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Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*

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Abstract Mutualistic interactions between aphids and ants are mediated by the honeydew produced by aphids. Previous work showed that when attended by the ant *Formica yessensis*, nymphs of the aphid *Tuberculatus quercicola* developed into significantly smaller adults with lower fecundity than when not ant-attended. This study tested the hypothesis that this cost of ant attendance arises through changes in the quality and quantity of honeydew. Ant-attended and ant-excluded aphid colonies were prepared in the field, and the sugar concentration and sugar composition of the honeydew of ant-attended colonies were compared with those of ant-excluded colonies. The frequency and amount of honeydew excretion were also quantified in the two types of colonies. The aphids excreted smaller droplets of honeydew more frequently in ant-attended colonies than in ant-excluded colonies. There was no significant difference in total sugar concentration between the honeydew of ant-attended aphids and ant-excluded aphids. However, ant-attended aphids produced honeydew containing a significantly lower proportion of glucose and higher proportions of sucrose and trehalose than did ant-excluded aphids. These results suggest that the enhanced rate of honeydew-excretion behavior under ant attendance led to changes in the aphid's physiological status. We suggest that the increase in the proportions of sucrose and trehalose in honeydew leads to a shortage of carbohydrates available for energy metabolism, resulting in lower performance of the aphids under ant attendance.

Keywords Aphid-ant mutualism · Behavioral plasticity · Phloem sap · Carbohydrate metabolism · *Quercus dentata*

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Introduction

Many homopterans and larvae of lycaenid butterflies produce carbohydrate- and nitrogen-rich secretions that play a critical role in mutualistic interactions with ants (Way 1963; Baylis and Pierce 1992). The attending ants collect the honeydew or secretion directly from the partner by tapping its anus or dorsal nectar organ. The partners, in return, benefit from protection by the ants against natural enemies and the sooty mold that grows on the excretion (Way 1954; Banks and Macaulay 1967; Tilles and Wood 1982; Bristow 1984; Pierce et al. 1987; Devries 1991; Itioka and Inoue 1996; Stechmann et al. 1996; Yao et al. 2000; but see Völkl 1992; Stadler and Dixon 1999). A growing body of evidence suggests that ant attendance has multiple effects on the partners' performance through behavioral or physiological changes in the partners. When attended by ants, some aphids exhibit enhanced feeding and excretion rates (Banks and Nixon 1958; Mittler 1958; Takeda et al. 1982) or increased reproductive and developmental rates (El-Ziady 1960; Stadler and Dixon 1999). Similarly, in some butterfly larvae, ant attendance results in an increase in the frequency of secretory behavior (Fiedler and Hummel 1995), a gain in adult weight (Wagner 1993; Cushman et al. 1994; Wagner and del Rio 1997), or a shortened duration of development (Pierce et al. 1987; Cushman et al. 1994). In contrast, several studies have indicated that ant attendance has negative effects on the partners' performance. These examples include prolonged developmental duration in lycaenid larvae or aphids (Pierce et al. 1987; Robbins 1991; Stadler and Dixon 1998), weight loss in lycaenid larvae or in aphid gonads (Pierce et al. 1987; Stadler and Dixon 1998), or reduction in adult body size and embryo numbers in aphids (Stadler and Dixon 1998; Yao et al. 2000). Yao et al. (2000) have suggested that these physiological and developmental costs may be due to a failure in the compensatory assimilation of the nutrients offered to the attending ants. Several authors have emphasized that the outcomes of aphid-ant interactions are mediated by the quality and quantity of the

honeydew offered to the ants (Addicott 1978; Sakata 1995; Völkl et al. 1999). These results imply that chemical analysis of aphid honeydew is indispensable for an understanding of the evolutionary outcomes of aphid-ant interactions.

Aphid honeydew contains a variety of saccharides: monosaccharides (glucose, fructose), disaccharides (sucrose, trehalose, maltose), and trisaccharides (melezitose, raffinose, fructomaltose) (Mittler 1958; Auclair 1963; Walters and Mullin 1988; Nemec and Stary 1990; Hendrix et al. 1992; Völkl et al. 1999), as well as several kinds of amino acids (Mittler 1953, 1958; Maltais and Auclair 1962; Barlow and Randolph 1978; Sasaki et al. 1990; Douglas 1993; Arakaki and Hattori 1998). Interestingly, the sugars present in aphid honeydew are not the same as those contained in the phloem sap in the host plants. Phloem sap obtained from the cut stylets of *Tuberolachnus salignus* contain sucrose alone, whereas the honeydew is composed of roughly equal amounts of sucrose, glucose, fructose, and melezitose (Mittler 1958). The primary sugar found in aphid honeydew is melezitose, which is considered to play a key role in aphid-ant interactions (Kiss 1981), aphid-parasitoid interactions (Wäckers 2000), plant-animal interactions (Owen 1978), and the osmoregulation of aphid hemolymph (Petelle 1980; Fisher et al. 1984; Walters and Mullin 1988).

