Tool selection during foraging in two species of funnel ants

István Maák[1], Gábor Lőrinczi[1], Pauline Le Quinquis[2], Gábor Módra[1], Dalila Bovet[3], Josep Call[4], Patrizia d'Ettore[2]

[1] Department of Ecology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary, *bikmakk@gmail.com*

[2] Laboratory of Experimental and Comparative Ethology, University Paris 13 – Sorbonne Paris Cité, France

[3] Laboratoire Ethologie Cognition Développement, Université Paris Ouest Nanterre La Défense, France

[4] School of Psychology & Neuroscience, University of St Andrews, UK

Corresponding authors:

István Maák – Department of Ecology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary, e-mail: *bikmakk@gmail.com*, tel: +36-70-2793398

Patrizia d'Ettore – Laboratory of Experimental and Comparative Ethology, University Paris 13, Sorbonne Paris Cité, 99 Avenue J.-B. Clément, 93430 Villentaneuse, France

e-mail: *dettorre@leec.univ-paris13.fr*, tel: +33-1-49403196

Tool selection during foraging in two species of funnel ants

Highlights

• Some ant species use debris as tools to transport liquid food to the nest.

• When given the choice between different kinds of potential tools ants are selective.

• They show preference for materials with optimal handling and/or soaking properties.

• They can employ artificial materials novel to them, thus tool use is flexible in ants.

1   **Tool selection during foraging in two species of funnel ants**

2   **ABSTRACT**

3   Tool use by non-human animals has received much research attention in the last couple of

4   decades. Nonetheless, research has focused mostly on vertebrates, particularly primates and

5   corvids, even though tool use has also been documented in insects. One of the best-

6   documented examples involves ants using debris (e.g., sand grains, mud, leaf fragments) to

7   collect and transport liquid food to their nest. However, little is known about the factors that

8   determine the selection of materials to be used as tool. We investigated tool selection in two

9   species of *Aphaenogaster* ants by giving them the choice between different kinds of potential

10   tools (natural and artificial objects). Ant workers showed a clear preference for certain

11   materials to be used as tool objects. Tool selection was also shaped by familiarity with the

12   material as ants developed a preference for artificial tools with a good soaking capacity that

13   cannot be found in their natural environment. Our results indicate that ants of this genus

14   evolved unique foraging strategies and show plasticity in their behaviour.

15   **Keywords:** ants, *Aphaenogaster*, foraging, food transport, tool use

INTRODUCTION

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 significantly 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 sub-goals 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, orangutans and capuchin monkeys) and passerine birds (New Caledonian crows, rooks, woodpecker finches) (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 qualifies as tool use. Although it is a fairly stereotyped behaviour, it is not completely fixed: antlions modify the characteristics of their trap according to 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), *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 characterised by a very chitinous gaster (abdomen) and by the lack of a distensible crop, which prevents the transportation 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 as a consequence 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 the absence of an expandable crop, may have favoured the evolution of tool using behaviour, which allows for an 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 non-food substances (Agbogba, 1985; Banschbach, Brunelle, Bartlett, Grivetti, & Yeamans 2006). Tool-assisted food transportation 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 and 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).

3

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 transportation. The aim of this study was to comprehensively investigate material selectivity in liquid food transportation 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 Bentley-Condit and Smith's (2010) definition of tool use, which in turn closely followed that of St Amant and Horton's (2008): "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" p.1203. 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). *A. subterranea* (Latreille, 1798) is a highly thermophilous species distributed in southern and central Europe (Czechowski et al., 2012; Seifert, 2007). It lives in moderately wet and warm

104 deciduous and pine forests, and builds nests under stones, in the soil, litter or occasionally in
105 fallen branches. Colony size can vary from several hundred up to 2000 workers (Czechowski
106 et al., 2012; Seifert, 2007; Stukalyuk & Radchenko, 2011). *A. senilis* (Mayr, 1853) inhabits
107 open, sunny locations such as forest edges, lawns, fields and sand dunes. The nests are built
108 into the soil, often sheltered by stones. Workers forage individually mostly at the ground
109 level, but they can occur on shrubs and trees. Since these habitats have scarce food sources,
110 workers can cover large areas with the help of their long legs. Colony size can vary between a
111 few hundred to a few thousand workers (Boulay et al., 2007).

112   Eight medium sized colonies of *A. subterranea* (two queenright and 6 queenless,
113 between 500-1500 workers) were collected in a black pine forest near the village of Litér
114 (Hungary) and kept under standard conditions (temperature 24 ± 4 ℃; relative humidity 42-
115 43%; 12 L: 12 D cycle) in the laboratory. Together with some material coming from the
116 original habitat (soil, dried pine needles and leaf fragments), the colonies were kept in plastic
117 boxes (44 cm × 31 cm × 23 cm) with their cover cut in a circular shape (diameter 15 cm) and
118 covered with a fine-wired metal mesh for ventilation and easy moistening of the nest. Every
119 box containing a colony was connected with a 10 cm long plastic tube to an arena (60 cm ×
120 30 cm × 15 cm). They were watered daily, and fed every second day with a commonly used
121 artificial diet (Bhatkar & Whitcomb, 1970) in a distant location of the foraging arena. During
122 the experimental period the colonies were not fed, to increase motivation for food found on
123 the baits. Water was always provided *ad libitum.*

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

Experimental setup

135 Ant colonies were presented with food baits and different types of potential tools in the
136 foraging arena (Fig. 1, 2). The methodology used in *A. subterranea* and *A. senilis* and
137 described below is slightly different because this study is the combined output of two initially
138 separate studies conducted independently in two different laboratories.

