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Organization of honeydew collection by foragers of different species of ants (Hymenoptera: Formicidae): Effect of colony size and species specificity

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Abstract. Aphid honeydew is one of the main energy sources for various ants in the temperate zone, nevertheless relatively little is known about the organization of the work of honeydew foragers (aphid milkers). This study focuses on the honeydew collecting strategies used by different ants in steppe and forest multi-species communities in Western Siberia. The behaviour of marked foragers of 12 species (*Formica* – 7, *Lasius* – 2, *Camponotus* – 1, *Myrmica* – 2) was recorded. Depending on the degree of the aphid milker specialization and degree of protection of the aphids five honeydew collecting strategies of various complexity were distinguished: unspecialized foragers in (I) "unprotected" aphid colonies (attended by ants <60% of time) and (II) "protected" colonies (attended >95% of time); (III) low "professional" specialization (ants "on duty" constantly attending aphid colonies); (IV) medium and (V) high "professional" specialization (clear division of tasks: honeydew collecting by "shepherds" and protection of trophobionts by "guards"; and honeydew transportation by "transporters" in V). Task specialization of the honeydew foragers is facultative: different ant taxa demonstrate a certain range of the honeydew collecting strategies of different complexities (*Formica* – I–V, *Lasius* – I–II, *Camponotus* – III, *Myrmica* – I–II) depending on the needs of their colony. The strategy used by ants did not depend on the species of aphid attended, but is strongly dependent on the species of ant, their colony size, available food resources and seasonality. In summer, the aphid milker specialization becomes more complex as ant colony size increases at both intra- and inter-specific levels and when food is scarce. In autumn *Formica* s. str. ants, which have the most advanced foraging strategy, adopt a simpler honeydew collecting strategy. Overall, the variety of honeydew foraging strategies seems to reflect the unequal contribution of different ants in forming trophobiotic interactions with aphids.

INTRODUCTION

Social insects (ants, bees, wasps and termites) dominate many terrestrial ecosystems (Wilson, 1971). Their largest colonies consist of thousands to millions of workers (Beshers & Fewell, 2001; Jeanne & Taylor, 2009). The great ecological success of social insects as manifested in their diversity and biomass is thought to be due to the complex organization of their societies based on a clear division of labour (Wilson & Hölldobler, 2005). There are three general patterns in the division of labour among workers in social insects based on: worker age polyethism, worker polymorphism and individual differences in task specialization (Robinson, 1992). In recent years the organization of work in social insect societies has been actively explored both at the colony level (division of labour) and within small groups of foragers that have the same goal (Jeanne, 1991; Ratnieks & Anderson, 1999). These small groups of foragers appear to be a good model for investigating the interactions between individual insects. So-called task partitioning (the division of discrete task among workers within a team) is quite often recorded for foragers of social insects, particularly, ants, when collecting and transporting food or other materials (Franks, 1986; Anderson et al., 2001; Robson & Traniello, 2002; Czaczkes & Ratnieks, 2013), but even the most successful ant worker teams (Anderson & Franks, 2001) usually disperse after finishing a job (Franks et al., 2001).

One of the most striking examples of a "team" functioning over a long period of time is the group of foragers tending a colony of a sap-feeding insect (trophobionts), particularly, aphids. Fidelity of foragers to definite trees and even branches (Dobrzańska, 1959; Rosengren, 1971; Rosengren & Sundström, 1987) enables the recording of the long-term relationships of the ants in relatively constant groups of workers tending separate aphid colonies (Novgorodova & Reznikova, 1996). Although aphid honeydew is one of the main energy resources for ants (Way, 1963; Delabie, 2001; Oliver et al., 2008), relatively little is known about the organisation of the work carried out by aphid milkers despite the great ecological effect of these symbiotic interactions on ecosystems (Stadler & Dixon, 2005; Styrsky & Eubanks, 2007). Based on the diversity of foraging strategies and communication systems used by foragers of different species of ants visiting sugar baits in a maze (the analogue of honeydew foragers in nature) (Reznikova & Ryabko, 1994; Reznikova, 2007, 2008) Reznikova hypothesized that the organization of honeydew collection by different species of ants in the field would also differ.

Investigations carried out on a limited number of ants and species of aphids have shown that the behaviour of foragers tending aphids (aphid milkers) differs significantly in different ants (Novgorodova & Reznikova, 1996; Reznikova & Novgorodova, 1998). The interactions of ants with aphids can be characterized by their different degrees of functional differentiation with the aphid milkers ranging from unspecialized to "professional" foragers (Novgorodova, 2007, 2008). Analysis of the preliminary data for six species of ants indicates there is a tendency for the specialization of the aphid milkers that tend separate aphid colonies to increase as ant colony numbers increase and as food resources become scarce (Novgorodova, 2007). However, the data available at that time were insufficient to analyse in detail the factors affecting the degree of functional differentiation of the honeydew foragers.

This study focuses on the honeydew collecting strategies of the different members of multi-species ant communities and the factors that affect the functional differentiation of aphid milkers. The main aims of this study are to: (i) analyse the behaviour of aphid milkers in 12 different species of ants belonging to four genera and describe their main foraging strategies; (ii) estimate the effect of various factors (aphid and ant species, ant colony size, seasonality) on the honeydew collecting strategy of ants.

MATERIAL AND METHODS

Functional differentiation of aphid milkers

Sites investigated

Investigations of ant-aphid interactions were carried out in 1994–1996, 1998–2003 and 2007–2010 in Western Siberia in pine and aspen-birch-pine forests (54°7´N, 83°06´E, alt. 200 m a.s.l., Novosibirsk) and mixed-grass-cereal steppes with aspen-birch groves (53°44´N, 78°02´E, alt. 110 m a.s.l., near Karasuk) in the Novosibirsk Region and coniferous forests in the north-eastern Altai (north end of Lake Teletskoe, 51°48´N, 87°17´E, alt. 434 m a.s.l.) in multispecies communities dominated by ants of the group *Formica* s. str.

