

Research Article

Trade-Off between Foraging Activity and Infestation by Nest Parasites in the Primitively Eusocial Bee *Halictus scabiosae*

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Diurnal activities of *Halictus scabiosae* bees and their nest parasites (major bee-flies, cuckoo wasps, ichneumon wasps, *Sphecodes* bees, and velvet ants) were investigated at a study site with 159 nests in Eastern Austria. Foraging activity correlated with ambient temperature only before midday and decreased in the afternoon. The activity of nest-infesting parasites increased during the day and correlated with ambient temperature. The match factor fm between the ratios of the foraging activities of *H. scabiosae* and the ratios of aspects of morning temperature was assessed on three consecutive days with different weather. The activity patterns of halictine bees and their nest parasites differed: the parasites exhibited only small time windows in which their activities were synchronised with those of their hosts. The bees exhibited an anticyclic behaviour and collected food in times of low parasite pressure and decreased foraging activity when parasite pressure increased.

1. Introduction

The way in which animals may alter their foraging behaviour under predator or parasite threat is a large and well-researched area of behavioural ecology [1, 2]. The interdependence of activity patterns between predators and their prey, as well as between parasites and their hosts, is affected by a diversity of factors. It has been observed for mammals and birds [3] in particular, that foragers cease feeding when the benefit of harvesting no longer outweighs the cost of foraging. Marginal costs of foraging include the risk of predation, also while the animals are carrying food items to their protective cover [4], and they also include the infestation of potential hosts by nest parasites. This paper reports on the principles of trade-off between foraging and the risk of infestation by parasites, using *Halictus scabiosae* bees, which need to juggle between keeping their nest entrances open to facilitate foraging traffic and closing the entrances in order to reduce parasite impact.

Halictus is a large genus of Halictidae, which is divided into 15 subgenera with well over 300 species, primarily in the Northern Hemisphere. Many species in the genus are

eusocial [5–7], with colony sizes ranging from very small (2–4 bees) to large (>200), showing division of labor and castes, and guards for defense. Nests are typically underground burrows [8], with several ovoid cells in which a mix of pollen and nectar is stored as food for the developing larvae. The cells may be arranged in clusters resembling a honeycomb, but constructed of soil rather than beeswax. The nests have a main entrance. The duct widens below the entrance, allowing the bypassing of a forager aside a guard bee, and reaches 20 to 30 cm into the soil. It is ramified by lateral ducts, cells, and emergency outlets. Due to the long flight season from the end of April until October [9, 10], halictine bees have evolved as a polylectic species. In the summer, they develop smaller sterile summer females with a low rate of production of males [8]. Queen females copulate in autumn and hibernate in polygyne associations [9]. Two to five summer bees stay in the maternal nest and provide food for the brood [10]. Females hibernate in the maternal nests and reuse them to rear the next generation. After the hibernation period the polygyne society turns into *semisocial*, and the egg-laying female, the queen, is engaged as a guard bee [10]. Later on, the queen chases off the other females [9] and lives with her



FIGURE 1: *Halictus scabiosae*. (a) Homing forager bee with pollen on its coxae and femurs reopening the nest entrance hole that had been previously closed by the queen. (b) Guard bee at the nest entrance.

filial generation turning the society to *primitive eusocial*. The females that have been chased away by the queen may found their own nests in the vicinity of their original home nest by usurping nests of other bees such as *Lasioglossum nigripes* [11, 12]. In this way, *Halictus scabiosae* societies are regularly observed in nest aggregations.

In halictine bees activity patterns of nectar and pollen foraging are determined by a set of major factors [13–15]. Microclimatic conditions primarily affect the life of the bees regarding flying ability, mating behavior, and the development and survival of larvae [16]. Ground-nesting insects, like many wasps, bees, and ants, likely depend more on soil temperature than on air temperature with regard to nest-site selection, daily activity patterns, foraging success, and sex allocation [16, 17]. This is also true for the social ground-nesting halictine bees with an annual life cycle. Soil temperature controls the duration of the development of helpers and the rate of provisioning [18], affecting the number of broods that can be produced during the limited flight season, and therefore also the colony size and the level of social complexity [17]. Furthermore, the amount of time a bee forages per day is associated with the amount of pollen she can gather. More specifically, the daily rate of foraging trips is correlated with the minimum temperature over daytime [19], with the shortage of food for their offspring [15], and with the quality and quantity of floral rewards available [13, 14].

Finally, foraging bees have to cope with insect predators and parasitoids. In general, the level of compatibility of a particular host-parasite combination depends not only on unsuitability but also on active resistance by the host against the parasite which implies a cost for the host [20, 21]. There are at least three main strategies that bees have evolved against nest parasites. (a) Many halictine species keep the nest entrances open during the foraging period during the day but seal them with soil daily after foraging activity ceases [10]. Pleometrotic species such as *Halictus scabiosae* with more intense flight traffic keep their entrances open for longer than haplometrotic species. In addition, social species close their nests after the provision of the larvae has been

terminated. In this case the entrances are not reopened again until the new generation hatches. (b) Sociality facilitates foraging activities while the nest remains guarded. This aspect is considered an important factor in the establishment of social behaviour [22], in particular in the evolution of larger social units among the halictine bees. On the other hand, there are arguments which make it unlikely that the protection against predators or parasites bestows any significant advantage to pleometrotic nests [23–25] (c) *Halictus scabiosae* societies are regularly observed in nest aggregations which may improve mating efficiency and nesting success. On one hand, a higher nest density may provide a visual stimulus for further nesting in a given locality by social facilitation [17, 26] but also allows females to enter foreign nests, along with their general tendency to guard nest entrances. On the other hand, a nest aggregation considerably increases parasite and predator pressures because aggregated hosts can be found more easily [22].