139 *Aphaenogaster subterranea*

140 The duration of the experiments was 3 hours, which was enough time to observe the transport
141 of tools into the nests. Each observation period lasted one minute, repeated every four minutes
142 until the end of the experiment. During this time we noted the number of tools transported to
143 the bait and from the bait into the nests. Tool preference was tested for tools found and used
144 in nature: small soil grains (diameter 1 mm), large soil grains (diameter 2-3 mm), and
145 fragments of pine needles and leaves. Additionally, we also used a fifth tool type of
146 anthropogenic origin with good soaking/weight ratio: small pieces of sponges (diameter 5
147 mm) (Fig. 1 and Fig. A1, appendix). Tools were placed at a distance of 12 cm from the baits
148 in a random order. To estimate the soaking properties, the weight of 10 tools of each type was
149 measured with a precision analytical balance (10 mg accuracy) before and after soaking them
150 in the different types of baits (Table 1). The objects were placed on the surface of the baits
151 and left there for 10 minutes. Three different baits were tested: water, honey diluted in water
152 (1:3 ratio; further honey-water) and honey (condensed with sugar powder; further honey)
153 placed in plastic plates (diameter 4 cm, Fig. 1 and A1). The baits were given to the colonies in
154 a random order.

155

156 *Aphaenogaster senilis*

157 The experiments lasted 3 hours, during which the activity of the colonies was videotaped. The
158 tapes were analysed as for *A. subterranea*. The experiments with the same setup were
159 repeated 10 times per each colony.

160 Tool use preference was tested for a total of 6 different, novel potential tools, such as pieces
161 of paper, sponges, artificial foam, twigs, string and parafilm (Fig. 2). Groups of 10 tools of

162 each type (average weight for each tool: 2.5 mg) were placed 12 cm from the food source

163 (diluted honey on a piece of aluminium foil). The soaking properties were estimates by

164 measuring the weight of 10 tools of each type with a precision analytical balance (10 mg

165 accuracy) before and after soaking them in diluted honey (Table 2).

166 *Ethical Note.*

167 Ant colonies were collected with care in the field and maintained in nearly natural conditions

168 in the laboratory. Ants were provided with suitable nesting sites, food and water thus

169 minimizing any adverse impart on their welfare. Our experimental designs include only

170 beahvioural observations, no insect was harmed or stressed during the experimental procedure

171 and all ant colonies were healthy at the end of the experiments.

172 *Statistical analysis*

173 In *A. subterranea,* we did not observe any transport of tools into the nests in the case of water

174 baits; furthermore, the transport rate towards the water baits was much lower than towards the

175 other baits, so we focused on the analysis of tool use in honey-water and honey baits. The

176 effect of the bait type on the total number of tools dropped into the baits was analysed using a

177 GLMM model (Poisson errors, maximum likelihood fit, log link). In the full model, bait type

178 was included as a factor and colony ID as a random factor ($N = 12$) to account for within

179 colony similarities. Differences in the number of tools dropped into baits by different colonies

180 according to different tool types were analysed with GLMM models (Poisson errors,

181 maximum likelihood fit, log link). In the full model, tool type was included as a fixed factor

182 and colony ID as a random factor ($N_{A.su}= 30$, $N_{A.se}= 18$).

183 The transport rate of tools towards the baits for the colonies of both species was analysed with

184 the help of Log-rank test with Montecarlo (100.000) simulations ($N_{A.su}= 60$ corpses, $N_{A.se}=$

185 30). The removal rate was tested until at most the 10th piece of tools was removed. The

186 different tool types were included as dummy variables, while colony ID was included as a

187 random factor. The transport rate of the different tools into the nests was analysed with the

188 same model construction in both species.

189   In *A. subterranea*, the transport of tools to the different baits was analysed in separate

190   models, and because 2 colonies did not transport anything to the baits, only 6 colonies were

191   used for the analysis. In *A. senilis*, the same model constructions were used for the analysis of

192   the 1st, the 5th and 10th experiment. The three-hour experimental period was enough in the

193   case of both species to observe the transport of tools into the nests. In *A. subterranea*, 3

194   colonies in the case of honey-water, and 6 colonies in the case of honey baits were actively

195   transporting tools into their nests, so only these colonies were used in the analysis with the

196   same model constructions as described before ($N_{honey-water}$= 15, N_{honey}= 30). All statistical

197   analyses were carried out in R (version 3.0.2, The R Foundation for Statistical Computing,

198   Vienna, Austria, http://www. r-project.org). GLMMs were performed using *glmer* function in

199   *lme4* package (Bates, Maechler, Bolker, & Walker, 2013). In the analyses, all tools used were

200   included. Log Rank Test was carried out with the *surv_test* function in *coin* package

201   (Hothorn, Hornik, van de Wiel, & Zeileis, 2008). Pairwise comparisons were performed with

202   separate Log Rank Tests and the *Relevel* function was used to carry out post-hoc sequential

203   comparisons among factor levels when performing GLMM analyses. We applied table-wide

204   sequential Bonferroni-Holm correction to reveal the exact significance levels in these cases.

205   **RESULTS**

206   *Aphaenogaster subterranea*: transport to the baits

207   Workers of *A. subterranea* dropped significantly more tools into honey-water (GLMM: $z =$

208   12.5, $N = 24$, $P < 0.001$) and honey ($z = 11.97$, $P < 0.001$) than into water, but no difference

209   was observed between the honey-water and honey ($z = -0.96$, NS). We therefore concentrate

210   on honey-water and honey baits in the following analyses.

211   Overall, small soil grains were the most frequently transported tool to the honey-water,

212   and their number was significantly different from the number of sponges ($z = -3.33$, $P <$

213 0.001) and leaf fragments (Fig. 3), with the latter being the least frequently dropped tools

214 compared to every other tool type ($z < $ -3.22, $P < 0.01$). Furthermore, leaf fragments were

215 transported at the lowest rate (Log Rank Test: $13 < \chi^2 < 29.76$, $P < 0.001$) while sponges were

216 transported at a slower rate than small soil grains ($\chi^2 = 29.76$, $P < 0.01$) (Fig A2).

217   In the case of honey baits, the preferred tools were the small soil grains and pine

218 needles, which were transported in significantly higher numbers than leaf fragments ($z < $ -

219 3.07, $P < 0.001$) and large soil grains ($z < $ -2.65, $P < 0.05$); sponges were also used more

220 frequently than leaf fragments ($z = $ -2.83, $P = 0.03$) (Fig. 3). Every tool type was transported

221 at a faster rate than leaf fragments ($11.5 < \chi^2 < 40.29$, $P < 0.003$) (Fig. A2).