Völkl et al. (1999) reported that when four aphid species were reared on the same host plant, the trisaccharides melezitose and raffinose were detected only in the honeydew of the two myrmecophilous aphid species, and suggested that interspecific differences in the sugar composition of honeydew are due to species-specific differences in the aphids' ability to transform ingested sucrose into other sugars. It has been reported that the sweet potato whitefly (Homoptera) can change both carbohydrate metabolism and feeding behavior to cope with changes in host-plant quality (Isaacs et al. 1998). These observations point to the possibility that aphids are capable of adjusting their carbohydrate metabolism depending on the presence or absence of the attending ants such that aphids invest more carbohydrates in the honeydew under ant attendance at the expense of their own assimilation of carbohydrates.

The aim of this study is to determine whether aphids exhibit plastic responses in honeydew excretion depending on the presence or absence of attending ants, using *T. quercicola* and the attending ant *Formica yessensis*. The specific questions addressed here are (1) to what extent do the proportions of sugars differ between the phloem sap of the host plant *Quercus dentata* and the honeydew of *T. quercicola* aphids, (2) whether the sugar concentration and sugar composition of honeydew of a clonal colony change after the onset or termination of ant-attendance treatment, and (3) whether aphids have the ability to change the amount and frequency of honeydew production depending on the status of ant attendance.

Materials and methods

Study area and aphids

Observations and experiments were conducted on the Ishikari Coast, Hokkaido, northern Japan, from May to September 1999. Colonies of the red wood ant, *F. yessensis*, were found throughout the dunes, along which grew bushy stands of the Daimyo oak, *Q. dentata*. Nests of *F. yessensis* were distributed contiguously along this coast.

T. quercicola colonies were obligatorily attended by *F. yessensis* workers on the oaks that grow on the dunes; preliminary experiments demonstrated that removal of attending ants always resulted in the extinction of *T. quercicola* colonies (Yao et al. 2000). During the summer, all nymphs develop into alate viviparous females.

Sugar composition of phloem sap and honeydew

Using microcapillaries of 0.5 µl volume, an exudation of the phloem sap of *Q. dentata* was collected from two trees by cutting shoots on 26 May when the trees were actively growing. Phloem sap from each tree was collected in one microcapillary until it was filled.

In late June, one branch bearing a pair of shoots was selected from a tree. On each of the selected shoots, all leaves except one were cut. On each of the two remaining leaves, one leaf cage consisting of a small plastic cup (3 cm upper diameter, 5 cm lower diameter) was attached along the midrib with water-repellent glue. The upper side of the leaf cage was cut circularly and bound loosely to the rim with plastic tape. This treatment prevented predators other than ants from invading the aphid colony. Two clonal 4th-instar nymphs were then collected, and each was transferred into a leaf cage and propagated clonally for 2 weeks. The aphid density in the leaf cages was kept at a constant level of 15–20 individuals by removing some individuals. In early July when the aphids were at the 3rd- to 4th-instar stadium, honeydew was collected directly from several aphids using a 0.5-µl microcapillary in each colony, where a total of 0.5 µl honeydew was collected so as to fill the microcapillary. After the collection, each of the microcapillaries was placed in a microtube containing 10 µl of distilled water.