The paper reports on activities recorded for a batch of 28 nests of an aggregation of 159 *Halictus scabiosae* nests that have been monitored continuously over three days during daytime. We first measured the foraging activity and nest-sealing behaviour of the *Halictus scabiosae* nest members, and second, we assessed the activity of parasites, which tried to infest the bee nests under observation. We gathered evidence for the hypothesis that the foraging activity of the worker bees in *Halictus scabiosae* is anticyclic to the diurnal activity of nest parasites. A positive proof of this hypothesis would suggest that halictine bees minimize the parasite impact on their nests by decreasing their foraging activities when parasite pressure is high.

2. Material and Methods

2.1. Species and Study Site. We observed an aggregation of nests of *Halictus scabiosae* (Figure 1) distributed along the edge of a forest in an area of 5×24 m (120 m^2) in Krobotek ($46^\circ 58' \text{N}$, $16^\circ 11' \text{E}$) in southern Burgenland, Austria, at an altitude of 300 m sea level (Figure 2). The nesting area was



FIGURE 2: Study site. A strip of 5×24 m (orange bar in the middle image and orange arrows) near the edge of a forest in Krobotek ($46^{\circ}58'N$, $16^{\circ}11'E$) in southern Burgenland (Austria) at an altitude of 300 m sea level was selected as the study site. An aggregation of 159 nests of *Halictus scabiosae* was found in a meadow with thermophilic vegetation on sandy-loamy soil. Yellow scale bars represent 100 m in length. The orange area and the arrows mark the study patch. The bottom image shows the four camcorders on tripods filming the activities at four selected batches as defined in Figure 3.

located on a slope that was slightly inclined to the south, with thermophilic vegetation on sandy-loamy soil. We counted 159 entrance holes of 4 to 6 mm diameter and 36 slightly bigger (7 to 10 mm) holes (Figure 3). All holes were active and used by outgoing and incoming bees, by guard bees or other bees that closed or reopened the holes, but no traffic was observed at or around the 36 bigger holes. The fact that the holes remained still open let us assume that they belonged to the nests in close vicinity. Possibly, these were “emergency outlets” which are known from *Halictus* nests [8].

2.2. Videotaping and Recording of Meteorological Data. The strip selected for observation was situated along the edge of a forest as documented in Figure 2. Here, four cameras on tripods were positioned to trace four sample batches with a total of 28 nests: (a) 22×22 cm with four nests; (b) 28×31 cm with eight nests; (c) 36×32 cm with twelve nests; (d)

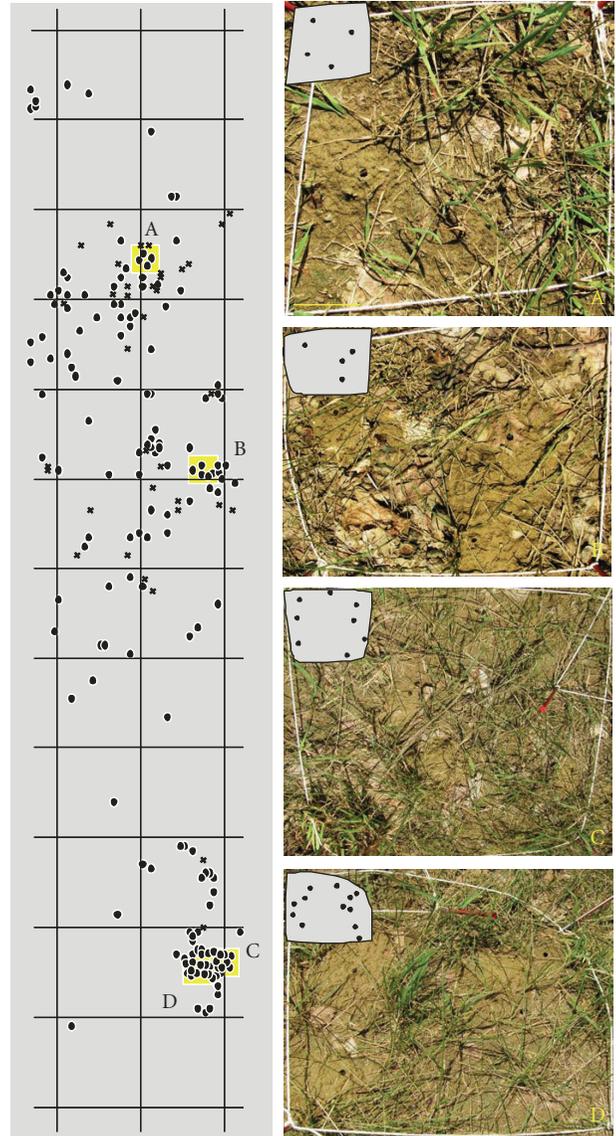


FIGURE 3: Mapping of the nests of *Halictus scabiosae* at the study site. Left: strip of 5×24 m (see Figure 2) with the four batches of nests (A–D, marked by yellow areas) where the activities of *Halictus scabiosae* and their parasites were videotaped. The grid indicates $2 \text{ m} \times 2 \text{ m}$. Right: batches of active nests; the insets show the batches on a smaller scale with the nest entrances shown as black full circles.