Methods of observation

Visual observations of insect behaviour were carried out under natural conditions from June to September / beginning of October. Foragers of 12 species of ants belonging to four genera [*Formica* – 7 (*Formica* s. str. – 4, *Serviformica* – 3), *Lasius* – 2, *Camponotus* – 1, *Myrmica* – 2] were observed in aphid colonies of different species (Table 1) located on plants at heights not exceeding 1.3 m (usually at a height of between 0.6 and 1.10 m above the ground). The aphid colonies consisted of 10 or more individuals. Almost all of the aphid species were attended by several different species of ants, except the aphid *Stomaphis quercus* (Linnaeus), which was only tended by *Lasius fuliginosus* (Latreille) in Siberia.

All the foragers that visited the aphid colonies studied were marked with nitrocellulose enamel. Various combinations of dots of different colours were painted on the abdomen, thorax and head of the ants. The number of dots varied (1–5 on abdomen, 1–2 on thorax, 0–1 on head) and depended on ant species, ant size and number of workers marked in the same aphid colony. Old marks were renewed if necessary. Furthermore, in most cases the specific combination of dots was recoverable if 1 (in some cases 2) dot was lost by an ant. This enabled the activity of some marked foragers to be observed for a long time (e.g. over a period of several weeks in the case of *Formica* and *Camponotus*).

All the observational data were recorded in a uniform way. The time spent by the foragers that exhibited one of seven behavioural features was recorded: (1) honeydew collection, (2) standing still ready to attack, (3) trophallaxis (mouth-to-mouth exchange of food between workers), (4) grooming, (5) aggressive behaviour (which includes alert poses, aggressive poses, body jerking, "hit-and-run attacks", biting and the "death grip"), (6) exploratory activity (investigation of a plant near to an aphid colony using their antennae), (7) antennal contacts between ants. In addition, in order to understand how much time foragers spent in an aphid colony (percentage of observation period) the time when foragers left and returned to a plant was recorded. The period of time between the ant leaving a plant with a full crop and returning to the plant was categorized as the time taken to transport food to the nest. The time of observation for each ant is a sum of the periods of continuous observation of their behaviour.

In addition, the potential degree of aggressiveness was estimated for all of the foragers of the genus *Formica* monitored. The reactions of each individual to an artificial irritant (a preparation needle, which was positioned about 1 cm from an ant) were recorded several times under quiet conditions. The aggressiveness of the foragers was quantified using a 9-level scale based on their reactions to various irritants: (0) avoidance – dropping down or running away; (1) tolerance – neutral reaction (ants do not react); (2) investigation of the irritant using antennae; (3) "an alert pose" – standing still with mandibles slightly open and antennae slightly extended towards the irritant; (4) an aggressive pose – the pose adopted by ants before an attack (stilt-legged posture; mandibles widely open, antennae directed towards the irritant or slightly upwards; in the ant species *Formica* s. str. with gaster extended forwards in order to spray acid); (5) body jerking – usually repeated rapid forward-and-back jerking with open mandibles, without any contact with the irritant; (6) "hit-and-run attack" – sudden attack on the irritant; (7) biting – short bites (less than 5 s); (8) the "death grip" – a prolonged biting/stinging fight (ant seizes the irritant and does not loosen its grip for more than 5 s). If an ant quickly changed its reaction, from one to another, only the most aggressive response was used in the analysis.

The number of ant colonies studied is presented in Table 1. Behaviour of the workers from each ant colony was observed for 3–10 aphid colonies of each of the species of aphid studied (Table 1). Observations on ant behaviour were usually carried out from 9.00–13.00 and 15.00–20.00, which were the periods when the species of ants studied were most active. The total period of continuous observation of ants at aphid colonies per day varied from 1–2 h in rainy weather up to 5–7 h on sunny days. About 200–800 workers of each species of ant were individually marked. In order to exclude the possibility of repeated marking of the same individuals (when marks are lost) only the data for the aphid milkers that remained permanently in an aphid colony or regularly visited the aphids were analyzed. Thus, the behaviour of ants attending aphids was observed in detail and then analyzed for that of about 20–30% of the marked individuals (40–225 workers of different species) was analyzed. The time spent observing ants in the different aphid colonies varied from 15 to 115 h. The number of individuals studied and time for which they were observed depended mainly on the behavioural complexity of the species of ant studied.

Data analysis

In order to determine whether there is a functional differentiation among aphid milkers and reveal the groups of foragers with the most similar time budgets, hierarchical cluster analysis (complete linkage, 1 – Pearson r) was used. The average time budgets for the foragers in the different clusters and presumably performing different tasks were analyzed using Spearman's Rank correlation: a positive correlation (R_s real > R_s critical 0.05 = 0.78, n = 7) between the data for foragers in different clusters indicates their time budgets and functions are significantly similar; otherwise the behaviour of the foragers in the groups compared differs significantly (Urbakh, 1964).

Each of the groups revealed is named based on the behaviour of the foragers (main functions) as aphid milkers: "shepherds" collect honeydew, "guards" protect aphids from competitors, "scouts" search for new aphid colonies, "transporters" transport honeydew to the nest; ants "on duty" are constantly present in a aphid colony, collect honeydew and/or protect aphids from various competitors. Unspecialized foragers search for new aphid colonies, collect honeydew and transport it to the nest.

In order to determine the main functions of foragers in the different groups of a particular species of ant, the percentage of time spent by these workers on a plant with trophobionts, the percentage of time spent by them doing different things (honeydew collection, aggressive behaviour, exploratory activity, trophallaxis) and their aggressiveness (in case of *Formica* ants) were compared using a Kruskal-Wallis ANOVA by Ranks (H) and Mann-Whitney U-test. Non-parametric tests were used since the data were non-normally distributed (Shapiro-Wilk test, $p < 0.05$). The data were analyzed using STATISTICA and Microsoft Excel.