222 *Aphaenogaster subterranea*: transport into the nests

223   In the case of honey-water baits, small soil grains were transported into the nest in

224 higher numbers than pine needles ($z = 3.43$, $N = 24$, $P < 0.001$), leaf fragments ($z = 3.56$, $P < $

225 0.001) and sponges ($z = 2.91$, $P < 0.001$) (Fig 4). Small soil grains were transported also at a

226 faster rate than every other tool type ($11.43 < \chi^2 < 29.26$, $P < 0.003$) (Fig. A3).

227   In the case of honey baits, sponges were transported into the nest in highest numbers,

228 which differed significantly from every other tool type ($z < $ -4.02, $P < 0.001$). The second

229 most preferred tools were small soil grains, which were transported significantly more often

230 than leaf fragments ($z = $ -2.79, $P = 0.03$) (Fig 4). Sponges were transported at a faster rate than

231 every other tool type ($8.56 < \chi^2 < 37.39$, $P < 0.01$). Leaf fragments were transported at the

232 slowest rate, which significantly differed from the small ($\chi^2 = 22.31$, $P < 0.001$) and large soil

233 grains ($\chi^2 = 7.07$, $P < 0.05$) (Fig A3).

234 *Aphaenogaster senilis*: transport to the baits

235   Overall, the number of tools used by *A. senilis* workers significantly changed across

236 trials (from the 1st, to the 5th and the 10th trials: GLMM: $z = 3.09$, $N = 36$, $P < 0.01$), and there

237 was no significant difference between the 5th and the 10th trial ($z = 0.95$, NS), therefore the use

238 of potential tools was analysed separately for each trail (Fig. A4).

239   In the 1st and 5th trials, there was no significant difference in the number of different

240   tools transported to the bait (between every tool type 1st $z > 0.2$, $N = 18$, NS; 5th $z > -2.2$, $N =$

241   18, NS) (Fig. 5), although in the 1st trial paper was transported at a significantly faster rate

242   than the artificial foam (Log Rank Test: $\chi^2 = 12.72$, $P < 0.01$; Fig. A5). In the 5th trial, the

243   sponges (Log Rank Test: $\chi^2 = -4.33$, $P < 0.05$; Fig. A5) and the pieces of paper (Log Rank

244   Test: $\chi^2 = -3.82$, $P < 0.05$) were transported significantly faster than every other tool types, but

245   there was no significant difference between these two tool types (Log Rank Test: $\chi^2 = -0.28$,

246   NS), and paper did not differ significantly from string (Log Rank Test: $\chi^2 = -2.33$, NS; Fig.

247   S5).

248   In the 10th trial, the preferred tools were paper and sponges ($\chi^2 = 0.89$, NS), which

249   were used more frequently than artificial foam ($z > 3.24$, $N = 18$, $P < 0.01$, Fig. 5) and

250   transported at a significantly faster rate than parafilm and twigs (respectively: $\chi^2 > 8.66$, $P <$

251   0.05 and $\chi^2 > 8.22$, $P < 0.05$, Fig. A5). Artificial foam was transported at a significantly

252   slower rate than every other tool type ($\chi^2 = 9.3$, $P < 0.05$, Fig. A5).

253   *Aphaenogaster senilis*: transport into the nest

254   Overall, the number of tools transported into the nest by *A. senilis* workers

255   significantly changed across trials (GLMM: $z = 2.79$, $N = 36$, $P < 0.01$), and there was no

256   significant difference between trial 5th and 10th ($z = 1.01$, NS), therefore the use of artificial

257   tools was analysed separately for the 1st, 5th and 10th trials (Fig. A4).

258   In the first trial, there was no significant difference in the number (GLMM: $1.82 > z$

259   >0.001, *NS*) and transport speed (Log Rank Test: $0 > \chi^2 > 1$, NS) of different tools brought

260   into the nest (Fig. 6 and Fig. A5). In contrast, in the 5th trial the pieces of papers and sponges

261   were transported in a significantly higher number than the foam ($z > -3.16$, $P < 0.05$) and

262   parafilm tools ($z > -3.38$, $P < 0.01$; Fig. 6). Moreover, paper and sponge (Log Rank Test: $\chi^2 =$

263   0.29, NS) were transported at a higher rate than every other tool type ($\chi^2 > 2.85$, $P < 0.05$;

264   Fig. S5). Strings and twigs ($\chi^2 = -0.91$, NS) were transported at a higher rate than foam ($\chi^2 > -$

265   6.24, $P < 0.01$) and parafilm ($\chi^2 > 2.48$, $P < 0.05$; Fig. A5).

266   In the 10th trial, paper was the most frequently transported tool to the nest, differing
267 significantly from the number of artificial foam ($z = 2.73$, $P < 0.05$) and parafilm tools ($z =$
268 2.73, $P < 0.05$) (Fig. 6). The fastest transport rate was observed for paper tools, which differed
269 significantly from every other tool type ($\chi^2 > 8.2$, $P < 0.05$, Fig. A5) except for sponges and
270 strings ($\chi^2 > 6.78$, NS, Fig. A5). Parafilm and artificial foam ($\chi^2 = 3.35$, NS) were transported
271 at a significantly slower rate than every other tool type ($\chi^2 > 8.66$, $P < 0.05$).

272 **DISCUSSION**

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

287   *A. senilis* could choose among six materials that differed in terms of soaking power
288 (paper, sponges, artificial foam, twigs, sting and parafilm). It is likely that ants of this species,
289 which live in sandy areas, were unfamiliar with most of these materials. Initially, *A. senilis*
290 showed no preference for any of the tools for dropping them into the baits, although paper
291 was transported faster to the bait compared to the artificial foam. However, along trials, ants
292 significantly preferred dropping paper and sponges into the baits, thus choosing in accordance
293 with the tools' soaking properties. A similar pattern was observed for tool transportation to

11

294 the nest: ants developed a preference for paper, followed by sponges, thus optimizing their

295 foraging effort by using the tools with the best soaking properties and ease of grasping

296 compared to other tool types.

