which differed significantly from every other tool type ($\chi^2 > 8.2$, *P* < 0.05; Fig. A5f) except for sponges and strings ($\chi^2 > 6.78$, NS; Fig. A5f). Parafilm and artificial foam ($\chi^2 = 3.35$, NS) were transported at a significantly slower rate than every other tool type ($\chi^2 > 8.66$, *P* < 0.05).

DISCUSSION

We observed the occurrence of tool use to transport liquid food in two ant species of the genus Aphaenogaster thus confirming previous observations (Agbogba, 1985). More importantly, ant workers were selective in both the materials they chose and the baits that they exploited. Aphaenogaster subterranea was mainly tested with natural materials. Small soil grains were the most preferred item and leaf fragments, despite having a superior soaking power than soil grains, the least preferred item. Ants showed a remarkable preference for sponges despite being novel (they were the only artificial material tested in this species). Interestingly, ant workers in more than 80% of the trials broke the sponges into smaller fragments, presumably to facilitate handling. Once tools were dropped into the baits, ants behaved differently depending on the bait's viscosity (honey-water or pure honey). Imbibed small soil grains were transported to the nest in the highest numbers and faster than other tool types from honey-water baits, while sponges were the preferred transported tools from the honey baits (small soil grains were the second preferred tool to be brought to the nest). Additionally, ants threw many fewer items into water baits than into those baits that contained honey.

Aphaenogaster senilis could choose among six materials that differed in terms of soaking power (paper, sponges, artificial foam, twigs, sting and parafilm). It is likely that ants of this species, which live in sandy areas, were unfamiliar with most of these materials. Initially, *A. senilis* showed no preference for any of the tools to drop into the baits, although paper was transported faster to the bait than the artificial foam. However, along trials, ants significantly preferred dropping paper and sponges into the baits, thus choosing in accordance with the tools' soaking properties. A similar pattern was observed for tool transport to the nest: ants developed a preference for paper, followed by sponges, thus optimizing their foraging effort by using the tools with the best soaking properties and ease of grasping compared to other tool types.

Our results suggest that using tools to transport liquid to the nest in the two studied ant species is not behaviourally fixed. Ants incorporated novel tools, many of them made of artificial materials, into their foraging activities. Moreover, *A. senilis* learned within 10 trials to select the best tool options available based on their soaking properties and *A. subterranea* chose tools in relation to the characteristic of the food itself, e.g. food viscosity. This means that ants can select tools in line with both food and tool properties. Nevertheless, other factors (besides a tool's soaking properties) may have played a role in determining ants' preferences. One possibility is that the low density of some materials in the natural habitat (e.g. leaf fragments are not abundant in the pine forest inhabited by these *A. subterranea* colonies) may have been the reason for largely ignoring this item. However, a lack of familiarity with potential

tools cannot explain why *A. subterranea* used sponge pieces, which were totally novel objects. Even more compelling are the data on *A. senilis*, which developed a preference for unfamiliar materials during testing.

Handling effort is another factor that could have played an important role in tool selection, perhaps in combination with the tool's soaking properties. During our observations, we noticed that A. subterranea workers had problems with grasping, handling and orienting the leaf fragments, while the pieces of sponge could be grasped more easily. In fact, ants may have torn apart sponges to reduce their size and facilitate handling. Although the soaking power of sponges was lower when dropped into honey than into honey-water, their relatively low weight made them more buoyant than other tool types and their irregular texture increased their potential for being grasped. We found that the preference for some items often depended on the type of task that ants carried out. Sponges might have been preferentially transported to the nest, but small soil grains were the tools most often dropped into the baits. This suggests that selection among different tool types occurred both when first encountered and at the food source after the tools had been dropped into the baits. This two-stage selection process might be especially important in the case of novel tools whose properties are still unknown. Indeed, A. senilis ants transported very few foam fragments to the bait in the first trial but subsequently increased their transport during the fifth trial. Nevertheless, foam fragments were rarely transported from the bait to the nest and by the 10th trial ant workers even reduced their transport to the baits. This suggests that tool selection, after familiarization with novel material, may have also occurred at the baits. Other authors observed that not all the tools dropped into the baits were transported into the nest (Fowler, 1982; McDonald, 1984), corroborating our interpretation that further tool selection can occur once the tools have been dropped into the liquid food source.