Honeydew collection strategies of ants

The variability in the honeydew collecting strategies was analyzed for the 12 species of ant studied (Table 1). Depending on the degree of functional differentiation of aphid milkers (the number of task groups revealed) and the degree of protection the ants provide the aphids, five main strategies were identified, which ranged from I to V in terms of increasing complexity: with the unspecialized foragers (I) in "unprotected" aphid colonies (attended by ants less than 60% of the time) and (II) in "protected" colonies [attended by ants almost all of the time (for more than 95% of the time they were observed) due to a regular change of unspecialized foragers]; (III) low "professional" specialization (ants "on duty" constantly attending aphid colonies); (IV) medium and (V) high "professional" specialization [clear division of a number of tasks among foragers (at least honeydew collection by "shepherds" and protection of trophobionts by "guards"), and also honeydew transportation by "transporters" in V].

Effect of the species of ant and their colony size, aphid species and seasonality

Ant colony size

Due to the impossibility of calculating the exact size of an ant colony during this investigation of insect behaviour, the size of each ant colony studied was estimated as follows (Table 1): hundreds of ants (10^2), thousands of ants (10^3), tens of thousands (10^4), hundreds of thousands (10^5) and more than a million (10^6). To provide a more accurate estimate, data in the literature (Dlusskiy, 1967; Reznikova, 1983; Beckers et al., 1989) were used to take into account both the characteristic features of ant nests (e.g. type, structure, diameter and height of mound, number of sections etc.) and foraging and territorial behaviour of the ants.

TABLE 1. Data collected on the ant species studied: number of ant colonies (N), their size class (Size), aphid species and honeydew collecting strategies (Strategies): I – unspecialized foragers in "unprotected" aphid colonies, II – unspecialized foragers in "protected" aphid colonies, III–V – "professional" specialization (III – low, IV – medium, V – high). The data collected in autumn (at the end of August, September and October) are marked with *.

Ant species	N	Size	Task groups	Strategies	Aphid species
Formica (*Formica*) *polyctena* Foerster	3	10^5	"Shepherds", "guards", "scouts", "transporters", ants "on duty"*, unspecialized foragers*	V; III*; I*	*Symydobius oblongus* (Heyden), *Chaitophorus populeti* (Panzer), *Aphis jacobaeae* Schrank, *A. grossulariae* Kaltenbach
	1	10^6			
F. (*F.*) *aquilonia* Yarrow	3	10^5	"Shepherds", "guards", "scouts", "transporters", ants "on duty"*, unspecialized foragers*	V; III*; I*	*S. oblongus*, *Ch. populeti*
	1	10^6			
F. (*F.*) *lugubris* Zettersted	1	10^4	"Shepherds", "guards", "scouts", "transporters"	V	*S. oblongus*, *Cinara laricis* (Hartig)
	2	10^5			
F. (*F.*) *pratensis* Retzius	4	10^4	"Shepherds", "guards", unspecialized foragers*	IV; II*; I*	*S. oblongus*, *Ch. populeti*, *A. jacobaeae*
F. (*Serviformica*) *cunicularia* Latreille	3	10^2	Unspecialized foragers	I	*Ch. populeti*, *Aphis craccivora* Koch
	8	10^3	Unspecialized foragers, ants "on duty", "shepherds", "guards", "scouts"	I; II; III; IV	
F. (*S.*) *fusca* Linnaeus	3	10^2	Unspecialized foragers	I	*S. oblongus*, *Ch. populeti*, *A. craccivora*
	2	10^2	Unspecialized foragers	I; II	
F. (*S.*) *candida* F. Smith	3	10^2	Unspecialized foragers	I	*S. oblongus*, *A. craccivora*
	2	10^3	Unspecialized foragers, ants "on duty"	I; II; III	
Camponotus saxatilis Ruzsky	3	10^3	Ants "on duty", "transporters"	III	*S. oblongus*, *A. craccivora*
Lasius niger (Linnaeus)	4	10^3	Unspecialized foragers	II; I	*Ch. populeti*, *A. viburni* Scopoli, *A. pomi* De Geer, *A. craccivora*
L. fuliginosus (Latreille)	3	10^5	Unspecialized foragers	II; II*; I*	*Ch. populeti*, *C. laricis*, *Stomaphis quercus* (Linnaeus)
Myrmica rubra (Linnaeus)	3	10^2	Unspecialized foragers	I	*Ch. populeti*, *A. pomi*
	2	10^3	Unspecialized foragers	I; II	
M. ruginodis Nylander	2	10^3	Unspecialized foragers	I	*Ch. populeti*, *A. jacobaeae*

Data analysis

The effects of various factors (species of ants and aphids, ant colony size, seasonality) on honeydew collection by ants were analyzed using Generalized Linear Models (STATISTICA). The effect of aphid species was additionally analyzed at an intra-species level for 4 species of ants of the genus *Formica* (*F. polyctena*, *F. aquilonia*, *F. pratensis*, *F. cunicularia*), both species of *Lasius* and 2 species of *Myrmica* (*M. rubra* and *M. ruginodis*) grouped together. The effect of seasonality (month of investigation) was estimated for *Formica* s. str. ants, which have the most complex honeydew collecting strategy.

At an intra-species level the effect of ant colony size on the honeydew collection strategy adopted by the foragers was analyzed in the case of *Serviformica* ants [*Formica* (*S.*) *cunicularia* and *F.* (*S.*) *candida*] using Fisher's exact test. The percentage of aphid colonies protected by foragers (using more complex strategies of honeydew collection, II–IV) of small (10^2 foragers) and large (10^3) ant colonies was compared.

In order to estimate the relationship between ant colony size and the degree of functional differentiation of aphid milkers (honeydew collecting strategy) Spearman's Rank correlation was used. To exclude the possible influence of seasonal differences in ant activity, September was not included in this analysis. The data were analyzed using STATISTICA and Microsoft Excel.