297   Our results suggest that tool use to transport liquid to the nest in the two studied ant

298 species is not behaviourally fixed. Ants incorporated novel tools, many of them made of

299 artificial materials, into their foraging activities. Moreover, *A. senilis* learned within 10 trials

300 to select the best tool options available based on their soaking properties and *A. subterranea*

301 chose tools in relation to the characteristic of the food itself, e.g., food viscosity. This means

302 that ants can select tools according to both food and tool properties. Nevertheless, other

303 factors (besides a tool's soaking properties) may have played a role in determining ants'

304 preferences. One possibility is that the low density of some materials in the natural habitat

305 (e.g., leaf fragments are not abundant in the pine forest inhabited by these *A. subterranea*

306 colonies) may have been the reason for largely ignoring this item. However, a lack of

307 familiarity with potential tools cannot explain why *A. subterranea* used sponge pieces, which

308 were totally novel objects. Even more compelling are the data on *A. senilis,* which developed

309 a preference for unfamiliar materials during testing.

310

311   Handling effort is another factor that could have played an important role in tool

312 selection, perhaps in combination with the tool's soaking properties. During our observations

313 we noticed that *A. subterranea* workers had problems with grasping, handling and orienting

314 the leaf fragments, while the pieces of sponges could be grasped more easily. In fact, ants may

315 have torn apart sponges to reduce their size and facilitate handling. Although the soaking

316 power of sponges was lower when dropped into honey than into honey-water, their relatively

317 low weight made them more buoyant than other tool types and their irregular texture

318 increased their potential for being grasped. We found that the preference for particular items

319 often depended on the type of task that ants carried out. In particular, sponges may have been

320 preferentially transported to the nest, but small soil grains were the most dropped tools into

321 the baits. This suggests that selection among different tool types occurred both when first

322 encountered and also at the food source after the tools had been dropped into the baits. This

323 two-stage selection process might be especially important in the case of novel tools whose

324 properties are still unknown. Indeed, *A. senilis* ants transported very few foam fragments to

325   the bait in the first trial but subsequently increased their transportation during the 5th trial.
326   Nevertheless, foam fragments were rarely transported from the bait to the nest and by the 10th
327   trial ant workers even reduced their transportation to the baits. This suggests that tool
328   selection, after familiarization with novel material, may have also occurred at the baits. Other
329   authors observed that not all the tools dropped into the baits were transported into the nest
330   (Fowler, 1982; McDonald, 1984), corroborating our interpretation that further tool selection
331   can occur once the tools have been dropped into the liquid food source.

332   Availability, weight, soaking properties, easy handling and the possibility of shaping
333   the material to a desired form are therefore important factors in tool selection. The assessment
334   of these characteristics appears to be the result of a familiarization and learning process, at
335   least for the novel objects offered to the ants in our experiments. As the natural availability of
336   certain kinds of tools varies both spatially and seasonally, plasticity in tool selection is likely
337   to be adaptive. Tool use behaviour in ants may have evolved from the tendency shown by
338   many species to cover with debris liquid or viscous substances, which may otherwise cause
339   drowning or entanglement of workers near their nest (Fellers & Fellers, 1976; McDonald,
340   1984). Note, however, that it has been experimentally shown that *Aphaenogaster* ants
341   typically drop debris in food substances (Agbogba 1985; Banschbach et al., 2006) and our
342   experiments confirm this. The adaptive advantage of tool use in *Aphaenogaster* ants is that,
343   by using tools, foragers are capable of efficiently exploiting ephemeral food sources by
344   transporting much larger quantities of liquid nutrients than they could do by internal transport
345   (Fellers & Fellers, 1976; Tanaka & Ono, 1978), given the non-expandable crop characteristic
346   of myrmicine ants. Tool use might help these ants to compete with more dominant ant species
347   which are able to monopolize food sources by numbers: if the food is quickly covered by
348   debris, other ant species cannot exploit it and tool users can take the necessary time to bring
349   the soaked debris back to their nest (Banschbach et al., 2006; Fellers & Fellers, 1976).

350   We began our paper by referring to the research on flexible tool use in vertebrates
351   hoping to establish a link with the existing observations on invertebrate tool use. Our
352   experiments showed some indication that ants preferred materials with good soaking power
353   although other aspects such as ease of grasp may have also contributed to their choices.
354   Chimpanzees create a vegetable mass with good absorbent properties by chewing leaves that

13

355 they use to extract liquid from crevices (Goodall, 1986). It is unknown, however, whether

356 chimpanzees select certain plant species for their high soaking power. In a liquid extraction

357 task in the laboratory, Lehner, Burkart and van Schaik (2011) reported that captive orangutans

358 developed liquid extraction techniques based on materials with high soaking power (e.g.,

359 wood wool, paper) over those with low soaking power (e.g., leafless branch). Although this

360 may indicate that orangutans, like ants, were sensitive to the properties of such materials, the

361 lack of a direct and systematic comparison between different kinds of materials considerably

362 reduces the conclusions that one may be able to draw from that study. Nevertheless, other

363 studies with vertebrates in non-liquid extractive foraging tasks have systematically varied the

364 materials presented and have found evidence of selectivity in terms of hardness and weight

365 (Visalberghi et al., 2009), weight (Bird & Emery, 2009) or rigidity (Manrique et al., 2010).

366 Our findings with ants are in some ways comparable to those studies although admittedly, our

367 results are less clear than those reported in vertebrates. This is partly understandable because

368 our study is only the second systematic study on material selectivity in ants (besides Tanaka

369 and Ono, 1978) and we may have not fully considered a number of factors. For instance,

370 dropping a substantial number of tools (e.g., pine needles) inside the liquid and abandoning

371 them there may seem puzzling from the point of view of efficiency. Note, however, that

372 dropping those materials on the liquid may have accomplished another function (e.g.,

373 preserving the liquid food against exploitation from other species). Also, the absorbed liquid

374 may have altered the potential for grasping the potential tool object or increased its weight in

375 excess.Another possibility is that ants can only identify suitable materials *after* observing its

376 effect on the substance to be collected instead of selecting them *prior* to their use (e.g.,

377 artificial foam). Although selecting materials to collect liquid has not been described in

378 corvids or primates, selecting and manufacturing tools prior to their use is well-documented in

379 those taxa (e.g., Manrique et al., 2010; Sanz , Call & Morgan, 2009; Visalberghi et al., 2009;

380 Wimpeny et al., 2009). Whether ants (or any other species) can also select new materials prior

381 to experiencing its absorbent properties is an open question that future studies should address.