28×22 cm with four nests. The entrances of these sample nests were videotaped on 3 days in July 2008 during daytime (between 08:30 and 15:00 h). Recording was only interrupted for few minutes when it was necessary to replace the used tape by a new one. The pauses were logged and considered in the assessment of the rate of nest activities.

Ambient temperature, irradiance, and relative humidity were recorded every 10 seconds using a HOBO data logger.

2.3. Assessment of Nest Activities. Typically, foraging bees flew off the nest within seconds. Guard bees only appeared with their heads at the nest entrance and stayed there. When

foragers came back and tried to enter the holes, the guards retreated and let the incoming forager pass [27]; afterwards the guards immediately reappeared at the entrance hole. The frequency of outgoing (A_{out}) and incoming (A_{in}) activities was assessed from the videos. The kind of activity, its time, and the nest concerned were recorded.

2.4. Assessment of the Impact of Parasites on the Nests of *Halictus scabiosae*. The presence of five groups of nest parasites was observed: the major bee-fly (*Bombylius major*, Asilomorpha, Bombyliidae), the cuckoo or gold wasps (subfamily Chrysidinae, family Chrysididae), ichneumon wasps, members of the genus *Sphcodes*, and velvet ants (Mutillidae). Each parasite that patrolled around the nests was logged and identified from the video tapes. The five nest parasites were grouped for statistical analysis. However, there were typically two categories of parasite impact (I_p). First, if parasites scanned unspecifically around the nests, equal impact values were assigned to all active nests in each batch. For example, if there were four nests, each of the nests was assigned an impact value of 0.25 for a single observation of a scanning parasite. Second, when a parasite visited the nest area specifically at the nest entrance, a parasite impact value of 1.0 was assigned to the visited nest.

2.5. Basic Statistics. Means and mean errors of all data were calculated. The data of nest activities (A_{out} , A_{in} , I_p) were normalized per nest and per observation period of 30 minutes. For that, nest entrances were selected on the first day of experimentation, which were active with regard to outflying and incoming frequency and guarding. Inactive nest holes were not considered in the normalizing of the rates of bee activities or parasite impact. For description of diurnal activity patterns and for comparison of the rates of nest parasites and bee activities the means of the normalized data were used to calculate the corresponding regression polynomials which were tested for significance using Sigmaplot.

2.6. Calculation of the Probability of the Match between Nest Activities and the Aspects of Morning Temperature. The question was investigated of whether and how the temperature conditions in the morning affect the activities of bees and their parasites later in the day. The morning temperature conditions could first, trigger the decision of the bee to start foraging and second, it may influence the rate of foraging throughout daytime. Similarly, the nest parasite could be influenced in its starting time to infest the nests of the hosts and in the infestation rates throughout daytime. In our model, we take into account that bees and parasites have to sense the following critical morning temperature aspects prior to their first bout. These include the temperature inside the nest (T_n), the ambient air temperature (T_a) outside the nest, the difference between both aspects (ΔT_{a-n}), and lastly, the change in ambient temperature within a given initial time interval $[t_0, t_1]$.

For calculating these morning aspects of temperature we considered the time interval between $t_0 = 8:50$ to $t_1 = 9:50$ h

in the three experimental days (d_1, d_2, d_3). The mean change in ambient temperature in this initial hour of experiment (ΔT_a) was calculated by averaging the changes in steps of 10 seconds. The initial temperature inside the nest T_n was mathematically assumed with a virtual range between 12° and 19°C. This allowed us to calculate a usable measure of the morning aspect of temperature according to the equations $\Delta T_m[d_i] = (T_a - T_n) + \Delta T_a$ and $r\Delta T_m[d_i] = \Delta T_m[d_i]/\Sigma\Delta T_m[d_{1+2+3}] * 100$ (for $i = 1$ to 3 experimental days). This relative value includes all crucial temperature aspects which could be important for the bee or the parasite to decide to start the first bout in the morning and is a useful measure for the correlation of behavioral traits of bees and parasites on sequential days under varying weather conditions, provided that similar mathematical procedures are applied. The outflyer rate A_{out} of the bees was taken as an estimate for the aspect of foraging activity A_{out} , and the impact rate of parasites I_p for the aspect of infestation. Both measures were normalized per nest and per 30 minutes observation time and related to the sum of the respective time interval of the three experimental days according to

$$\begin{aligned} rA_{out}[k, d_i] &= A_{out}[k, d_i]/\Sigma A_{out}[d_{1+2+3}] * 100, \\ rI_p[k, d_i] &= I_p[k, d_i]/\Sigma I_p[k, d_{1+2+3}] * 100 \end{aligned} \quad (1)$$

for each time interval k of observation of the day d_i (for $i = 1$ to 3).