RESULTS

Functional differentiation of aphid milkers

The aphid colonies studied were attended by relatively constant groups of foragers regardless of ant species with more than 87% of the individuals marked regularly tending the aphid colonies. The cluster analysis made it possible to group together the ants with the most similar time budgets. The number of clusters varied from one to four for the ants studied. In five species of ants (*L. fuliginosus*, *L. niger*, *F. fusca*, *M. rubra* and *M. ruginodis*) the time budgets of the aphid milkers were similar and characterized as unspecialized foragers in each of these species. Different levels of functional differentiation were recorded for the aphid milkers of *Formica* s. str. ants (4 species), *Serviformica* (2 species) and *Camponotus saxatilis* (Table 1). The average time budgets of the foragers in the different clusters in each case were not closely linked (Spearman's Rank correlation, Rs real < Rs critical 0.05 = 0.78, n = 7), i.e. foragers in these groups performed different functions. Key behavioural differences, including the prevailing behaviour (in aphid colonies) of foragers in the different clusters, are presented in Fig. 1. The percentage of time spent by aphid milkers of the different task groups in an aphid colony and/or the time spent by foragers doing different things (honeydew collecting, standing still ready to attack, aggressive behaviour, exploratory activity and trophallaxis; Fig. 1) varied significantly (Kruskal-Wallis ANOVA by Ranks, $p < 0.05$–0.0001; the Mann-Whitney U-test with Bonferroni correction, $p < 0.008$ (*Formica rufa* group), $p < 0.017$ (*F. cunicularia*), $p < 0.05$ (*F. pratensis*, *F. candida*, *F. cunicularia*, *C. saxatilis*). Furthermore, the potential aggressiveness of "guards" and ants "on duty" of *Formica* irrespective of ant species was significantly greater than that of other task groups (Fig. 2). Since the number of task groups did not depend on the species of aphid (Table 2) the results in Figs 1–2 are presented only for the ant colonies tending the obligate myrmecophilous aphids *Symydobius oblongus* and *Chaitophorus populeti*, in order to save space. Similar results were obtained for all ant colonies (of the same size class and in the same season) of the same species irrespective of the aphid species. The list of task groups revealed for each ant species studied is presented in Table 1.

Honeydew collecting strategies of ants

Strategy I (unspecialized foragers in "unprotected" aphid colonies) was found to be typical mainly for the ants *Myrmica* and *Serviformica*, which live in small colonies of 10^2 workers (Table 1). Strategy II (unspecialized foragers in "protected" aphid colonies) was usually recorded in aphid colonies tended by ants of the genus *Lasius* (*L. niger*, *L. fuliginosus*) and also in some aphid colonies tended by

TABLE 2. The level of task specialization of the ant workers that collected honeydew in the different species of ants studied associated with their colony size, aphid species attended and seasonality.

Response variables	Distribution	Explanatory variables	d.f.	χ^2	p
Honeydew collecting strategy (in 12 ant species explored)	Ordinal multinomial	Aphids	9	9.55	0.39
		Ants	11	156.52	< 0.0001
		Ant colony size	3	98.41	< 0.0001
Honeydew collecting strategy in: *F. polyctena*	Ordinal multinomial	Aphids	3	3.69	0.30
F. aquilonia	Ordinal multinomial	Aphids	1	0	1
F. pratensis	Ordinal multinomial	Aphids	2	4.81	0.09
F. cunicularia	Ordinal multinomial	Aphids	1	1.74	0.19
L. niger	Binomial	Aphids	2	0	1
L. fuliginosus	Binomial	Aphids	2	4.43	0.11
Myrmica	Binomial	Aphids	1	2.09	0.15
Honeydew collecting strategy in *Formica* s. str.	Ordinal multinomial	Aphids	3	4.11	0.25
		Month	3	99.68	< 0.0001
		Aphids × Month	5	0.56	0.99