382   Some authors may question our use of the term tool use to describe the behaviour of

383 the ants. Tool use is after all a contentious term in the literature typically characterized by

384 rather long definitions, a number of clear examples, and some areas of substantial

385 disagreement (see Bentley-Condit & Smith, 2010; Shumaker et al., 2011). Although we
386 would be ready to accept a less contentious terminology such as 'material use', it is difficult
387 to do so when chimpanzees using leaves to extract liquid from crevices is considered a
388 classical example of tool use (Goodall, 1986). More importantly, we think that the behaviour
389 of the ants meets one of the most important criteria that define tool use in foraging contexts
390 (Beck, 1980; Bentley-Condit & Smith, 2010; St Amant & Horton, 2008), i.e., the use of an
391 external object to affect the position or location of another object or substance. Obviously
392 one could argue that nest building materials affect the position of other nesting materials,
393 which is why some authors object to the idea of tool use and would perhaps like to see either a
394 more restricted use of the term, or its complete abandonment for a wider term such as
395 construction behaviour (Hansell & Ruxton, 2008). Although we understand this position and
396 the reasons for it, given the above considerations, we still prefer to refer to the behaviour of
397 the ants as tool use, or the similar denomination of object use.

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

405

406

407 **REFERENCES**

408 Agbogba, C. (1985). Observations sur la récolte de substances liquids et de sucs animaux chez
409   deux espèces d'*Aphaenogaster*: *A. senilis* et *A. subterranea* (Hym. Formicidae).
410   *Insectes Sociaux*, *32*, 427–434.

411 Auersperg, A. M. I., Szabo, B., von Bayern, A. M. P., & Kacelnik, A. (2012). Spontaneous
412   innovation in tool manufacture and use in a Goffin's cockatoo. *Current Biology*, *22*,
413   903–904.

414   Banschbach, V. S., Brunelle, A., Bartlett, K. M., Grivetti, J. Y., & Yeamans, R. L. (2006).
415   Tool use by the forest ant *Aphaenogaster rudis*: ecology and task allocation. *Insectes*
416   *Sociaux*, *53*, 463–471.

417   Barber, J. T., Ellgaard, E. G., Thien, L. B., & Stack, A. E. (1989). The use of tools for food
418   transportation by the imported fire ant, *Solenopsis invicta*. *Animal Behaviour*, *38*, 550–
419   552.

420   Bates, D., Maechler, M., Bolker, B., & Walker, S. (2013). lme4: Linear mixed-effects models
421   using Eigen and S4. R package version 1.0-5 http://CRAN.R-
422   project.org/package=lme4, published online 25 October 2013.

423   Beck, B.B. (1980). *Animal tool behavior: the use and manufacture of tools by animals*. New
424   York, Garland Publishing STPM Press.

425   Bentley-Condit, V. K., & Smith, E. O. (2010). Animal tool-use: current definitions and an
426   updated comprehensive catalog. *Behaviour, 147*, 185–221.

427   Bird, C. & Emery, N., J. (2009). Insightful problem solving and creative tool modification by
428   captive nontool-using rooks. *Proceedings of the National Academy of Sciences USA*,
429   *106*, 10370–10375.

430   Bhatkar, A. & Whitcomb, W. H. (1979). Artificial diet for rearing various species of ants.
431   *Florida Entomologist, 53*, 229–232.

432   Boulay, R., Hefetz, A., Cerdá, X., Devers, S., Francke, W., Twele, R., & Lenoir, A.
433   (2007). Production of sexuals in a fission-performing ant: dual effects of queen
434   pheromones and colony size. *Behavioral Ecology & Sociobiology*, *61*, 1531–1541.

435   Chappell, J. & Kacelnik, A. (2002). Tool selectivity in a non-mammal, the New Caledonian
436   crow (*Corvus moneduloides*). *Animal Cognition, 5,* 71–78.

437   Czechowski, W., Radchenko, A., Czechowska, W., & Vepsäläinen, K. (2012). *The ants*
438   *(Hymenoptera, Formicidae) of Poland with reference to the myrmecofauna of Europe*.
439   Warszawa, Poland: MIZ PAS.

440   Davidson, D. W., Cook, S. C., & Snelling, R. (2004). Liquid feeding performances of ants
441   (Formicidae): ecological and evolutionary implications. *Oecologia*, *139*, 255–266.

16

442 Delage, B. & Jaisson, P. (1969). Etude des relations sociales chez des Fourrnis du genre
443   *Aphaenogaster*. *Comptes Rendus de l'Académie des Sciences Paris, 268*, 701–703.

444 Devetak, D & Arnett, A. (2015). Preference of antlion and wormlion larvae (Neuroptera:
445   Myrmeleontidae; Diptera: Vermileonidae) for substrates according to substrate particle
446   sizes. *European Journal of Entomology, 112*, 500-509.

447 Evans, H. E. & Eberhard, M. J. W. (1970). The wasps. Ann Arbor, USA: The University of
448   Michigan Press.

449 Fellers, J. H. & Fellers, G. M. (1976). Tool use in a social insect and its implications for
450   competitive interactions. *Science*, *192*, 70–72.

451 Fowler, H. G. (1982). Tool use by *Aphaenogaster* ants: a reconsideration of its role in
452   competitive interactions. *The Bulletin of New Jersey Academy of* Science, *27*, 81–82.

453 Goodall, J. (1986). *The chimpanzees of Gombe. Patterns of behavior*. Cambridge, MA: The
454   Belknap Press of Harvard University Press

455 Hansell, M. & Ruxton, G. D. (2008). Setting tool use within the context of animal
456   construction behaviour. *Trends in Ecology & Evolution, 23*, 73-78.

457 Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2008). Implementing a class of
458   permutation tests: the coin package. *Journal of Statistical Software*, *28*, 1–23,
459   http://www. jstatsoft.org/v28/i08/.

460 Hölldobler, B. & Wilson, E. O. (1990). *The ants*. Cambridge, USA: Belknap Press of Harvard
461   University.

462 Hunt, G. R. & Gray, R. D. (2002). Species-wide manufacture of stick-type tools by New
463   Caledonian crows. *Emu*, *102*, 349–353.