We then compared the ratios of the relative aspects of morning temperature $r\Delta T_m[d_1] : r\Delta T_m[d_2] : r\Delta T_m[d_3]$ with the ratios of the relative aspects of foraging activity of the bees $rA_{out}[k, d_1] : rA_{out}[k, d_2] : rA_{out}[k, d_3]$, and that of the infesting activity of their parasites $rI_p[k, d_1] : rI_p[k, d_2] : rI_p[k, d_3]$ regarding the time intervals of observation on the three experimental days. The chi-square test and the F -test were used to estimate the probability with which the daytime-dependent relations of the behavioural activities of each host and parasites match the morning aspect of temperature. In this paper, this probability of matching P_m is taken as the crucial measure to estimate whether and how behavioural activities remain linked to the morning aspect of temperature throughout daytime. P_m values of $>.05$ signify that the ratios of foraging activities of the bees and the infesting activities of the parasites match the morning aspect of temperature, whereas P_m values smaller than $.05$ document a contrast between the ratios of behaviors and the morning aspect of temperature.

3. Results

3.1. Diurnal Activity Patterns of Foraging. We recorded the activities of four batches of *H. scabiosae* nests at the study site over three days and assessed the number of bees departing (F_{out}) or homing (F_{in}). Although the weather conditions differed considerably from day to day, the activity patterns were uniform insofar as they peaked between 10 and 12 am and declined in the early afternoon, mostly before the daily ambient temperatures reached their maximum values (Figure 4(b)). Day 1 was representative for this tendency; the