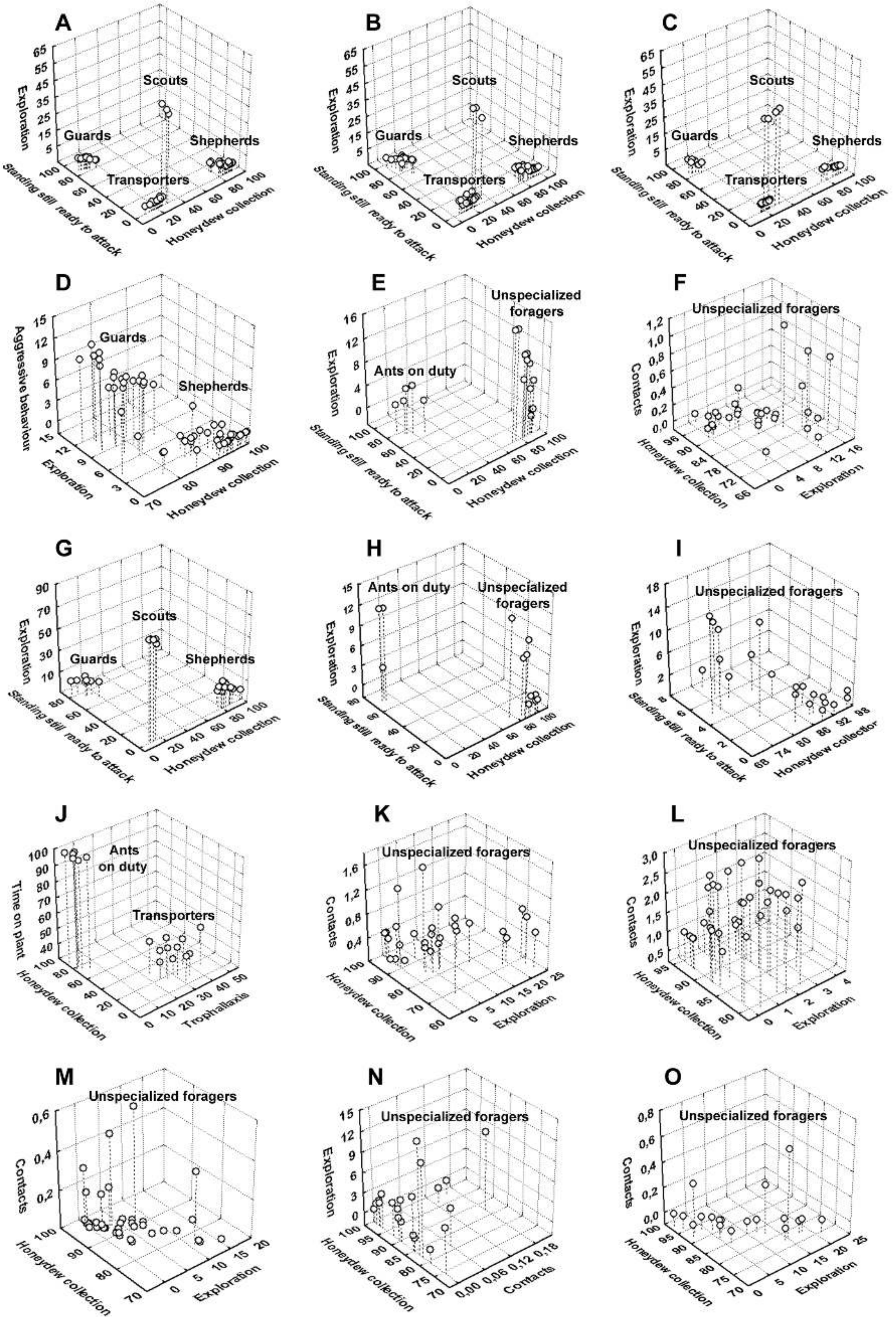

Fig. 1. Key behavioural features of the honeydew foragers of the different species of ants studied. The percentage of time spent by aphid milkers of the different task groups in exhibiting different types of behaviour and spent by foragers in aphid colonies (in the case of *C. saxatilis*). A – *F. polyctena*, B – *F. aquilonia*, C – *F. lugubris*, D – *F. pratensis*, E – *F. candida* (colony size 10^3), F – *F. candida* (colony size 10^2), G – *F. cunicularia* (colony size 10^3), H – *F. cunicularia* (colony size 10^3), I – *F. cunicularia* (colony size 10^2), J – *C. saxatilis*, K – *L. fuliginosus*, L – *L. niger*, M – *M. rubra*, N – *M. ruginodis*, O – *F. fusca*. The data for the different task groups differed significantly (the Mann-Whitney U-test with Bonferroni correction, A–C – $p < 0.008$, G – $p < 0.017$, D–E, H, J – $p < 0.05$).

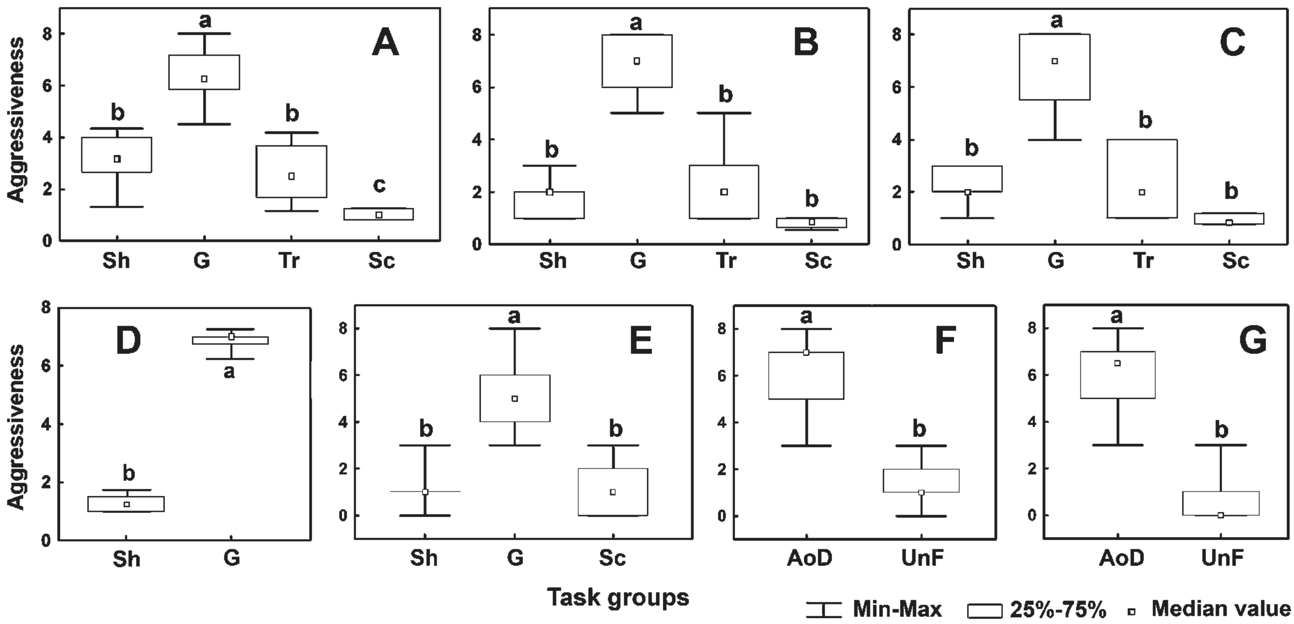

Fig. 2. The ordinal measure of the aggressiveness of the aphid milkers of the seven species of *Formica* compared for the different task groups within each species. Ants: A – *F. polyctena*, B – *F. aquilonia*, C – *F. lugubris*, D – *F. pratensis*, E–F – *F. cunicularia* (colony size 10^3), G – *F. candida* (colony size 10^3). Task groups: Sh – "shepherds", G – "guards", Tr – "transporters", Sc – "scouts", AoD – ants "on duty", UnF – unspecialized foragers. The results marked with different letters are significantly different (the Mann-Whitney U-test with Bonferroni correction: A–B – $p \leq 0.008$, E – $p < 0.017$, D, F–G – $p < 0.05$).

ants of the subgenus *Serviformica* living in larger colonies (10^3), usually of more than 1500–2000 workers.