Plasticity in the sugar concentration and sugar composition of honeydew

Four trees averaging 1.7 m in height were used for the experiments. A total of 20 shoots were selected from the four trees and used for the experiment. To eliminate the effect of genetic differences among aphids, prior to the experiments one aphid clone was reared on each of the study trees. On each shoot, all leaves except one were cut and all aphids found on the remaining leaf were removed. On each study tree, 10–20 clonal 3rd- to 4th-instar nymphs were transferred onto each of the remaining leaves on 8 July. After the transfer, each of the leaves was bagged with a nylon net (33×22 cm). On one half of the selected shoots, two plastic tubes, each 6 cm long and 4 mm inside diameter, were attached with plastic tape along the petiole, and a net was bound over the plastic tubes. This treatment enabled ants alone to approach the aphid colonies directly through the tubes, and is referred to as an *ant-attendance treatment*. In an *ant-exclusion treatment*, a net was bound directly over the petiole to prevent ant visitation. The two treatments were randomly arranged on a study tree. For each of the netted colonies, honeydew was collected twice. The first collection of honeydew was conducted from 9 July (1 day after aphid transfer) to 24 September. During this period of time, aphid density in a colony was kept at a constant level of 10–20 individuals by removing some individuals at each census. In each colony, an average of 6.05 (±2.82, SD) aphids were found along the midrib, and honeydew was collected from 2–14 aphids using a 0.5-µl micro-

capillary so as to fill the microcapillary. Five to six ants were always found attending the aphids at each census in ant-attended colonies, where the honeydew of an aphid was always collected just after an attending ant touched the aphid with its antennae to induce excretion. In ant-excluded colonies, honeydew was collected directly from aphid anuses just before the aphids kicked their honeydew droplets. After the collection of honeydew, each colony was subjected to the other treatment: for ant-excluded colonies, ant visitation was then permitted; in contrast, ant visitation was prohibited in the ant-attended colonies by netting. Within 24–72 h after the re-bagging, honeydew was again collected in the same manner as in the first collection. Aphid colonies that became extinct due to accidental invasions of predators or deterioration in host quality were not included in the analysis. Consequently, this experiment used 17 clonal colonies, of which 8 replicates switched from ant attendance to ant exclusion and 9 replicates switched from ant exclusion to ant attendance.

Plasticity in excretion behavior

This experiment quantified (1) the volume of a single honeydew droplet excreted on one occasion, (2) the frequency of excretion behavior per aphid per hour, and (3) the total volume of honeydew produced per aphid per hour, using part of the netted aphid colonies described above. In September 1999, six replicates were used for the treatment that switched from ant-attendance to ant-exclusion and five replicates for the treatment that switched from ant-exclusion to ant-attendance. In each colony, the volume of a single honeydew droplet was quantified using one aphid that was chosen randomly from aphids feeding along the midrib. The volume of a honeydew droplet was estimated by measuring the height of honeydew absorbed in a 0.5-µl microcapillary, 30 mm long. This measurement was made in each colony for the aphid used first in the honeydew collection mentioned above, and then collection was continued for other aphids using the same microcapillary. Thus the procedure for collecting honeydew for both treatments was the same as that described in the previous section.

The frequency of excretion behavior for each colony was determined by counting the number of feeding aphids along the midrib and by recording, for them, the total number of excretion behavior observed in 1 h, during which a single colony was observed by the naked eye. The total volume of honeydew produced per aphid per hour was estimated for each colony by multiplying the volume of a honeydew droplet by the frequency of excretion behavior.

HPLC sugar analysis

The sugar concentration and sugar composition in the honeydew were measured by a column (Carbopac PA-1, 4×250 mm) with a Dionex Bio LC series apparatus using pulsed amperometric detection (PAD). The gradient elution was established by mixing eluant A (0.15M NaOH) with eluant B (0.5M sodium acetate in 0.15M NaOH) at various ratios using a flow rate through the column of 1.0 ml min^{-1}. The concentration of sodium acetate was changed over time: 0.025M (0–1 min), 0.025–0.05M (1–2 min), 0.05–0.2M (2–20 min), 0.5M (20–22 min), and 0.025M (22–30 min). The applied PAD potentials for E1 (300 ms), E2 (120 ms), and E3 (300 ms) were 0.04, 0.06, and – 0.80 V, respectively, and the output range was 1 µC.

Nine sugars (mannitol, trehalose, mannose, galactose, xylose, glucose, fructose, sucrose, and melezitose) of known concentration were analyzed using this method, and the retention time of each sugar was measured. Sugars in phloem sap or honeydew were identified by comparing the retention times of sugars in the samples with those of the standard sugars. This comparison revealed the presence of three sugars, glucose, fructose and sucrose, in phloem sap and additionally trehalose and melezitose in honeydew. The actual amount of the five sugars in the samples was estimated by comparing the peak areas of the samples with those of the standard sugars of known concentration.

