Age dependent spatio-temporal learning in the ant *Myrmica sabuleti* (Hymenoptera : Formicidae)

Marie-Claire CAMMAERTS

Faculté des Sciences, DBO, Université Libre de Bruxelles, 50, A. F. Roosevelt, 1050 Bruxelles. Belgium
(e-mail : mtricot@ulb.ac.be ; phone n° : 32 2 673 49 69 ; fax : 32 2 650 2445)

Abstract

Spatio-temporal learning has been examined separately in young, middle aged and old workers of the ant *Myrmica sabuleti*. Young workers could not acquire such learning. Middle aged ants could perfectly acquire spatio-temporal learning, essentially for sugar food. Old workers, though still able to acquire spatio-temporal learning, however forage nearly everywhere, at any times o'clock. Therefore, comparatively with middle aged ants, old ants are less efficient for collecting food continuously or periodically available on given places, but are more able to detect new food sources.

Keywords : food collection, ontogenesis, time place learning, training, Formicidae.

Introduction

In ant societies, each member recognizes its congeners, can communicate with them and perform, often stereotypically, more or less complex tasks, in accordance with its caste and age. We studied such social tasks, e.g. recruitment (CAMMAERTS, 1978), spatial and temporal learning (CAMMAERTS, 2004), navigation (CAMMAERTS & RACHIDI, 2009 ; CAMMAERTS *et al.*, 2012 ; CAMMAERTS, 2012) and recently examined spatio-temporal learning, in three *Myrmica* species (CAMMAERTS, 2013). These ants could acquire spatio-temporal learning but their collective score was low, generally equaling 40%. Thus, not all members of the colony may acquire time place learning. Such ability might be not innate, but progressively acquired during the worker's life. It might emerge at the same time as – or later than – the recognition of the specific nest odor (BOS & D'ETTORRE, 2012). Suspecting some ontogenesis for spatio-temporal learning ability, we isolated, from two colonies of *Myrmica sabuleti* Meinert, 1861, young, middle aged and old workers and tempted on them spatio-temporal learning.

Material and methods

Collection and maintenance of ants

Twelve colonies of *M. sabuleti* were maintained in the laboratory in artificial nests and under rearing conditions identical to those described in our previous work (CAMMAERTS, 2013). Their brood development was examined every day until many callows and, later on, newly emerged workers appeared, at the same time, in two colonies (labeled I and II) collected at Marchin (Condroz, Belgium) and containing queens, brood and more than 500 workers.

From each of these two colonies, we withdrew 50 workers being 3 to 6 months old which were located inside of the nest, 50 workers being 1 to 2 years old which were moving at the nest entrance and on the foraging area, as well as 50 very pigmented workers which were moving on the foraging area far from the nest. Six larvae were also withdrawn, two ones being added to each three groups of differently aged ants. Each of these groups was maintained in a small nest made of one glass tube, half filled with water and set in a tray (34 cm x 23 cm x 3.5 cm). Food used for tempting to obtain spatio-temporal learning consisted of a piece of cornet beef and one of a *Tenebrio molitor* Linnaeus, 1758 larvae on one hand and of a droplet of sugar water on the other, each kind of food being delivered at a precise time o'clock, on a glass slide (7.6 cm x 2.6 cm) located at a given place (see below).

Table 1. Experimental planning used to study spatio-temporal learning ability of young, middle aged and old ants. Details are given in the 'Material and Methods' section. The experimental protocol is schematically presented in previous work (CAMMAERTS, 2013, Fig. 1). I, II : the two colonies from which ants were removed ; Tr : training ; *control* : control experiment ; test : test experiment ; M b : marking in blue the ants coming on the meat site ; C b : counting, on the sugar site, the blue marked and not marked ants ; M y : marking in yellow the ants coming on the sugar site ; C y : counting, on the meat site, the yellow marked and not marked ants.