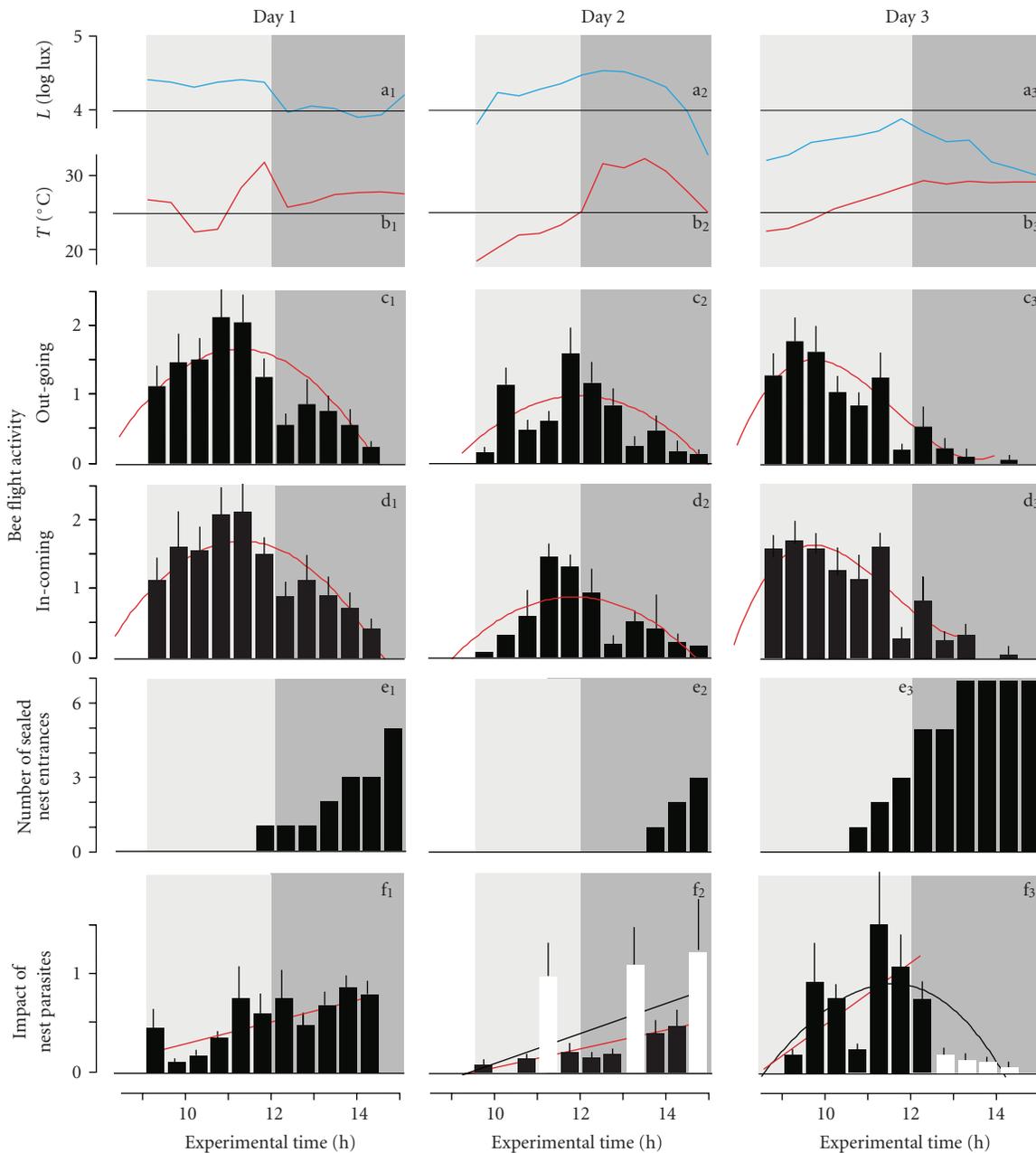


FIGURE 4: Activities observed at 28 nests (four batches) of *Halictus scabiosae* nests over three days (1–3). (a) Irradiance in lux and (b) ambient temperature in °C measured in the shade. (c) Out-flying and (d) incoming activity pooled over all 28 nests; data were normalized per nest and 30 minutes of observation time; red curves show the regression functions, which allow to extrapolate the flight rates before the start of the observation sessions (which are coded by the grey background; am, bright grey; pm, dark grey); (e) sealing activity at the study site, given by the number of sealed nests in steps of 30 minutes; (f) the impact of nest parasites (for measurement, see Methods) on the experimental nests of *H. scabiosae*, given by the number of parasites per nest and 30 minutes of observation time. The regression curves (red) refer to all means coded in black columns which are used for further analysis (for equations of the regression functions see Tables 1 and 2 and text).

foraging activities reached the maximum level of 2 individuals per nest and 30 minutes observation time (Figures 4(c₁) and 4(d₁)). The activity patterns on all experimental days are displayed in Figures 4(c) and 4(d) and their regressions are defined in Table 1 (line 1–6).

The daily activities of the bees reflect, to some extent, the weather situation. For instance, the morning temperature prior to experimentation triggered the emergence of the bees for their first bouts. Correspondingly, the lower temperatures in the morning of the second day (Figure 4(b₂)) triggered

TABLE 1: Regression functions of foraging activities of *Halictus scabiosae*. F_{out} and F_{in} : outgoing and incoming foraging activity (per nest and per 30 minutes observation session); $F = (F_{out} + F_{in})/2$; t : daytime in hours; T_{amb} , ambient temperature in °C; a–d: regression coefficients, r : correlation coefficient. *Additional virtual values (marked with +) were introduced for the calculation of the regression function.

Line	<i>Halictus scabiosae</i> reference	Regression	Day	a	b	c	d	r	n	P
1	Figure 4(c ₁)	$F_{out} = a * t^2 + b * t + c$	1	-0.145	3.230	-16.517		0.844	12	.004
2	Figure 4(d ₁)	$F_{in} = a * t^2 + b * t + c$	1	-0.159	3.576	-18.486		0.901	12+2	<.001
3	Figure 4(c ₂)	$F_{out} = a * t^2 + b * t + c$	2	-0.110	2.531	-13.601		0.742	13	.033
4	Figure 4(d ₂)	$F_{in} = a * t^2 + b * t + c$	2	-0.108	2.556	-14.339		0.742	13	.033
5	Figure 4(c ₃)	$F_{out} = a * t^2 + b * t + c$	3	0.053	-1.809	20.100	-71.450	0.761	12 + 3*	.014
6	Figure 4(d ₃)	$F_{in} = a * t^2 + b * t + c$	3	0.058	-1.965	21.777	-77.358	0.669	13 + 2*	.048
7	Figure 5(a)	$F = a * t^3 + b * t^2 + c * t + d$	1–3	0.0022	-0.077	0.704	-0.743	0.940	15	<.001
8		$F_{out} = a * T_{amb} + b$	1	0.020	0.592			0.087	11	.800
9		$F_{out} = a * T_{amb} + b$	2	0.005	0.488			0.045	11	.893
10		$F_{out} = a * T_{amb} + b$	3	-0.079	2.914			0.363	11 + 2*	.223
11	all day	$F_{out} = a * T_{amb} + b$	1–3	0.024	0.1089			0.139	39	.396
12	pre-noon	$F_{out} = a * T_{amb} + b$	1–3	0.107	-1.4523			0.615	15	.015
13		$T_{amb} = a * t + b$	1–3	1.239	12.392			0.680	39	<.001
14		$F_{in} = a * F_{out} + b$	1–3	0.152	0.878			0.826	988	<.001

foraging flights later and at a lower rate (Figures 4(c₂) and 4(d₂)). On the third day, the moderately warm temperature caused earlier foraging activity which reached levels higher than that on the previous day, in spite of lower irradiance due to overcast sky (Figure 4(a₃)). This higher foraging level on the third day was probably caused by a shortage in food provisioning for the larvae due to the lower temperatures on the day before.

The rates of outgoing (F_{out}) and incoming bees (F_{in}) correlated significantly ($r = 0.826$, $P < .001$; Table 1: line 14), and there was no time lag between the patterns of outgoing and incoming rates. This indicates that the departing individuals were identical with the homing bees, and that the foraging flights were on average shorter than 30 minutes.

In Figure 5(a) the activities of all 28 nests at the study site were pooled for a more integrative view. The regression of the mean values of this distribution ($r = 0.940$; $P < .001$; Table 1: line 7) confirms that there was a peak in foraging activity between 10 and 12 am. However, this summarization does not consider the weather conditions which changed during daytime and from day to day. The question is how the foraging activities in *H. scabiosae* were affected by the weather conditions; the most important measure for weather appeared to be ambient temperature, which is discussed in the next section.