Statistical analysis

Randomized block ANOVA was used to test variation in all dependent variables, which included the concentrations and proportions of sugars in honeydew, the volume of a honeydew droplet, and the frequency of excretion behavior and the total volume of honeydew. The effect of ant treatments (ant attendance or ant exclusion) on each variable was primarily tested, as well as the effects of the sequence of treatments and the month of honeydew collection. The month was included as a main effect because the quality of the phloem sap might change depending on the time of the experiment. The ANOVA model contained "tree" and "shoot nested within tree" as blocks. In this randomized block design, the interaction terms including those relating to shoots and trees were included in the error term (Sokal and Rohlf 1995). The main effects, treatment, sequence, and month, were treated as fixed variables. The proportions of sugars in honeydew were transformed to arcsine square-root in order to satisfy the requirement of normality. These proportions, as well as the concentrations of sugars, are apparently variables that are correlated with one another. Therefore, sequential Bonferroni tests (Rice 1989) were applied to both the sugar concentrations and the sugar proportions to adjust the *P*-value of each effect to a level of 0.05 throughout the ANOVAs for each set of variables. Computations using the SAS program package (SAS 1990) were made in the Computing Center, Hokkaido University.

Results

Sugar composition of phloem sap and honeydew

The HPLC analysis revealed that sucrose was the predominant carbohydrate in the phloem sap of *Q. dentata*, whereas the honeydew of the aphids contained a large proportion of melezitose, which was not detected in the phloem sap (Table 1). When combined, the five sugars, trehalose, glucose, fructose, sucrose, and melezitose, accounted for approximately 90% of the total volume of sugar in the honeydew.

Plasticity in the sugar concentration and sugar composition of honeydew

Randomized block ANOVA indicated that the concentration of the five sugars combined was not significantly different between ant-attended colonies (on average, 180.6 µg $µl^{-1}$ honeydew) and ant-excluded colonies (on average, 192.6 µg $µl^{-1}$ honeydew) (Table 2). However, the presence of attending ants resulted in a significantly lower concentration of glucose as compared to sugar concentration under ant exclusion (Fig. 1a; Table 3). No other sugars varied significantly between treatments in concentration. The sequence of treatments, month and the interactions had no significant effects on the concentration of the five sugars or that of each sugar (Tables 2, 3).

The ANOVAs for the proportion of each sugar revealed that the presence or absence of attending ants radically changed the sugar composition of the honeydew; the honeydew under ant attendance consisted of a significantly lower proportion of glucose and significantly higher proportions of trehalose and sucrose as compared

Table 1 Sugar composition of the phloem sap of *Quercus dentata* and of the honeydew of *Tuberculatus quercicola* aphid. Mean percentage±SD

	T. quercicola honeydew (*n*=2)	*Q. dentata* phloem sap (*n*=2)
Trehalose	5.5±3.5	0
Mannose	0	5.5±0.6
Glucose	5.0±4.4	6.5±5.5
Fructose	25.5±1.2	2.7±3.8
Sucrose	11.9±1.6	62.7±10.6
Melezitose	40.6±3.2	0
Total of unidentified monosaccharides	11.6±3.8	22.6±1.9