days	I young	I middle aged	I old	II young	II middle aged	II old
1	Tr			Tr		
2	Tr *control*			Tr *control*		
3	Tr	Tr		Tr	Tr	
4	Tr *control*	Tr *control*		Tr *control*	Tr *control*	
5	Tr test	Tr	Tr	Tr test	Tr	Tr
6	Tr *control*	Tr *control*	Tr *control*	Tr *control*	Tr *control*	Tr *control*
7	Tr test	Tr test	Tr	Tr test	Tr test	Tr
8	Tr *control*	Tr *control*	Tr *control*	Tr *control*	Tr *control*	Tr *control*
9	Tr test	Tr test	Tr test	Tr test	Tr test	Tr test
10	Tr	Tr *control*	Tr *control*	Tr	Tr *control*	Tr *control*
11	Tr test	Tr test	Tr test	Tr test	Tr test	Tr test
12	Tr	Tr	Tr *control*	Tr	Tr	Tr *control*
13	M b	Tr test	T test	M b	Tr test	Tr test
14	C b M y	Tr	Tr	C b M y	Tr	Tr
15	C y	M b	Tr test	C y	M b	Tr test
16		C b M y	Tr		C b M y	Tr
17		C y	M b		C y	M b
18			C b M y			C b M y
19			C y			C y

Experimental design and protocol

Each nest tube was set so that it opened in the middle of the tray. There were two food sites consisting, each one, of a glass slide deposited along the tray border, one slide on the left, the other on the right of the nest opening.

The experimentation ought to be done as soon as possible to avoid social regulation in each group of differently aged ants. The final marking manipulations being rather long lasting and difficult, the three differently aged groups of ants began to be experimented not strictly at the same time, but with a time-lag of two days between each other (Tab. 1). A two day period is a short time, at any time of an ant's life, comparatively to the duration of that ant's life.

The experimental protocol is schematically presented in a previous paper (CAMMAERTS, 2013, Fig. 1) and partly illustrated in Fig. 1.

For each group, during 12 consecutive days, a piece of cornet beef and one of a *Tenebrio molitor* larvae was delivered on the glass slide located on the left of the nest opening from 10:45 to 11:00 and sugar water was provided on the glass slide located on the right of the nest opening from 21:45 to 22:00. During each food presentation, the ants present on each glass slide were counted every 30 sec, during 15 minutes, a total of 30 counts being obtained for each colony and food site. The mean of these counts were calculated for each of the two colonies and each food site, and the mean number of ants present on each kind of food site was established (Fig. 2).

Control experiments were made on days 2, 4, 6 and 8 at 15:00. The ants present on each glass side were then counted 15 times and the mean number of ants present on each food site established (Tab. 2). In the same way, test experiments were performed on days 5 (= after a minimum learning time period), 7, 9 and 11 at 10:38 and 21:38. The mean number of ants present on each food site when food ought to be delivered was so established (Tab. 2). When a test was made, the food delivery – i.e. the training session – occurred just after.

After the experimentation, on day 13, an additional experiment was performed as follows. At 10:38, the ants of colony I coming onto the meat food site during 15 min were collected and marked in blue (Airfix® enamel), then put back onto their foraging area and meat food was delivered on the appropriate glass slide until 11:08. At 21:38, the ants of colony II coming onto the sugar site during