464 Lehner, S.R., Burkart, J.M., & van Schaik, C.P. (2011). Can captive orangutans (*Pongo*
465   *pygmaeus abelii*) be coaxed into cumulative build-up of techniques? *Journal of*
466   *Comparative Psychology, 125*, 446-455.

467 Lőrinczi, G. (2014). Some notes on the tool-using behaviour of the ant, *Aphaenogaster*
468   *subterranea* (Hymenoptera: Formicidae). *Tiscia*, *40*, 17–24.

469 Manrique, H. M., Gross, A. N., & Call, J. (2010). Great apes select tools based on their
470   rigidity. *Journal of Experimental Psychology: Animal Behavior Processes, 36,* 409–
471   422.

472 Martin-Ordas, G., Schumacher, L., & Call, J. (2012). Sequential tool use in great apes. *PLoS*
473   *One, 7*, e52074.

474 McDonald, P. (1984). Tool use by the ant, *Novomessor albisetosus* (Mayr). *Journal of New*
475   *York Entomological Society*, *92*, 156–161.

476 Morrill, W. L. (1972). Tool using behaviour of *Pogonomyrmex badius* (Hymenoptera:
477   Formicidae). *Florida Entomologist*, *55*, 59–60.

478 Mulcahy, N. J., Call, J., & Dunbar, R. I. M. (2005). Gorillas and orangutans encode relevant
479   problem features in a tool-using task. *Journal of Comparative Psychology*, *119*, 23–
480   32.

481 Pierce, J. D. (1986). A review of tool use in insects. *Florida Entomologist, 69*, 95–104.

482 Sanz, C., Call, J., Boesch, C. (2013). Tool Use in Animals: Cognition and Ecology. New
483   York, USA: Cambridge University Press.

484 Sanz, C., Call, J., & Morgan, D. (2009). Design complexity in the tool use of chimpanzees
485   (*Pan troglodytes*) in the Congo basin. *Biology Letters, 5*, 293–296.

486 Scharf, I., Lubin, Y. & Ovadia, O. (2011). Foraging decisions and behavioural flexibility in
487   trap-building predators: a review. *Biological Reviews, 86*, 626–639.

488 Seifert, B. (2007). *Die Ameisen Mittel- und Nordeuropas.* Görlitz, Germany: Lutra Verlags
489   und Vertriebsgesellschaft.

490 Shumaker, R. W., Walkup, K. R., & Beck, B. B. (2011). *Animal tool behavior: The use and*
491   *manufacture of tool by animals*. Baltimore, USA: The Johns Hopkins University Press.

492 St. Amant, R. & Horton, T. (2008). Revisiting the definition of animal tool use. *Animal*
493   *Behaviour, 75*, 1199-1208.

494 Stukalyuk, S. V. & Radchenko, V. G. (2011). Structure of multispecies ant assemblages
495   (Hymenoptera, Formicidae) in the Mountain Crimea. *Entomological Review*, *91*, 15–
496   36.

497 Tanaka, T. & Ono, Y. (1978). The tool use by foragers of *Aphaenogaster famelica*. *Japanese*
498   *Journal of Ecology*, *28*, 49–58.

499 Visalberghi, E., Addessi, E., Spagnoletti, N., Truppa, V., Ottoni, E., Izar, P., & Fragaszy, D.
500   (2009). Selection of effective stone tools by wild capuchin monkeys. *Current Biology*,
501   *19*, 213–217.

502 Wimpenny J. H., Weir A. A. S., Clayton L., Rutz C., & Kacelnik, A. (2009). Cognitive
503   Processes Associated with Sequential Tool Use in New Caledonian Crows. *PLoS One*,
504   *4*, e6471.

505

506 **Table 1.** Soaking properties of the different tools used by *Aphaenogaster subterranea.*

	Imbibed /Initial weight (weight ratio)	
Tool type	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

507 **Table 2.** Soaking properties of the different tools used by *Aphaenogaster senilis.*

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

508

Figure legends

510 **Figure 1**. Experimental setup and tool types offered to *Aphaenogaster subterranea*.

511 **Figure 2.** Experimental setup and tool types offered to *Aphaenogaster senilis.*

512 **Figure 3.** Number of different tools transported to honey-water (a) and honey (b) baits by
513 *Aphaenogaster subterranea* workers. Box plots show the median (internal line), quartiles and
514 range (whiskers). Different letters above boxes represent groups that differ significantly from
515 each other.

516 **Fig. 4.** Number of different tools transported into the nest from the honey-water (a) and honey
517 (b) baits by *Aphaenogaster subterranea* workers. Box plots show the median (internal line),
518 quartiles and range (whiskers). Different letter s above boxes represent groups that differ
519 significantly from each other.

520 **Figure 5.** Number of different tools transported to the baits in the 1st, 5th and 10th trials by
521 *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and range
522 (whiskers).

523 **Figure 6.** Number of different tools transported into the nest in the 1st, 5th and 10th experiment
524 by *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and
525 range (whiskers).

526 **Appendix - Figure legends**

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

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

533   **Figure A3.** Estimated functions of tool transport time into the nest from the honey-water (a)

534   and honey (b) baits by *Aphaenogaster subterranea* workers (Log-rank test). Different tool

535   types are depicted by different colours.

536   **Figure A4.** Summed number of tools transported on the baits and into the nest during the

537   three trials (box plots show: median, quartiles and range). Different letters above boxes

538   represent groups that differ significantly from each other.

539   **Figure A5.** Estimated functions of tools transport time on the baits (a; c; e) and into the nests

540   (b, d, f) in the 1st (a, b), 5th (c, d) and 10th (e, f) experiment by *Aphaenogaster senilis* workers

541   (Log-rank test). Different tool types are depicted by different colours.