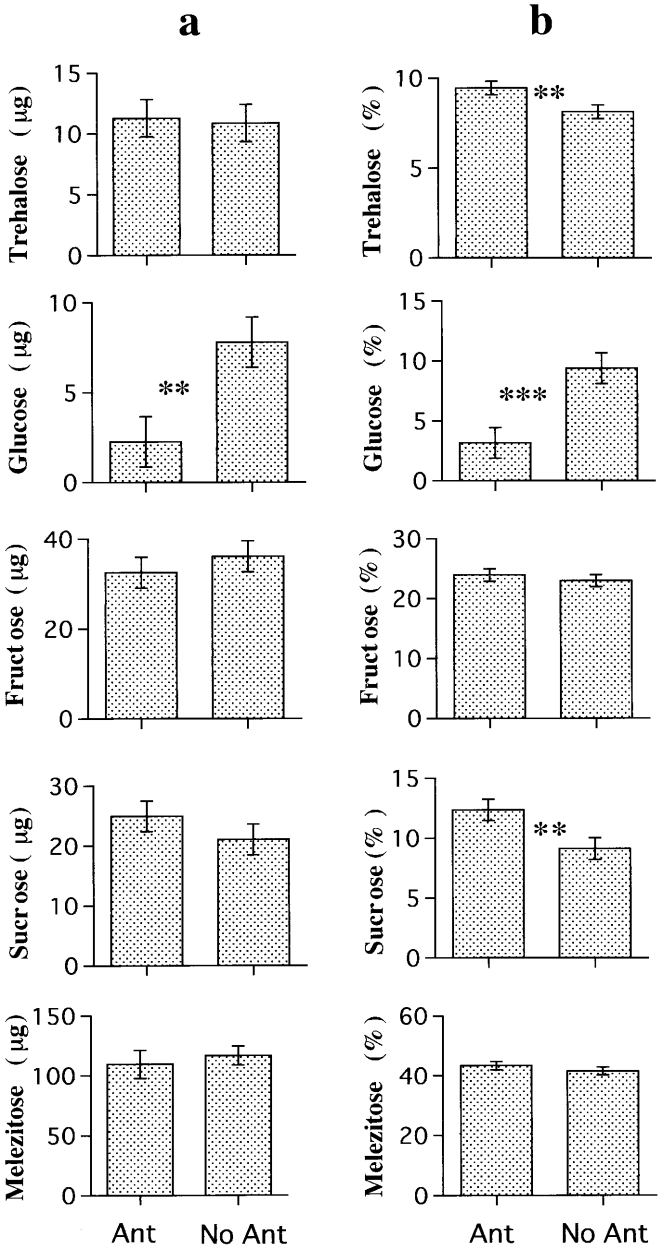

Fig. 1 a Sugar concentration (μg μl^{-1} honeydew) and **b** sugar composition of the honeydew under ant attendance (*Ant*) and ant exclusion (*No ant*), mean±SE (**P<0.01, ***P<0.001, see Tables 3, 4)

Table 2 Randomized block ANOVA for the effects of ant treatments (ant-attendance or ant-exclusion) on the concentration of five sugars combined in the honeydew

	df	SS	MS	*F*	*P*
Tree	3	7097.61	2365.87	1.27	0.323
Shoot (Tree)	9	80752.60	8972.51	4.82	0.005
Sequence	1	300.33	300.33	0.16	0.694
Ant treatment	1	827.66	827.66	0.44	0.516
Month	2	2355.06	1177.53	0.63	0.546
Month×Treatment	2	3454.80	1727.40	0.93	0.418
Sequence×Treatment	1	419.06	419.06	0.23	0.643
Error	14	26060.99	1861.50		

to sugar composition under ant exclusion (Fig. 1b; Table 4). The sequence of treatments and the interaction between sequence and treatment had no significant effects on the proportions of any sugars (Table 4). The effect of month was significant only in the proportion of glucose (Table 4). The proportion of glucose in the honeydew decreased consistently from July (on average, 8.7%) to September (on average, 5.0%). The proportions of fructose and melezitose were not significantly different between treatments.

Plasticity in excretion behavior

Randomized block ANOVA indicated that the aphids changed their excretion behavior depending on whether or not they were attended by ants. When attending ants were removed, the aphids periodically produced honeydew droplets that were ejected by kicking. In contrast, in ant-attended colonies, in response to ants' solicitations, the aphids extruded honeydew droplets, which were retained on the anus until the attending ants collected them. The aphids excreted honeydew more frequently when being attended by ants than when not being attended, but the droplets excreted by the ant-attended aphids were significantly smaller (Fig. 2; Table 5). The total volume of honeydew per hour under ant attendance did not differ significantly from that under ant exclusion (Fig. 2; Table 5). The treatment sequence had no effect on the volume of a honeydew droplet, the frequency of excretion behavior, or the total volume of honeydew. No significant effect was found in the interaction between sequence and treatment for any of the variables.

Table 3 Randomized block ANOVA for the effects of ant treatments on each sugar concentration (μg μl^{-1} honeydew)