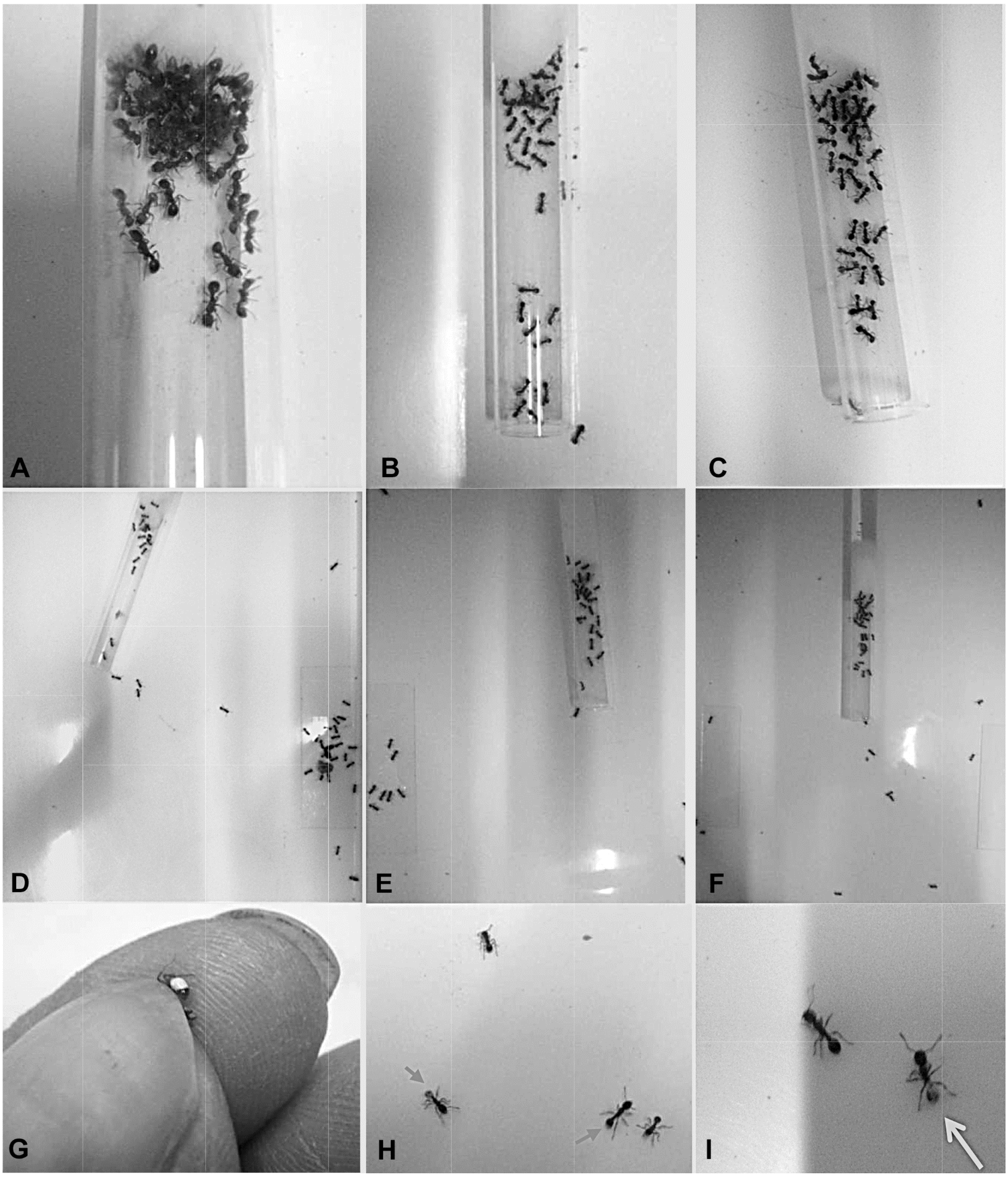

Fig. 1. **A**: a group of young workers. **B**: a group of middle aged workers. **C**: a group of old workers. **D**: a training experiment at 10:45, the meat delivery time, on young workers. **E**: a training experiment at 21:45, the sugar water delivery time, on old workers. **F**: a test experiment at 10:38, the meat delivery time, on middle aged workers. **G**: an ant marked in blue. **H**: middle aged ants coming on the sugar site after that those having visited the meat site were marked in blue: the 'blue' ants (blue arrows) know the place and time of the two food deliveries. **I**: middle aged ants coming on the meat site after that those having visited the sugar site were marked in yellow: the 'yellow' ant (yellow arrow) knows the place and time of the two food deliveries.

15 min were collected and marked in yellow, then put back into their colony. While doing so, the ants of colony I marked and not marked, coming on the sugar site, were counted during 15 min (Tab. 3). Just after that, sugar water was delivered on the appropriate glass slides, to the two colonies, and withdrawn at 22:08. The following day, at 10:45, the ants of colony II, marked and not marked, coming on the meat food site, were counted during 15 min (Tab. 3). For each group of ants, the proportion of marked and not marked ants seen on each food site was established.

Statistical analysis

To compare the four counts obtained for a given food site to the corresponding counts obtained for the other food site as well as to the corresponding four control counts, the Wilcoxon test for related samples (SIEGEL & CASTELLAN, 1989) was used for information only, as explained in CAMMAERTS (2013). The difference between the two compared sets of four numbers was accepted as being highly significant when T = 10, slightly significant when T = 9 and not significant when T < 9.

Results

Ants coming on the food sites during training experiments

Only few young ants came onto the two food sites : the maximum of individuals ever seen on a site was five. At the beginning of the experimentation, they nearly did not come on the food sites and, at the end, they ceased to visit the meat site (Fig. 2 upper graphs).

As soon as the experiment began, middle aged ants almost only visited the sites where food was delivered. During the ten first days, they were more numerous on the meat site and after, they became equally numerous on the two food sites (Fig. 2 middle graphs).

Since the beginning of the experiment, old ants visited the sites where food was delivered, mostly the meat site. But they also foraged on the sites where no food was offered and left them after a few minutes (Fig. 2 lower graphs).