Low "professional" specialization of aphid milkers (III) was typical for the ant *Camponotus saxatilis* and *Serviformica* ants living in large colonies (10^3). In both cases ants "on duty" constantly attended aphid colonies and very rarely left the plant, thereby protecting aphid-symbionts to some extent from various competitors (Fig. 1E, H, J). However, in the case of *C. saxatilis* they also actively collected honeydew (Fig. 1J). In addition to ants "on duty" groups of foragers tending separate aphid colonies included "transporters" (in the case of *C. saxatilis*) or unspecialized foragers (*Formica cunicularia*, *F. candida*). The "transporters" differed from the unspecialized foragers mainly by the manner in which they harvested honeydew. They actively contacted ants "on duty" and solicited honeydew (about 35% of time they were observed; Fig. 1J), whereas unspecialized foragers collected honeydew from aphids (about 85%; Fig. 1E, H) and rarely interacted with other aphid milkers (< 2%).

Medium "professional" specialization (IV) was usually recorded for *F. pratensis* and was sometimes noted for *F. cunicularia* living in large colonies (10^3 workers) (Table 1, Fig. 1D, G). High "professional" specialization (V) was typical only for highly social species of ants such as *Formica* s. str. (Table 1; Fig. 1A–C).

Effect of the species of ant, their colony size, aphid species and seasonality

The honeydew collecting strategy used by 12 species of ants was significantly affected by ant species and ant colony size (Table 2). Aphid milker specialization becomes more complex as ant colony size increases (Fig. 4). There is a positive correlation between colony size and the complexity of the honeydew collecting strategy used by the ants of the species studied ($Rs = 0.80$, $p < 0.0001$).

Significant effect of ant colony size on the behaviour of aphid milkers was also recorded at the intra-species level, for example in *Formica* ants of the subgenus *Serviformica*. The percentage of aphid colonies protected by foragers using more complex strategies of collecting honeydew (II–IV) was much higher for large ant colonies (10^3 individuals) than for small (10^2) colonies (the Fisher's exact test: *F. cunicularia*, $p = 0.003$; *F. candida*, $p = 0.039$). Aphid colonies protected by these ants made up about 5–13% of the aphid colonies attended by ants from the same ant colony and were usually located at a short distance from the nest ($r < 0.8$ m). Aphid milkers from small colonies (10^2) never protected their symbionts and exhibited only the simplest strategy I (Table 1).

Aphid species had no significant effect on the honeydew collecting strategy used by the ants (Table 2). Similar results were obtained for 4 species of ants of the genus *Formica* (*F. polyctena*, *F. aquilonia*, *F. pratensis*, *F. cunicularia*), *Lasius fuliginosus*, *L. niger* and also for the two *Myrmica* ants. Moreover, as shown in the case of *Formica* s. str. ants, it is clear that neither aphid species nor its combination with seasonality (month) had a significant effect on honeydew collection by ants (Table 2). The functional differentiation of aphid milkers of these ants, however, differed greatly depending on the time of the year (Table 2). A simplification of the organization of honeydew collection by the red wood ants *Formica rufa*-group and *F. pratensis* was recorded in autumn (Fig. 3; Table 1).

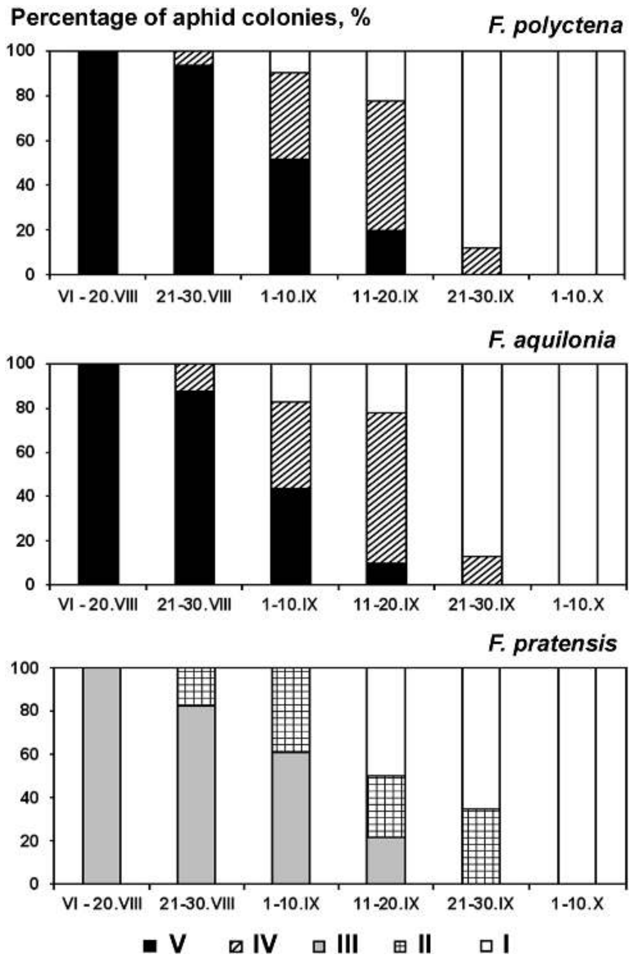

Fig. 3. The simplification of the organization of honeydew collection by ants of *Formica* s. str. in autumn. Honeydew collecting strategies of the ants: unspecialized foragers in (I) "unprotected" aphid colonies and (II) "protected" colonies; (III) low, (IV) medium and (V) high "professional" specialization.