	df	SS	MS	*F*	*P*
Trehalose					
Tree	3	80.61	26.87	2.04	0.154
Shoot (Tree)	9	463.84	51.54	3.91	0.011
Sequence	1	26.10	26.10	1.98	0.181
Ant treatment	1	1.01	1.01	0.08	0.785
Month	2	12.01	6.01	0.46	0.643
Month×Treatment	2	46.12	23.06	1.75	0.209
Sequence×Treatment	1	8.49	8.49	0.64	0.435
Error	14	184.32	13.17		
Glucose					
Tree	3	88.62	29.54	2.67	0.088
Shoot (Tree)	9	245.82	27.31	2.47	0.063
Sequence	1	21.72	21.72	1.96	0.183
Ant treatment	1	175.87	175.87	15.90	0.001*
Month	2	83.75	41.88	3.79	0.049
Month×Treatment	2	99.88	49.94	4.51	0.031
Sequence×Treatment	1	0.37	0.37	0.03	0.858
Error	14	154.86	11.06		
Fructose					
Tree	3	419.28	139.76	2.07	0.150
Shoot (Tree)	9	3,483.39	387.04	5.74	0.002
Sequence	1	28.22	28.22	0.42	0.528
Ant treatment	1	73.94	73.94	1.10	0.313
Month	2	34.97	17.48	0.26	0.775
Month×Treatment	2	252.61	126.30	1.87	0.190
Sequence×Treatment	1	25.81	25.81	0.38	0.546
Error	14	943.61	67.40		
Sucrose					
Tree	3	672.43	224.14	6.09	0.007
Shoot (Tree)	9	1,698.80	188.76	5.13	0.003
Sequence	1	71.72	71.72	1.95	0.185
Ant treatment	1	86.78	86.78	2.36	0.147
Month	2	199.17	99.59	2.71	0.102
Month×Treatment	2	16.22	8.11	0.22	0.805
Sequence×Treatment	1	26.03	26.03	0.71	0.415
Error	14	515.38	36.81		
Melezitose					
Tree	3	2,072.28	690.76	0.90	0.468
Shoot (Tree)	9	26,550.88	2950.10	3.83	0.012
Sequence	1	177.39	177.39	0.23	0.639
Ant treatment	1	296.90	296.90	0.39	0.545
Month	2	1,292.61	646.31	0.84	0.453
Month×Treatment	2	728.43	364.21	0.47	0.633
Sequence×Treatment	1	288.00	288.00	0.37	0.551
Error	14	10,794.67	771.05		

*Significant difference between ant treatments at $P \leq 0.05$ after application of the sequential Bonferroni method

Table 4 Randomized block ANOVA for the effects of ant treatments on the proportion of each sugar

	df	SS	MS	*F*	*P*
Trehalose					
Tree	3	0.010	0.003	9.660	0.001
Shoot (Tree)	9	0.035	0.004	10.955	<0.0001
Sequence	1	0.000	0.000	0.388	0.543
Ant treatment	1	0.006	0.006	15.514	0.002*
Month	2	0.002	0.001	3.279	0.068
Month×Treatment	2	0.011	0.005	15.223	0.0003*
Sequence×Treatment	1	0.000	0.000	1.100	0.312
Error	14	0.005	0.000		
Glucose					
Tree	3	0.056	0.019	7.572	0.003
Shoot (Tree)	9	0.064	0.007	2.889	0.037
Sequence	1	0.004	0.004	1.570	0.231
Ant treatment	1	0.061	0.061	24.934	0.0002*
Month	2	0.053	0.027	10.923	0.001*
Month×Treatment	2	0.016	0.008	3.240	0.070
Sequence×Treatment	1	0.000	0.000	0.149	0.705
Error	14	0.034	0.002		
Fructose					
Tree	3	0.018	0.006	6.222	0.007
Shoot (Tree)	9	0.024	0.003	2.798	0.041
Sequence	1	0.000	0.000	0.248	0.626
Ant treatment	1	0.001	0.001	0.861	0.369
Month	2	0.001	0.001	0.713	0.507
Month×Treatment	2	0.002	0.001	0.883	0.435
Sequence×Treatment	1	0.000	0.000	0.083	0.778
Error	14	0.013	0.001		
Sucrose					
Tree	3	0.014	0.005	2.820	0.077
Shoot (Tree)	9	0.027	0.003	1.775	0.162
Sequence	1	0.001	0.001	0.370	0.553
Ant treatment	1	0.019	0.019	11.061	0.005*
Month	2	0.014	0.007	4.185	0.038
Month×Treatment	2	0.001	0.001	0.431	0.658
Sequence×Treatment	1	0.004	0.004	2.394	0.144
Error	14	0.023	0.002		
Melezitose					
Tree	3	0.032	0.011	9.333	0.001
Shoot (Tree)	9	0.048	0.005	4.619	0.006
Sequence	1	0.001	0.001	0.579	0.459
Ant treatment	1	0.002	0.002	1.943	0.185
Month	2	0.007	0.004	3.192	0.072
Month×Treatment	2	0.001	0.001	0.613	0.556
Sequence×Treatment	1	0.001	0.001	0.551	0.470
Error	14	0.016	0.001		