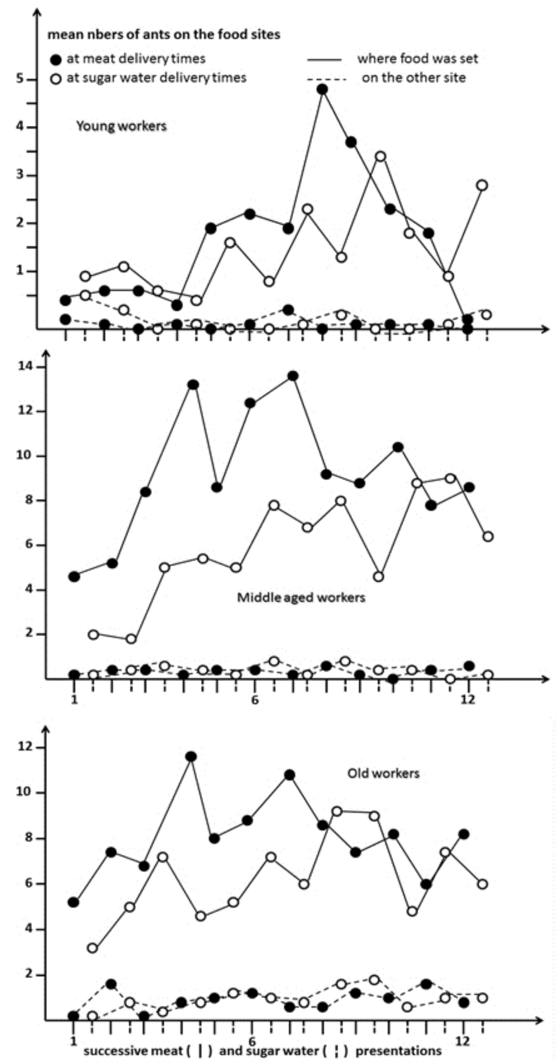

Fig. 2. Mean numbers of ants present on the meat and the sugar sites at meat (*filled circles*) and sugar (*empty circles*) delivery times, where food was offered (*solid lines*) and on the other empty site (*dotted lines*). These numbers, collected during training experiments, allow presuming potential acquisition of time place learning by the three differently aged ants.

Ants coming on the food sites during control and test experiments

During the control experiments, meanly 0.2 and 0.2 young ants visited the meat and the sugar sites respectively. No increase occurred in the course of time. The number of these ants visiting the meat site at the correct time was not larger than the control number as well as than the number of ants then visiting the sugar site. The number of young ants present on the sugar site at the correct time was not larger than the control number but somewhat larger than the number of ants then visiting the meat site (P = 0.0625) (Tab. 2 upper part). Young ants seemed thus unable to acquire spatio-temporal learning, seemed able to learn where meat and sugar were offered and preferred sugar food.

The number of middle aged ants coming on the meat and sugar sites in the course of the control experiments did not increase and meanly equaled 0.5 and 0.2 respectively. The number of these ants present on the meat site at the correct time was larger than the control number as well as that of ants then present on the sugar site (P = 0.0625). The number of these ants seen on the sugar site at the correct time was larger than the control number and that of ants then seen on the meat site (P = 0.0625) (Tab. 2 middle part). Middle aged ants had thus an obvious tendency in acquiring spatio-temporal learning.

Table 2. For each kind of ants, mean number of ants of two colonies present on the meat and the sugar sites during four controls (made on days 2, 4, 6, 8) and four tests (made on days 5, 7, 9, 11).

Nº of the experiment	site where ants were counted	control experiment at 15:00 – 15:15	test experiment at 10:38 – 10:53 meat time	test experiment at 21:38 – 21:53 sugar time
Young ants				
1	meat	0.2	0.4	0.4
	sugar	0.3	0.0	0.5
2	meat	0.4	0.3	0.0
	sugar	0.0	0.3	0.3
3	meat	0.4	0.1	0.0
	sugar	0.3	0.1	0.1
4	meat	0.0	0.5	0.0
	sugar	0.1	0.0	0.1
mean	meat	0.2	0.3	0.1
	sugar	0.2	0.1	0.3
Middle aged ants				
1	meat	0.4	1.4	0.5
	sugar	0.1	0.6	1.3
2	meat	0.6	2.0	0.3
	sugar	0.0	0.1	1.3
3	meat	0.4	2.8	0.0
	sugar	0.6	0.6	1.4
4	meat	0.6	1.6	0.4
	sugar	0.3	1.0	2.0
mean	meat	0.5	1.9	0.3
	sugar	0.2	0.6	1.5
Old ants				
1	meat	1.4	1.6	0.4
	sugar	1.3	1.1	0.9
2	meat	1.3	2.4	0.8
	sugar	0.8	1.0	0.9
3	meat	2.3	1.5	1.4
	sugar	0.6	1.6	1.1
4	meat	0.9	1.5	1.0
	sugar	1.3	2.0	1.3
mean	meat	1.4	1.8	0.9
	sugar	1.0	1.7	1.0