3.2. Do Diurnal Foraging Patterns Correlate with Ambient Temperature? We correlated the foraging activities of all experimental days with the ambient temperature over the entire experimental time when halictine bees were actively foraging (9:00–15:00 h). The resulting match ($r = 0.139$; $P = .396$; Table 1, line 8–10, 11) was much lower than the correlation of the ambient temperature with the daytime ($r = 0.680$; $P < .001$; Table 1: line 13). These data, together

with Figures 4(c) and 4(d), strongly suggest that foraging in halictine bees is not dependent on ambient temperature alone. The bees are affected by daytime, which could, at least partly, be independent of ambient temperature. We therefore investigated whether a time window exists in which the bees would have decided their foraging strategy of the day, for example, when to emerge first and at which rate foraging should proceed. A possible answer comes from the correlation of the foraging rate with the ambient temperature, if merely the time intervals before noon are considered. In this time period, the foraging rate is directly correlated with ambient temperature ($r = 0.615$; $P = .015$; Table 1: line 12). After the midday foraging peaks, the bees apparently organized themselves according to other principles. Most of the colonies decreased their foraging activities and retreated to the nest; some nests were closed (Figure 4(e)).

In a second approach, we investigated if the weather conditions in the morning are crucial for the bees to control the consecutive foraging later in the day. The question is to which extent this *aspect of morning temperature* (for definition, see Methods) correlates with the subsequent foraging activity of the same day. The large differences of the three experimental days regarding weather and foraging compromised the feasibility of the analysis of mean behavioural processes. This variability of the experimental conditions, however, allowed us to compare the ratios of the *aspects of morning temperature* with the ratios of the foraging activities of the bees and the infestation activity of parasites. The respective probability of matching P_m evaluates this comparison of all three experimental days (see Methods) under two surmises (Figure 6): first, the worker bees compare, in particular before they emerge for their first foraging bout, the current temperature inside the nest (defined virtually in the model in the range from 12° to 19°C) with the ambient temperature outside of the nest.

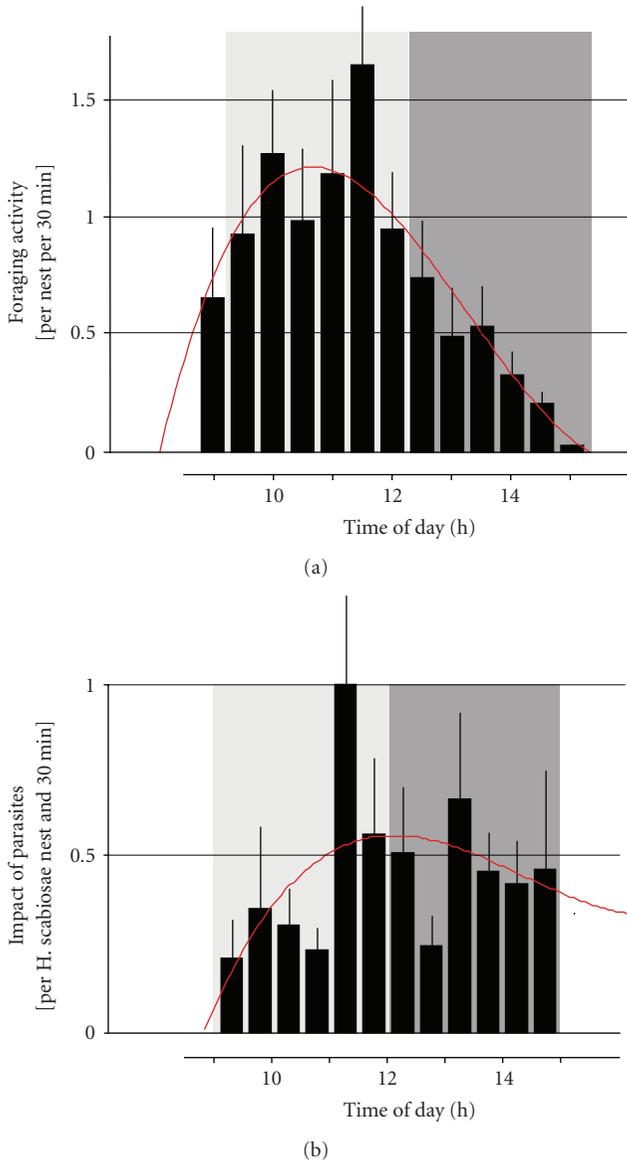


FIGURE 5: Summary of (a) mean foraging activities of 28 nests over three days and (b) mean impact rate of parasites as assessed at the experimental nests of the study site. Red curves, regression functions of the means (for equations, see Tables 1 and 2 and text).

Second, the bees remain affected by this *aspect of morning temperature* while they continue foraging. This surmise could be important if the morning temperature conditions were not sufficiently appropriate to predict the weather later in the day. In fact, the probability P_m with which the temperature conditions in the morning (T_m) would allow a prediction of the ambient temperature between 10:00 h and 14:00 h on the experimental days d_1 – d_3 decreased over daytime and was lower than 0.05 for nest temperatures greater than 15°C (Figure 6(d)), which turns the morning aspect of temperature into an unreliable predictor of ambient temperature relations later in the day.