*Significant difference between ant treatments at $P \leq 0.05$ after application of the sequential Bonferroni method

Discussion

This study established that viviparous females of *T. quercicola* are capable of changing excretion behavior and carbohydrate metabolism depending on whether they are attended by the ant *F. yessensis* or not. This behavioral and physiological plasticity in *T. quercicola* was not influenced by the sequence of the ant treatments (ant-attendance or ant-exclusion), suggesting that tapping by the attending ants or chemical stimuli from them directly induced the changes in aphid behavior and physiology. The aphids' ability to recognize the presence of ants is definitely demonstrated by the frequency of excretion behavior which was markedly enhanced under ant attendance. When attending ants were experimentally removed, *T. quercicola* females excreted honeydew less frequently and disposed of it by kicking the droplets away. This behavior is effective in preventing sooty

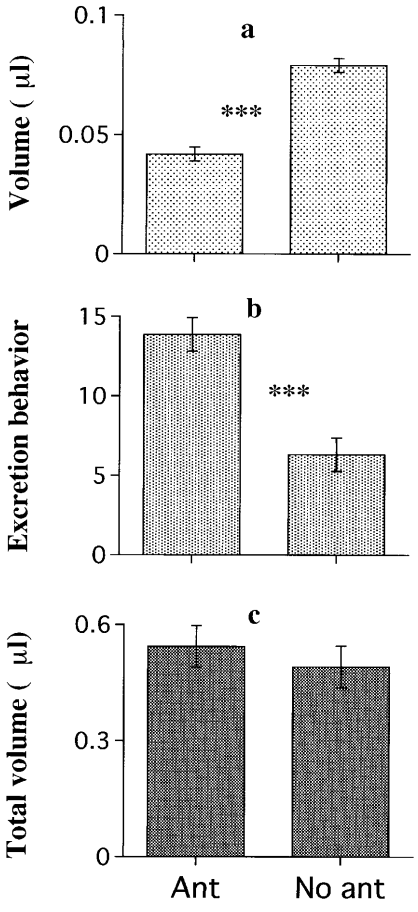

Fig. 2a–c Honeydew excretion under ant attendance (*Ant*) and ant exclusion (*No ant*). **a** The volume of a honeydew droplet per excretion behavior (µl), **b** excretion behavior per hour and **c** total volume of honeydew per hour (µl), mean±SE (***P<0.0001, see Table 5)

Table 5 Randomized block ANOVA for the effects of ant treatments on the volume of a honeydew droplet per excretion behavior, excretion behavior per hour, and the total volume of honeydew per hour

	df	SS	MS	*F*	*P*
Volume of a honeydew droplet					
Tree	2	0.0009	0.0004	6.927	0.010
Shoot (Tree)	4	0.0005	0.0001	1.911	0.173
Sequence	1	0.0001	0.0001	0.899	0.362
Ant treatment	1	0.0074	0.0074	118.336	<0.0001
Sequence×treatment	1	0.0001	0.0001	2.038	0.179
Error	12	0.0008	0.0001		
Excretion behavior					
Tree	2	19.9165	9.9582	1.202	0.334
Shoot (Tree)	4	38.2937	9.5734	1.156	0.378
Sequence	1	0.8649	0.8649	0.104	0.752
Ant treatment	1	315.2480	315.2480	38.060	<0.0001
Sequence×treatment	1	2.0675	2.0675	0.250	0.626
Error	12	99.3958	8.2830		
Total volume of honeydew					
Tree	2	0.0024	0.0012	0.055	0.947
Shoot (Tree)	4	0.1536	0.0384	1.762	0.201
Sequence	1	0.0064	0.0064	0.294	0.598
Ant treatment	1	0.0161	0.0161	0.739	0.407
Sequence×treatment	1	0.0017	0.0017	0.078	0.785
Error	12	0.2616	0.0218		

mold from growing on and around the colonies when the density of the attending ants is low. While field observations have indicated that colonies of *T. quercicola* are always attended by any ant species (I. Yao, unpublished work), this behavioral plasticity is adaptive to occasional absences of attending ants, implying that *T. quercicola* is not always dependent obligatorily on the attending ants.