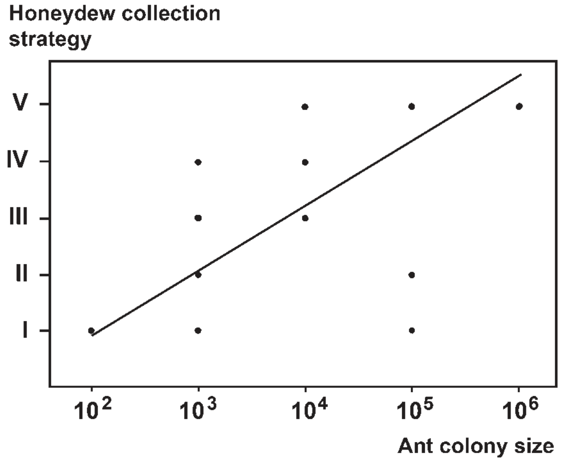

Fig. 4. The relationship between the degree of functional differentiation of the aphid milkers (honeydew collecting strategy) and the colony size of the 12 species of ants studied (see Table 1). Honeydew collecting strategies of the ants: unspecialized foragers in (I) "unprotected" aphid colonies and (II) "protected" colonies; (III) low, (IV) medium and (V) high "professional" specialization.

DISCUSSION

The foraging strategies of ants gathering protein food vary widely and range from solitary hunting to different levels of co-operation during search and food retrieval (Hölldobler & Wilson, 1990). There is little information in the literature, however, on the behaviour of ants foraging for honeydew. Aphid milkers of the genus *Formica* are described as passive individuals with a few functions, the collection and transport of honeydew (Dobrzańska, 1959), and a strong route/site fidelity (Dobrzańska, 1959; Rosengren, 1971).

Observations of marked aphid milkers of 12 species of ants belonging to four genera (*Formica*, *Lasius*, *Camponotus* and *Myrmica*) indicate that honeydew foragers usually visit definite aphid colonies. The groups of aphid milkers attending separate aphid colonies, however, can be quite heterogeneous: the number of groups with different tasks varies from one (lack of functional differentiation) to four among the ants studied and are not affected by species of aphid tended. The groups of specialized aphid milkers include both "passive" and "active" foragers (Novgorodova, 2008). The functions of the first (e.g. "shepherds" and "transporters") are usually restricted to collecting and transporting honeydew. "Active" foragers (e.g. ants "on duty", "guards" and "scouts") are usually multifunctional. In addition to the common functions (collecting and transporting honeydew) the aphid milkers can guard trophobionts, search for new aphid colonies and in the case of scouts even mobilize passive "shepherds" to work at newly found aphid colonies (Reznikova & Novgorodova, 1998; Novgorodova, 2008).

The ants studied used five different strategies to collect honeydew from aphids [from unspecialized foragers in "unprotected" aphid colonies (I) to a high level of "professional" specialization (V)], which reflect the variability in ant behaviour from solitary to group foraging. In the case of the III–V strategies the groups of aphid milkers that tend aphid colonies work as "teams" (Anderson & Franks, 2001) and coordinate their activities. In the case of I and II honeydew collecting strategies unspecialized aphid milkers rarely encounter one another. The only exceptions are ants of the genus *Lasius* (*L. fuliginosus* and *L. niger*), which use strategy II. They also coordinate their activities to a slight extent, e.g. if a honeydew forager is alone in an aphid colony, before leaving the plant to carry honeydew to the nest it usually waits until another aphid milker arrives (Novgorodova, 2005). This feature of the genus *Lasius* sometimes leads to the wrong conclusion on whether an aphid colony is exploited or not. For instance, Devigne & Detrain (2005) consider an aphid colony is exploited by *L. niger* if they observed at least one ant foraging on it, which ignores the possibility that some aphid colonies are attended by ants using strategy I.

The strategy used by the honeydew foragers was not significantly associated with the species of aphid attended. It is to be noted that this result concerns, first of all, species of aphids that live in exposed colonies and do not form galls. The degree of functional differentiation of honeydew foragers is, however, closely associated with the lifestyle of trophobionts, namely whether there was direct contact between ant foragers and their symbionts (Novgorodova & Biryukova, 2011). During interactions with trophobionts that cannot be directly contacted by ants, e.g. sawfly larvae of *Blasticotoma filiceti* living in fern fronds, honeydew foragers show a lower degree of functional differentiation, unspecialized foragers (II) and low "professional" specialization, than when attending open aphid colonies (Novgorodova & Biryukova, 2011).

The foraging strategy for collecting honeydew shown by ants is dependent on the species of ant. The most complicated strategies (IV, V) with clear divisions between a number of tasks, at least honeydew collection by "shepherds" and protection of trophobionts by "guards", are recorded for *Formica* s. str. ants that dominate multi-species communities. Only these ants strongly protect their trophobionts from natural enemies (Novgorodova & Gavrilyuk, 2012): the number of aphid colonies with aphidophages among colonies tended by these ants is significantly lower than in colonies tended by other species of ants. The major difference between the high and the medium "professional" specialization of the aphid milkers are the "transporters" that carry the honeydew to the nest. This enables ants from larger colonies (10^5–10^6) to station a definite number of foragers ("shepherds" and "guards") on plants with trophobionts, which seems to increase the efficiency with which they can collect honeydew.

Other species of ants usually have simpler strategies [low "professional" specialization (*Camponotus saxatilis*), and unspecialized foragers in (I) "unprotected" and (II) "protected" aphid colonies (*Formica* ants of the subgenus *Serviformica*, *Lasius* and *Myrmica*)] and provide their symbionts less protection from aphidophages (Novgorodova & Gavrilyuk, 2012).

The organization of honeydew collection is also greatly affected by ant colony size. The number of task-groups among aphid milkers is strongly positively correlated with increase in the size of ant colonies. Behaviour of social insects, e.g. the choice of foraging strategy and division of labour, is known to be closely associated with the size of their colonies (Anderson & McShea, 2001; Mailleux et al., 2003; Jeanson et al., 2007). The highest level of division of labour is typical of the larger colonies of various social insects including wasps (Jeanne & Taylor, 2009) and ants (Anderson & McShea, 2001; Thomas & Elgar, 2003; Holbrook et al., 2011). However, the effect of ant colony size on the behaviour of honeydew foragers is investigated here for the first time.