Availability, weight, soaking properties, ease of handling and the possibility of shaping the material to a desired form are therefore important factors in tool selection. The assessment of these characteristics appears to be the result of a familiarization and learning process, at least for the novel objects offered to the ants in our experiments. As the natural availability of certain kinds of tools varies both spatially and seasonally, plasticity in tool selection is likely to be adaptive. Tool use behaviour in ants may have evolved from the tendency shown by many species to cover with debris liquid or viscous substances, which may otherwise cause drowning or entanglement of workers near their nest (Fellers & Fellers, 1976; McDonald, 1984). Note, however, that it has been experimentally shown that Aphaenogaster ants typically drop debris in food substances (Agbogba, 1985; Banschbach et al., 2006) and our experiments confirm this. The adaptive advantage of tool use in Aphaenogaster ants is that, by using tools, foragers are capable of efficiently exploiting ephemeral food sources by transporting much larger quantities of liquid nutrients than they could do by internal transport (Fellers & Fellers, 1976; Tanaka & Ono, 1978), given the nonexpandable crop characteristic of myrmicine ants. Tool use might help these ants to compete with more dominant ant species which can monopolize food sources by numbers: if the food is quickly covered by debris, other ant species cannot exploit it and tool users can take the necessary time to bring the soaked debris back to their nest (Banschbach et al., 2006; Fellers & Fellers, 1976).

We began our paper by referring to the research on flexible tool use in vertebrates hoping to establish a link with the existing observations on invertebrate tool use. Our experiments showed some indication that ants preferred materials with good soaking power although other aspects such as ease of grasp may have also contributed to their choices. Chimpanzees create a vegetable mass with good absorbent properties by chewing leaves that they use to extract liquid from crevices (Goodall, 1986). It is unknown, however, whether chimpanzees select certain plant species for their high soaking power. In a liquid extraction task in the laboratory, Lehner, Burkart, and van Schaik (2011) reported that captive orang-utans developed liquid extraction techniques based on materials with high soaking power (e.g. wood wool, paper) over those with low soaking power (e.g. leafless branch). Although this may indicate that orang-utans, like ants, were sensitive to the properties of such materials, the lack of a direct and systematic comparison between different kinds of materials considerably reduces the conclusions that one may be able to draw from that study. Nevertheless, other studies with vertebrates in nonliquid extractive foraging tasks have systematically varied the materials presented and have found evidence of selectivity in terms of hardness and weight (Visalberghi et al., 2009), weight (Bird & Emery, 2009) or rigidity (Manrique et al., 2010). Our findings with ants are in some ways comparable to those studies although, admittedly, our results are less clear than those reported in vertebrates. This is partly understandable because ours is only the second systematic study on material selectivity in ants (besides Tanaka & Ono, 1978) and we may not have fully considered a number of factors. For instance, dropping a substantial number of tools (e.g. pine needles) inside the liquid and abandoning them there may seem puzzling from the point of view of efficiency. Note, however, that dropping these materials on the liquid may have accomplished another function (e.g. preserving the liquid food against exploitation from other species). Also, the absorbed liquid may have made the potential tool object too difficult to grasp or too heavy to carry. Another possibility is that ants can only identify suitable materials after observing its effect on the substance to be collected instead of selecting them prior to their use (e.g. artificial foam). Although selecting materials to collect liquid has not been described in corvids or primates, selecting and manufacturing tools prior to their use is well documented in these taxa (e.g. Manrique et al., 2010; Sanz et al., 2009; Visalberghi et al., 2009; Wimpenny et al., 2009). Whether ants (or any other species) can also select new materials prior to experiencing its absorbent properties is an open question that future studies should address.

Some authors may question our use of the term tool use to describe the behaviour of the ants. Tool use is, after all, a contentious term in the literature typically characterized by rather long definitions, a few clear examples, and some areas of substantial disagreement (see Bentley-Condit & Smith, 2010; Shumaker et al., 2011). Although we would be ready to accept a less contentious terminology such as 'material use', it is difficult to do so when chimpanzees using leaves to extract liquid from crevices is considered a classic example of tool use (Goodall, 1986). More importantly, we think that the behaviour of the ants meets one of the most important criteria that define tool use in foraging contexts (Beck, 1980; Bentley-Condit & Smith, 2010; St. Amant & Horton, 2008), i.e. the use of an external object to affect the position or location of another object or substance. Obviously one could argue that nest-building materials affect the position of other nesting materials, which is why some authors object to the idea of tool use and would perhaps like to see either a more restricted use of the term or its complete abandonment for a wider term such as construction behaviour (Hansell & Ruxton, 2008). Although we understand this position and the reasons for it, given the above considerations, we still prefer to refer to the behaviour of the ants as tool use, or the similar denomination of object use.