Cherix (1987) reported that four species of aphids were observed being attended by *F. yessensis* on the Ishikari Coast. The diets of *F. yessensis* consisted primarily of aphid honeydew rather than protein sources, implying that these aphid species could compete for ant attendance (Cherix 1987). Competition for ant services among coexisting aphids including *T. quercicola* may have promoted the evolution of plastic responses to attending ants in order to maintain ant visitation.

The increased rate of honeydew-excretion behavior in *T. quercicola* under ant attendance was consistent with the results of previous studies on *Aphis fabae* (Banks and Nixon 1958), *Tuberolachnus salignus* (Mittler 1958), and *A. craccivora* (Takeda et al. 1982). The rates of excretion behavior of homopterans have often been considered to reflect the feeding rates (Stadler and Dixon 1999). However, this study reveals that even though the frequency of excretion behavior is enhanced by ant attendance, the aphid feeding rate itself may not increase as much, because the difference in excretion frequency has no significant effect on the total amount of honeydew excreted per hour (Fig. 2; Table 5). This suggests that the aphids are always feeding at an optimal rate, with the excretion frequency being altered according to ant demands.

The enhanced rate of excretion behavior had a consistent effect on the carbohydrate metabolism of *T. quercicola*. Although the total sugar concentration did not change between the treatments (Table 2), the honeydew of ant-attended aphids was characterized by the decreased proportion of glucose and the increased proportions of sucrose and trehalose. There is no evidence that these changes in the sugar composition are adaptive for attracting attending ants, because melezitose, a trisaccharide most effective in attracting ants (Völkl et al. 1999), does not vary between the treatments in concentration or in proportion. An abundance of melezitose in honeydew, despite its absence in the phloem sap of the host plant, is commonly found in aphids and other homopterans (Bacon and Dickinson 1957; Mittler 1958; Byrne and Miller 1990; Völkl et al. 1999). It is likely that melezitose is synthesized from sucrose and glucose (Auclair 1963) not only for osmoregulation but in particular for the attending ants, as suggested by Kiss (1981). The constancy of melezitose in the honeydew suggests a physiological mechanism by which melezitose is kept at a constant concentration.

Sucrose, the primary component of the phloem sap, is transported into the aphid' s alimentary canal, where it is hydrolyzed by sucrase to the monosaccharides glucose and fructose (Srivastava and Auclair 1962; Blum 1985; Srivastava 1987). Along a concentration gradient of glucose from the midgut to the hemolymph, glucose passively diffuses through the midgut wall into the hemolymph and then is converted to the disaccharide trehalose in the fat body (Wyatt 1967; Blum 1985; Turunen and Crailsheim 1996). Trehalose, a storage carbohydrate characteristic of insect hemolymph, is hydrolyzed into glucose in tissues for energy metabolism (Wyatt 1967).

Sucrose in honeydew is regarded as an undigested part of the excess sugar ingested (Srivastava 1987). Thus, an increased proportion of sucrose in honeydew implies that the efficiency of its hydrolysis in the alimentary canal is lowered under ant attendance. This may be partly attributable to the ingested phloem sap passing through the alimentary canal at such a higher rate that catalysis by sucrase is partially hindered (Srivastava 1987). The lowered efficiency of sucrose hydrolysis would simultaneously lower the proportion of glucose in the midgut. The change in the proportion of glucose in honeydew was marked (Fig. 1) and also sensitive to the season (Table 4). However, glucose comes from the phloem sap and from the hydrolysis of sucrose while it is used for the synthesis of oligosaccharides, so that it is difficult to understand the direct cause of the changes. The decrease in glucose towards autumn seems to reflect the decrease of sucrose in the phloem sap, but it is not clear why sucrose in honeydew showed no seasonal change. Trehalose sometimes escapes from the hemolymph to the lumen of the midgut due to an occasional reversal of a glucose gradient, finally appearing in the honeydew (Wyatt 1967). Ant attendance seems to have promoted the escape of trehalose into the midgut lumen. Probably, the leakage of trehalose leads directly to a shortage of energy metabolic substrates.

In conclusion, despite limited information regarding the precise mechanisms of carbohydrate metabolism, the changes in sugar composition in honeydew as a whole suggest that the amount of carbohydrates available for energy metabolism was more limited under ant attendance than under ant exclusion. It is most likely that these chemical changes in honeydew provide a material basis for the cost of ant attendance to aphids.

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