Table 3. Proportion of ants, marked while coming on a food site at the correct time, among those visiting the other site at the correct time. For each kind of ants, ants of colony I and II coming on the meat and the sugar sites respectively were marked (second column). Thereafter, at the next feeding time, marked and not marked ants present at the correct times on the sugar and the meat sites respectively were counted and the means established (fourth column). Knowing the total mean number of ants present on a site (third column), the percentages of those having been marked at the other feeding site were established (fifth column).

Kinds of ants	Colony (No. of marked ants)	Ants present on the other food site: mean No. present	mean No. marked	% marked
Young ants	I (2 on meat site)	1.3 on the sugar site	1.1	10.1
	II (2 on sugar site)	1.8 on the meat site	0.3	14.3
Middle aged ants	I (14 on meat site)	2.5 on the sugar site	1.4	54.0
	II (17 on sugar site)	3.2 on the meat site	2.3	70.8
Old ants	I (9 on meat site)	1.7 on the sugar site	1.0	57.9
	II (9 on sugar site)	1.5 on the meat site	0.9	57.9

In the course of the control experiments, the old ants were rather numerous, meanly equaling 1.4 and 1.0 for the meat and the sugar sites respectively. Their number on the meat site at the correct time was not larger than the control number (P = 0.3125) nor than that of ants then visiting the sugar site. The number of these ants seen on the sugar site at the correct time was not larger neither than the control number nor than that of ants then seen on the meat site (P = 0.5625) (Tab. 2 lower part). Consequently, old workers' potential spatio-temporal learning could not be detected due to the pronounced foraging behavior of these ants.

Proportion of ants correctly visiting the two food sites

If the ants had not learned the two food sites (but only one, for instance), the number of ants marked on one site, then seen on the other site, would be at the most equal to half of the control number obtained for this food site. The different control numbers and their means are given in Tab. 2.

Only 10% of the young ants present on the sugar site had previously visited the meat site at the correct time and about 14% of those seen on the meat site at the correct time were previously present on the sugar site at the correct time (Tab. 3 upper part). These few ants have a tendency in acquiring spatio-temporal learning but, in general, the young workers did not know where nor when which kind of food was available.

Fourteen middle aged ants coming on the meat site at the correct time were marked. Among the 2.5 ants coming on the sugar site at the correct time, 1.4 appeared marked. So, 54% of the ants knowing the sugar delivery characteristics have also learned those of the meat offering. Seventeen middle aged ants visiting the sugar site at the correct time were marked. Among the 3.2 ants coming on the meat site at the correct time, 2.3 were marked. So, 71% of the ants knowing the meat delivery characteristics also memorized those of the sugar offering (Tab. 3 middle part). Middle aged workers acquired thus spatio-temporal learning with a mean score of 62.5%. We observed that these ants foraged nearly exclusively at the correct times, during the adequate period of time and in the surroundings of the correct food sites. They are efficient in collecting food periodically available on given places.

Nine old ants correctly visiting the meat site were marked. At the next sugar time, 1.0 among 1.7 ants coming on the site appeared to be marked. So, among the old ants knowing the sugar delivery characteristics, about 58% have also learned those of the meat offering. Nine old ants coming on the sugar site at the correct time were marked. At the next meat time, 0.9 among 1.5 ants visiting the meat site were found to be marked. So, among the ants knowing the meat delivery characteristics, 58% also memorized those of the sugar ones (Tab. 3 lower part). Nearly 60% of the old foragers could thus acquire spatio-temporal learning. However, these ants continuously foraged everywhere, seldom coming back into their nest. They are thus not very efficient in collecting localized and periodically available food, but may be very efficient in collecting occasional, unexpected food.