In conclusion, we observed *A. senilis* and *A. subterranea* using natural and artificial debris to collect and transport liquid food to their nests. We also documented the development of a preference for materials with optimal soaking properties in *A. senilis* although other factors such as familiarity and ease of grasping may have played a role in determining ants' choices. Additional studies are needed to better characterize the extent and limits of this form of flexible tool use particularly in relation to the existing forms of flexible tool use displayed by vertebrates.

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References

- Agbogba, C. (1985). Observations sur la récolte de substances liquids et de sucs animaux chez deux espèces d'Aphaenogaster: A. senilis et A. subterranea (Hym. Formicidae). Insectes Sociaux, 32, 427–434.
- Auersperg, A. M. I., Szabo, B., von Bayern, A. M. P., & Kacelnik, A. (2012). Spontaneous innovation in tool manufacture and use in a Goffin's cockatoo. *Current Biology*, 22, 903–904.
- Banschbach, V. S., Brunelle, A., Bartlett, K. M., Grivetti, J. Y., & Yeamans, R. L. (2006). Tool use by the forest ant *Aphaenogaster rudis*: Ecology and task allocation. *Insectes Sociaux*, 53, 463–471.
- Barber, J. T., Ellgaard, E. G., Thien, L. B., & Stack, A. E. (1989). The use of tools for food transportation by the imported fire ant, *Solenopsis invicta*. *Animal Behaviour*, 38, 550–552.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2013). Ime4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5 http://CRAN.R-project.org/ package=lme4. published online 25 October 2013.
- Beck, B. B. (1980). Animal tool behavior: The use and manufacture of tools by animals. New York, NY: Garland Publishing STPM Press.
- Bentley-Condit, V. K., & Smith, E. O. (2010). Animal tool-use: Current definitions and an updated comprehensive catalog. *Behaviour*, 147, 185–221.
- Bhatkar, A., & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. Florida Entomologist, 53, 229–232.
- Bird, C., & Emery, N. J. (2009). Insightful problem solving and creative tool modification by captive nontool-using rooks. *Proceedings of the National Academy of Sciences United States of America*, 106, 10370–10375.
- Boulay, R., Hefetz, A., Cerdá, X., Devers, S., Francke, W., Twele, R., et al. (2007). Production of sexuals in a fission-performing ant: Dual effects of queen pheromones and colony size. *Behavioral Ecology & Sociobiology*, 61, 1531–1541.
- Chappell, J., & Kacelnik, A. (2002). Tool selectivity in a non-mammal, the New Caledonian crow (Corvus moneduloides). Animal Cognition, 5, 71–78.
- Czechowski, W., Radchenko, A., Czechowska, W., & Vepsäläinen, K. (2012). The ants (Hymenoptera, Formicidae) of Poland with reference to the myrmecofauna of Europe. Warszawa, Poland: MIZ PAS.
- Davidson, D. W., Cook, S. C., & Snelling, R. (2004). Liquid feeding performances of ants (Formicidae): Ecological and evolutionary implications. *Oecologia*, 139, 255–266.
- Delage, B., & Jaisson, P. (1969). Etude des relations sociales chez des Fourrnis du genre Aphaenogaster. Comptes Rendus de l'Académie des Sciences Paris, 268, 701–703.
- Devetak, D., & Arnett, A. (2015). Preference of antlion and wormlion larvae (Neuroptera: Myrmeleontidae; Diptera: Vermileonidae) for substrates according to substrate particle sizes. *European Journal of Entomology*, 112, 500–509.
- Evans, H. E., & Eberhard, M. J. W. (1970). The wasps. Ann Arbor, MI: The University of Michigan Press.
- Fellers, J. H., & Fellers, G. M. (1976). Tool use in a social insect and its implications for competitive interactions. *Science*, 192, 70–72.
- Fowler, H. G. (1982). Tool use by Aphaenogaster ants: A reconsideration of its role in competitive interactions. Bulletin of New Jersey Academy of Science, 27, 81–82.
- Goodall, J. (1986). *The chimpanzees of Gombe. Patterns of behavior*. Cambridge, MA: The Belknap Press of Harvard University Press.
- Hansell, M., & Ruxton, G. D. (2008). Setting tool use within the context of animal construction behaviour. *Trends in Ecology & Evolution*, 23, 73–78.
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Cambridge, MA: Belknap Press of Harvard University.
- Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2008). Implementing a class of permutation tests: The coin package. *Journal of Statistical Software*, 28, 1–23. http://www.jstatsoft.org/v28/i08/.