Arena entrance

Test arena

Aluminium paper

1.Water, 2. Diluted honey
3. Honey

$7\ cm^3$ tiny soil grains

$7\ cm^3$ leaf fragments

12 cm

$7\ cm^3$ sponges
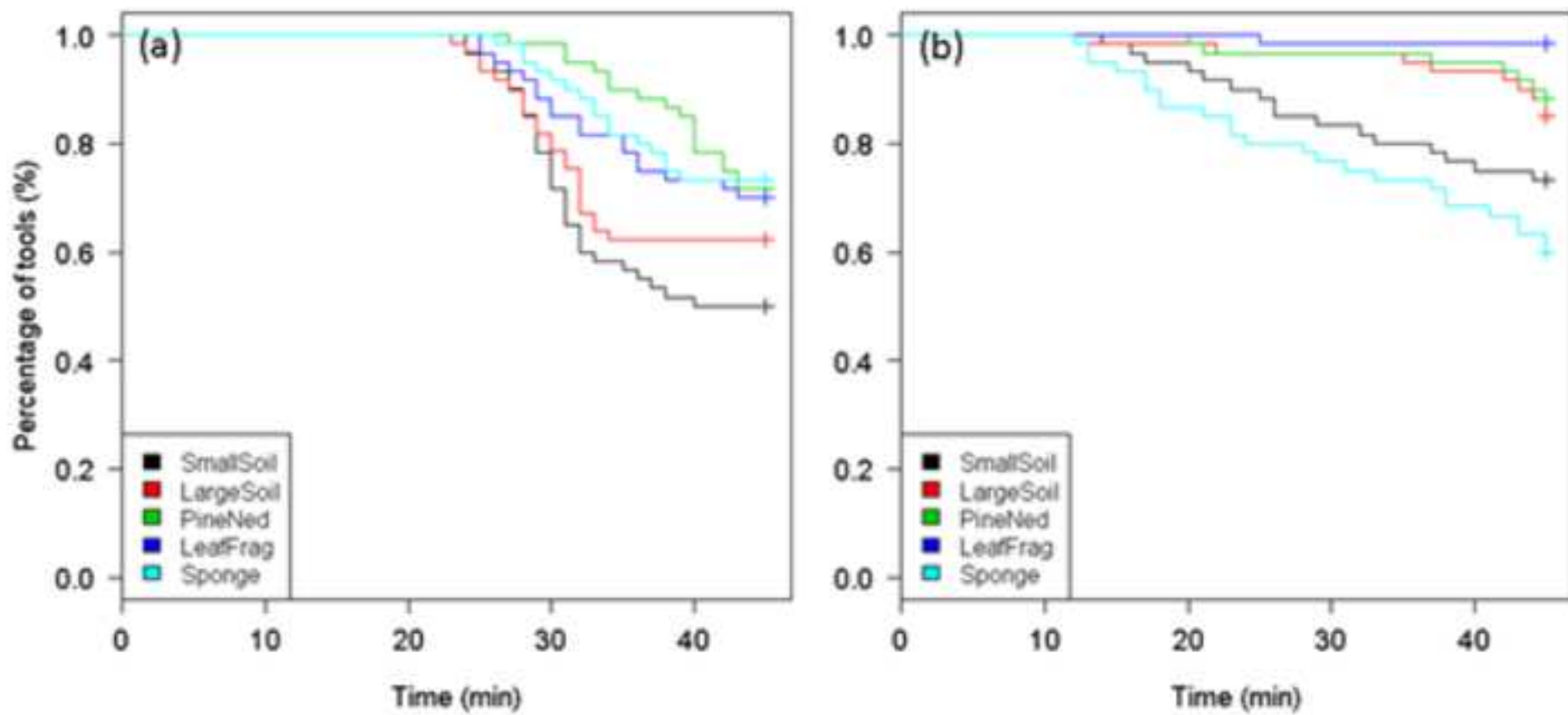
$7\ cm^3$ large soil grains