Figure 6(a) shows the probability value P_m between the aspect of morning temperature and the foraging activity. The probability P_m was calculated for each time step of 30 minutes of the entire observation time and detects matched ($P_m > .05$, chi-square test) or significantly diverse ($P_m < .05$) relations. There were two peaks in the P_m curves for the foraging bees; the first prominent peak occurred before noon when the foraging activities also peaked. The second peak in the P_m curves occurred after midday, between 13:00 and 14:00 h, and was weaker and shorter than the first one. The results let us assume that the aspect of morning temperature could be important for a bee's decision on her consecutive foraging activity at least in her pre-noon foraging activity.

3.3. Nest Closing and Guarding. In halictine bee colonies the nest entrances are usually closed (Figure 7(a)) as soon as the flight activity is terminated for the day. During nighttime or rainy weather the nests were also kept closed [10]. The nest entrance normally ends at the surface as a funnel with elevated rims, because some of the material gained by digging the nest tubes accumulates around the entrance hole. The females take material from the inside of the tube and close the entrance hole with their abdomen. Females that arrived after their home nests had already been sealed off were able to reopen it by digging at the right place (Figure 7(b)). For that, the homing foragers circled around with their body and removed soil parts with their mandibles. We counted the number of entrances that were initially open and also those that were sealed later in the day. In Figure 4(e), the cumulative numbers of closed entrances on each of the experimental days are shown. In the four batches of 28 nests, we counted five closing activities on day 1, three on day 2, and seven on day 3. These ratios of closing fit with the ratios of ambient temperatures on all experimental days in the time before midday ($P > .05$, F test); later, the data match at ($P < .05$, F test). Therefore, it is likely that the temperature aspect before noon determines the activity of closing the nest entrances.

Generally, all active nests provided guarding at least at certain periods of the day. Guard bees were initially present at the entrance, in particular before midday. Later on in the afternoon, they only appeared at the entrance hole, as soon as a parasite had approached. We did not observe any guard bee that flew out during her guarding. It happened several times that homing bees came to the nest entrance and competed against the guard bee which kept the entrance closed with her body. Sometimes the arriving forager bee succeeded to drag the guard out of the entrance hole, but some seconds later the guard bee had returned to her place.

3.4. Nest Parasites. The presence of five groups of nest parasites was observed. (1) The major bee-fly (*Bombylius major*, Asilimorpha, Bombyliidae) generally feeds on nectar and pollen in the adult stage; thus they are pollinators. The larval stages are predators or parasitoids of other insect eggs and larvae. We observed adult females that scanned around the nest sites and deposited eggs in the vicinity of the open entrances. *Bombylius major* has been described