The greater functional differentiation among the aphid milkers with increase in ant colony size is also revealed at the intra-specific level in *F. cunicularia* and *F. candida*. Unlike the honeydew foragers from small colonies (10^2) that only adopt the simplest strategy (I), those from colonies of the next size class (10^3) adopt more complex strategies (II–IV) in addition to strategy I. As a result, the percentage of the aphid colonies protected by their foragers is much higher for ant colonies of thousands of ants (10^3). The increase in the colony size of *Serviformica* ants (*Formica cunicularia* and *F. candida*) up to 10^3 workers is associated with a reorganization of the work of the honeydew foragers within these species. This is confirmed by the fact that the complexity of both the social structure of the ant colony and the territorial behaviour of these ants, increases in colonies of more than one thousand workers (Reznikova, 1983). At this colony size ants begin to build a mound nest and (at least partly) to protect their foraging territory. The foraging territory of *F. cunicularia*, however, does not enlarge with increase in colony size (Reznikova, 1983). Thus, these ants solve the problem of the increased demand for carbohydrate food not by expanding their territory, but by their honeydew foragers adopting low or medium levels of "professional" specialization in some aphid colonies, which increases the efficiency with which they can collect honeydew.

The increase in the number of ants in a colony of up to one or several thousand appears to trigger quantitative and qualitative changes in ant behaviour. However, it is to be noted that the species-specific peculiarities also play an important role in the choice of the honeydew collecting strategy. This relates, first of all, to ants of the genus *Lasius* in which task specialization among honeydew collectors is not recorded despite the large size of their colonies (10^3 – *L. niger* and 10^5 – *L. fuliginosus*). For honeydew collection they depend on unspecialized foragers visiting "unprotected" and "protected" aphid colonies (I and II). Strategy II is adopted only in aphid colonies located quite close to ant nests, or close to the main trails in the case of *Lasius fuliginosus*. The effectiveness of aphid milkers improves due to the high number of unspecialized foragers visiting aphid colonies. In the case of *L. fuliginosus* this is possibly due to the trunk trail system typical of this ant (Beckers et al., 1989). Furthermore, it has been shown experimentally that aphid milkers of both these species of ants demonstrate highly aggressive behaviour towards aphidophages (Novgorodova & Gavrilyuk, 2012).

The behaviour of ants is also known to be affected by the availability and abundance of carbohydrate resources (Sakata, 1995). The availability of an alternative source of carbohydrate can result in ants eating their symbionts (Offenberg, 2001). A chance observation in the field indicates that an insufficient supply of food can lead to the reorganization of the work of the aphid milkers, which results in the constant protection of aphid colonies by *F. fusca*, *F. cunicularia* and *L. niger*. This was recorded when the majority of the aphid colonies tended by these ants were destroyed when the grass was mowed: the number of unspecialized foragers and their aggressiveness significantly increased on those plants where a few aphid colonies survived. As a result, these aphid colonies were attended almost all of the time by foragers with strategies II or III (*F. fusca* – 2 aphid colonies, *F. cunicularia* – 3, *L. niger* – 5) rather than type I. In the laboratory a decrease in the number of the aphid colonies (from 10 to 1) available for *F. cunicularia* results in this ant adopting more complex strategies of honeydew collection (types III and IV) rather than type I recorded at the beginning of the experiment (Novgorodova, 2007, 2008). It is to be noted that all of the above observations and experiments were carried out in summer when the foraging activity of the ants is high.

However, the foraging behaviour of ants is known to depend on the season (Cook et al., 2011). Seasonality appears to be another important factor affecting the honeydew collection strategy used by ants. A simplification of the work organization of honeydew foragers is recorded in autumn even in the highly social red wood ants *Formica* (*F. rufa*-group) living in large colonies (10^5–10^6 workers). In September–October groups of these ants tending aphid colonies characteristically have a smaller number of task groups, with only non-aggressive passive foragers (previously worked as "shepherds" and "transporters") recorded in aphid colonies, which both collect and transport the honeydew, and protect the aphids.

The simplification of the work organization of aphid milkers in autumn could be a result of changes in the quantity or quality of the honeydew and decrease in the activity of the ants. The production of honeydew by a particular species of aphid is known to be influenced by various factors including their age and physiological condition (Auclair, 1963; Fischer et al., 2005), the quality of the host plant and the aphid's interaction with ants (Del-Claro & Oliveira, 1993; Fischer et al., 2001, 2005). Despite the large number of papers on this problem (Lundgren, 2009), it is still unclear whether the honeydew production by a particular species of aphid feeding on a particular host plant depends on the season. Unfortunately, the sugar composition and the volume of honeydew produced by the species of aphids studied were not recorded in this study. Nevertheless, if the productivity of the aphids was the main reason for changes in ant behaviour, we could expect the marked ants to switch to another food resource located not far from the aphid colonies studied. However, the marked ants which left the aphid colonies were not recorded elsewhere in the feeding territory. They did not switch to another food source or perform other functions.

As for the activity of ants, the traffic of foraging wood ants (*Formica rufa* group) on trees infested with trophobionts is known to be significantly lower in autumn (September) than at similar temperatures in summer (Domisch et al., 2009). Since the activity of aphids and other Hemiptera also tend to decrease with the autumnal decline in temperature (Richardson et al., 2002) it is assumed that the simplification in the honeydew collecting strategy used by foragers is explained by a decrease in both the activity of foraging ants throughout their territory and the productivity of aphids. However, this hypothesis needs to be tested.

Overall, the functional differentiation of the honeydew foragers is species specific and facultative for the species of ants studied. Ants demonstrate a certain range of honeydew collecting strategies of different complexity and use them according to the circumstances and ant colony needs. The honeydew collecting strategy used by ants is strongly dependent on species and colony size of the ants, available food resources and seasonality (at least in the highly social ant species of *Formica* s. str.). The aphid milker specialization becomes more complex as ant colony size increases at both intra- and inter-specific levels and when food is scarce. The variety of honeydew foraging strategies seems to reflect the unequal contribution of different ants in the forming of trophobiotic interactions with aphids.

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