$7\ cm^3$ pine needles

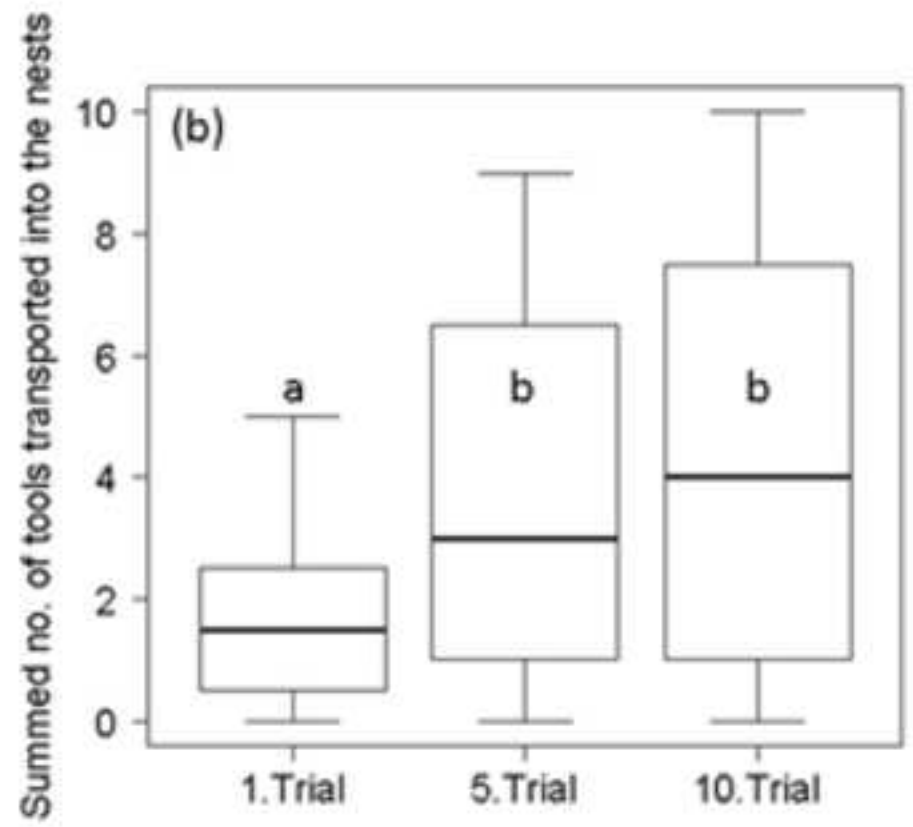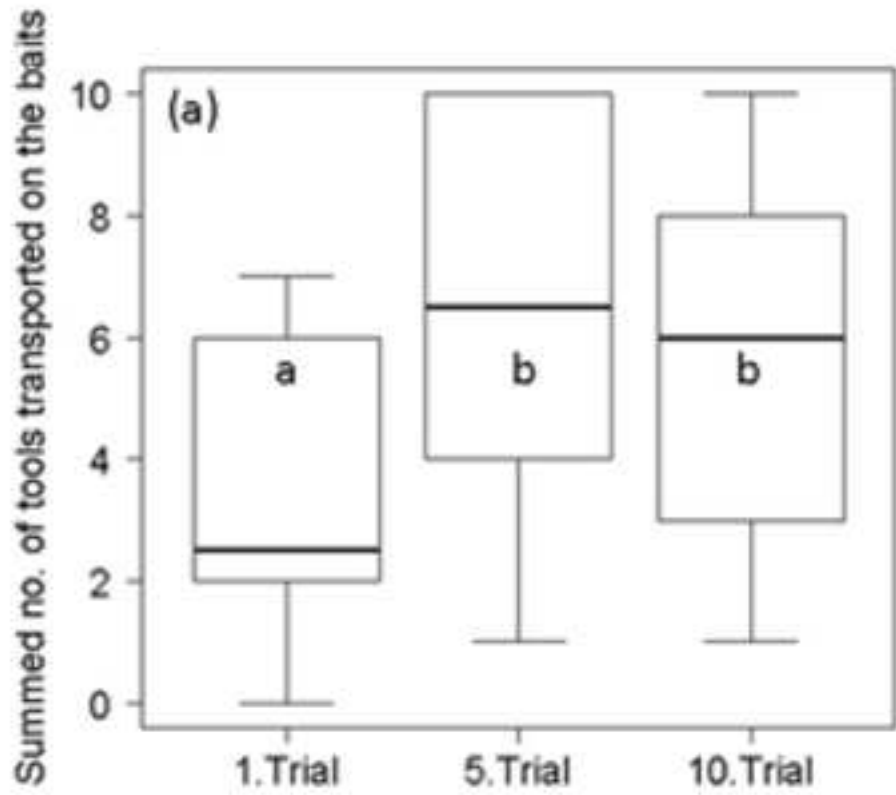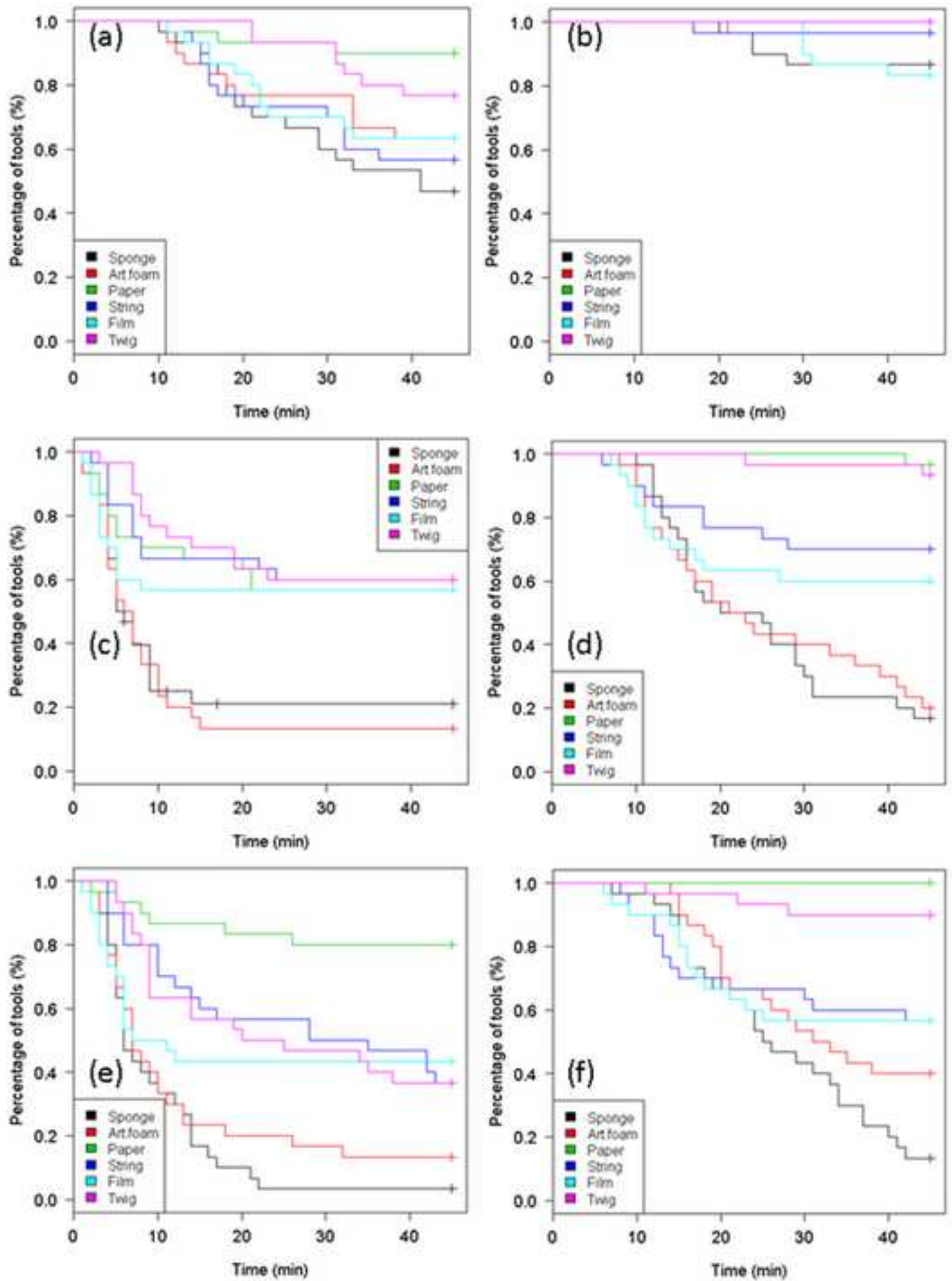**Figure 2**
Click here to download high resolution image

Figure 2

Figure 5

Figure 6