Supplementary observations

Unquantified personal observations showed that young workers often stay at rest, did not perform trophallaxis and had difficulties in returning to their nest and coming back to a food site. They were unable to deposit a trail. When they apparently did so, it could be stated, on the basis of congeners' locomotion, that they wrongly deposit together their poison and their Dufour glands' content. They did not recruit congeners and did not attack a living prey. In fact, they were unable to perform most of the social tasks.

Middle aged workers were very efficient in collecting food, going directly onto the sites, quickly returning to their nest. They strongly took a living prey in their mandibles, eat it while still alive and sometimes began to transport it towards the nest. They soon regulated their small society into younger ants staying in the nest near the larva, other ones staying in the middle of the nest, ready to go out when necessary, and a few ones staying at the nest entrance and going out from time to time. The latter ants went, without delay, just at the correct food delivery times on the adequate food sites. None of the middle aged ants died during the eighteen experimenting days.

Most of the old workers were nearly continuously foraging. They did not well care for the larva. They engaged in trophallaxis, laid trails and recruited only few congeners since these were already 'at work' on the foraging area. They stung living preys, often did not eat them but transported them towards the nest. Among the 100 used old ants, six died and surprisingly, the living ants eat their dead congeners.

Discussion

The present work shows that :
- Young ants have not yet the notion of their surrounding space and of the running time and so, have only a slight tendency in acquiring spatio-temporal learning.
- Middle-aged ants have a precise notion of their surrounding space and of running time ; they can thus acquire spatio-temporal learning, essentially for the sugar food. They forage nearly only on the adequate sites, at appropriate times o' clock. They rapidly recruit nestmates. They are efficient in collecting food available on given places, at precise times, during a limited time period.
- Old ants have a correct notion of space and running time. They can acquire spatio-temporal learning, but are not efficient in it since they forage nearly all the time everywhere. Seldomly returning to their nest, they quickly attack living prey and recruit only few congeners. They are appropriate for collecting unexpected, occasional food.

The production of mandibular glands (alarm, attractive pheromone), Dufour gland (attractive and area marking pheromone) and poison gland (trail pheromone) secretions by differently aged workers as well as the behavioral reactions of these differently aged ants to these secretions have been studied in the ant *M. rubra* (CAMMAERTS-TRICOT, 1974 ; CAMMAERTS-TRICOT & VERHAEGHE, 1974). The amount of secretion produced increases in the course of the workers' life and the ants' reactions to these secretions also change along the ants' life. Another work (CAMMAERTS-TRICOT, 1975) demonstrated that young ants do not lay down a trail while middle aged ants lay down a trail along long distances and old ants do so slightly. Young workers do not attack prey, nor come back to their nest ; middle aged ants essentially return to their nest, recruit congeners and come back to prey ; old ants essentially attack prey and seldomly go back to the nest. These previous observations are in agreement with the present ones.

Present and previous results thus show that a progression occurs in the course of the workers' life as for the production of pheromones, the reaction to these pheromones, the behavior in front of a living prey (attack, recruitment) and the learning of the most probable places and times o' clock for food occurrences. In fact, several social behaviors are not innate, but acquired in the course of the workers' life, from basic ones – as trophallaxis (personal observation) and nest odor recognition (ERRARD & HEFETZ, 1997 ; BOS & D'ETTORE, 2012) – to sophisticated ones, as the learning of time periods and places of food occurrences (present work). Many social knowledge and tasks performing might have their own ontogenesis.

In general, e.g. in non-social species, some of the animals' abilities are innate while other ones are learned in the course of life (due to operant conditioning, for instance). In social species, innate acts should exist, but learned abilities may be predominant (GOLDBERG, 1998). Moreover, learning may have a social character, e.g. a social individual may better learn (or only learn) while living inside of its colony than when living alone. As a matter of fact, learning may be enhanced thanks to an efficient communication between the members of the colony. This was demonstrated by GOLDBERG (1975) on termites as for their learning of a maze travelling. It is also known that, generally, a social individual performs, in the course of its life, successively different tasks what leads to an age-based division of labor. A first step may be the learning of the odor or other distinctive characters of the group and species. In ants, studies on this topic are only in their early stages. Imprinting process has been demonstrated for brood recognition (JAISSON, 1975) and nest odor learning (BOS & D'ETTORRE, 2012). The critical time period for such learning should be short (HINDE *et al.*, 1956 ; GUITON, 1959), the phenomenon being thus rather difficult to examine. However, the ontogenesis of the ants' cognitive abilities is our future research topic.

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