- Lehner, S. R., Burkart, J. M., & van Schaik, C. P. (2011). Can captive orangutans (Pongo pygmaeus abelii) be coaxed into cumulative build-up of techniques? Journal of Comparative Psychology, 125, 446–455.
- Lőrinczi, G. (2014). Some notes on the tool-using behaviour of the ant, Aphaenogaster subterranea (Hymenoptera: Formicidae). Tiscia, 40, 17–24.
- Manrique, H. M., Gross, A. N., & Call, J. (2010). Great apes select tools based on their rigidity. Journal of Experimental Psychology: Animal Behavior Processes, 36, 409–422.
- Martin-Ordas, G., Schumacher, L., & Call, J. (2012). Sequential tool use in great apes. *PLoS One*, 7, e52074.
- McDonald, P. (1984). Tool use by the ant, Novomessor albisetosus (Mayr). Journal of New York Entomological Society, 92, 156–161.
- Morrill, W. L. (1972). Tool using behaviour of Pogonomyrmex badius (Hymenoptera: Formicidae). Florida Entomologist, 55, 59–60.
- Mulcahy, N. J., Call, J., & Dunbar, R. I. M. (2005). Gorillas and orangutans encode relevant problem features in a tool-using task. *Journal of Comparative Psychol*ogv. 119, 23–32.
- Pierce, J. D. (1986). A review of tool use in insects. Florida Entomologist, 69, 95–104. Sanz, C., Call, J., & Boesch, C. (2013). Tool use in animals: Cognition and ecology. New York, NY: Cambridge University Press.
- Sanz, C., Call, J., & Morgan, D. (2009). Design complexity in the tool use of chimpanzees (*Pan troglodytes*) in the Congo basin. *Biology Letters*, 5, 293–296.
- Scharf, I., Lubin, Y., & Ovadia, O. (2011). Foraging decisions and behavioural flexibility in trap-building predators: A review. *Biological Reviews*, 86, 626–639.

- Seifert, B. (2007). Die Ameisen Mittel- und Nordeuropas. Görlitz, Germany: Lutra Verlags und Vertriebsgesellschaft.
- Shumaker, R. W., Walkup, K. R., & Beck, B. B. (2011). Animal tool behavior: The use and manufacture of tool by animals. Baltimore, MA: The Johns Hopkins University Press.
- St. Amant, R., & Horton, T. (2008). Revisiting the definition of animal tool use. Animal Behaviour, 75, 1199–1208.
- Stukalyuk, S. V., & Radchenko, V. G. (2011). Structure of multispecies ant assemblages (Hymenoptera, Formicidae) in the Mountain Crimea. *Entomological Re*view, 91, 15–36.
- Tanaka, T., & Ono, Y. (1978). The tool use by foragers of Aphaenogaster famelica. Japanese Journal of Ecology, 28, 49–58.
- Visalberghi, E., Addessi, E., Spagnoletti, N., Truppa, V., Ottoni, E., Izar, P., et al. (2009). Selection of effective stone tools by wild capuchin monkeys. *Current Biology*, 19, 213–217.
- Wimpenny, J. H., Weir, A. A. S., Clayton, L., Rutz, C., & Kacelnik, A. (2009). Cognitive Processes Associated with sequential tool Use in New Caledonian crows. *PLoS One*, 4, e6471.

APPENDIX





Figure A1. Different tool types used for experiments with *Aphaenogaster subterranea* (a) from left to right: small soil grains, sponges, pine needles, large soil grains, leaf fragments; (b) the dynamics of bait covering with tools.



Figure A2. Estimated functions of tool transport time to (a) honey-water and (b) honey baits by workers of *Aphaenogaster subterranea* (log rank test). Different tool types are depicted by different colours.



Figure A3. Estimated functions of tool transport time into the nest from the (a) honey-water and (b) honey baits by *Aphaenogaster subterranea* workers (log rank test). Different tool types are depicted by different colours.



Figure A4. Summed number of tools transported (a) to the baits and (b) into the nest by *Aphaenogaster senilis* workers during the three trials (box plots show median, quartiles and range). Different letters represent groups that differ significantly from each other.



Figure A5. Estimated functions of tool transport time (a, c, e) to the baits and (b, d, f) into the nests in the (a, b) first, (c, d) fifth and 10th (e, f) trials by *Aphaenogaster senilis* workers (log rank test). Different tool types are depicted by different colours.