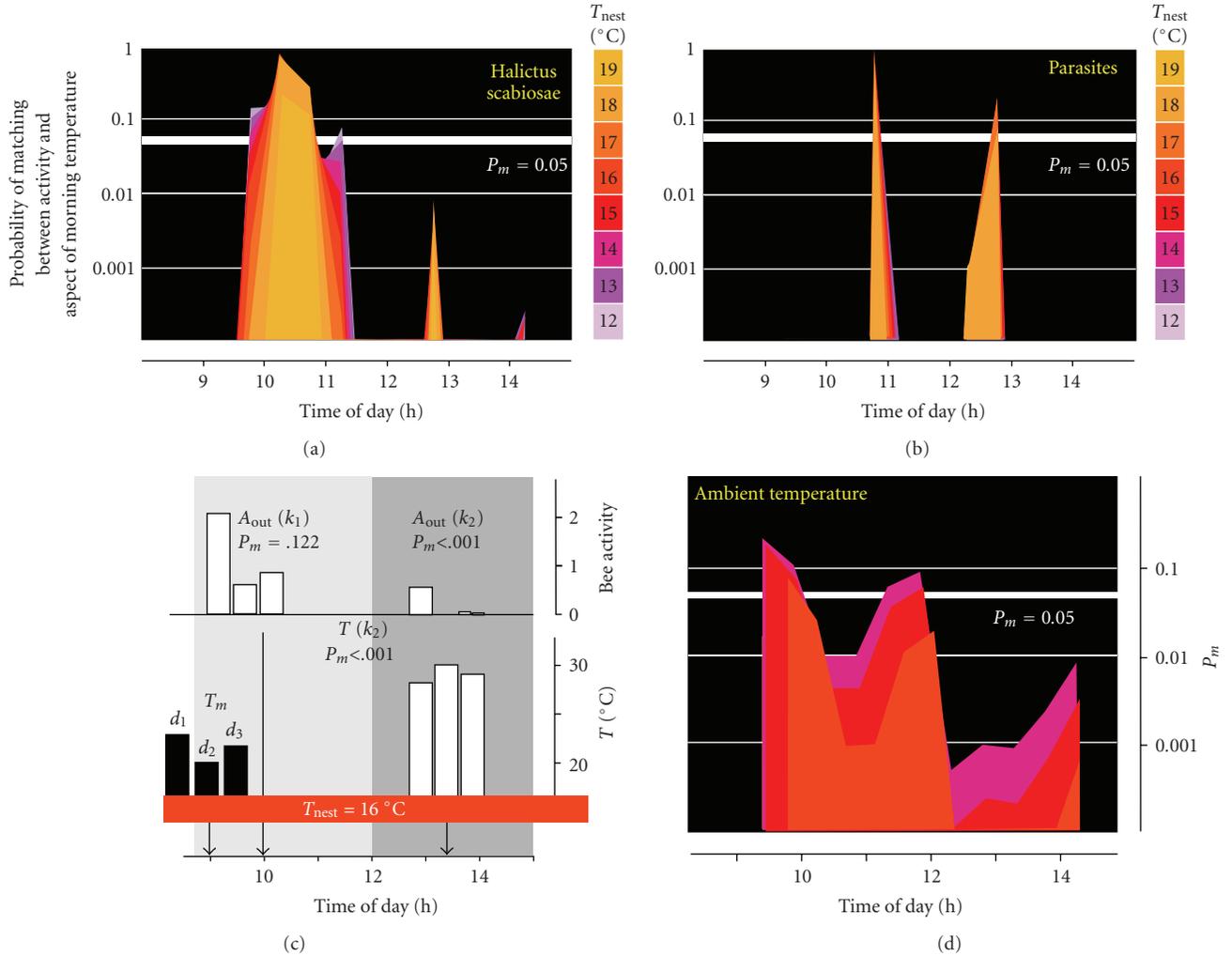


FIGURE 6: Support for the hypothesis that the aspect of morning temperature (for definition see Methods) determines the foraging activity of *Halictus scabiosae* and partially also the impact of nest parasites at the *H. scabiosae* nest over daytime. The abscissa gives the time of the day in hours and the ordinates show the probability of the match (P_m) with which the morning temperature conditions affect (a) the foraging activity rate in *Halictus scabiosae*, and (b) the ratio of the impact rates of parasites at *Halictus scabiosae* nests. The temperature conditions in the morning for the bees or parasites (for definition, see Methods) refer to the ambient temperature which was measured outside of the nest as well as to the temperature inside of the nest, which has been introduced into the model as a virtual parameter in the range from 12°C (blue violet) to 19°C (bright orange). The probability P_m was calculated by chi-square tests for each time step of 30 minutes of the entire observation time. (c) explains the assessment of the probability P_m ; $P_m > .05$ (chi-square test) signifies similarity, and $P_m < .05$ signifies contrast. T_m : morning aspect of temperature on the three successive experimental days (d_1, d_2, d_3) defined for the initial hour of experiments; $A_{\text{out}}(k_1)$ and $A_{\text{out}}(k_2)$: the relations of outflyer activities in the observation intervals k_1 (9:30–10:00 h) and k_2 (13:30–14:00 h) on the three successive experimental days (d_1, d_2, d_3) with the $P_m(k_1)$ -values 0.122 and $P_m(k_2) < .001$; $T(k_2)$: the temperature relations in the early afternoon in the observation interval k_2 with $P_m < .001$; the examples refer to a virtual nest temperature of $T_{\text{nest}} = 16^\circ\text{C}$ (d) gives the probability P_m by comparing the morning aspect of temperature on the days d_1 – d_3 with the ambient temperature later in the day. The graph shows that the match is decreasing over the day with $P_m < .05$ for nest temperatures greater than 15°C.

for *Andrena*, *Colletes*, *Osmia*, and *Megachile* spp. but not for *Halictus* sp. [8, 11]. (2) The cuckoo or gold wasps (subfamily Chrysidinae, family Chrysididae) are typically associated with solitary bees and are generally cleptoparasites [11], laying their eggs in host nests where their young larvae consume the host eggs or larvae, later also consuming the provisions. (3) Some species of ichneumon wasps lay their eggs in the ground, but most inject them directly into a host's

body, typically into a larva or pupa of solitary bees [28]. At the study site, ichneumon wasps were observed scanning around the nests of *Halictus scabiosae*. (4) Members of the genus *Sphecodes* are solitary parasitic bees; the larvae of this species are parasites of other solitary bees [8, 11]. (5) The velvet ants (Mutillidae) are a family of wasps whose wingless females resemble ants. The male wasp flies around searching for females. After mating, the female searches for a suitable



FIGURE 7: Nest closing and reopening. (a) Closing of an entrance hole from the inside of the nest by an egg-laying female; the sealing itself took 8 seconds while the whole closing activity continued over a further five minutes; images a_1 to a_6 were taken every seconds. (b) Opening of the nest entrance by circling movements by a worker bee from outside. This process took 8 minutes. The nest entrance was closed up at 11:20 h; at 11:28 h the worker bee in the image reopened the nest from outside. Four minutes later, the entrance hole was closed again. The images b_1 to b_6 were taken in the last minute before the forager bee succeeded to enter the nest.

host, typically a bee's nest, and lays her eggs near the larvae or pupae. The mutillid larvae are idiobiont ectoparasitoids that eventually kill and eat their immobile host.

The activities of nest parasites at the nests of *H. scabiosae* are displayed in Figure 4(f). The first experimental day

appeared to be representative regarding the impact of nest parasites. The rates with which parasites appeared at the nest per 30 minutes interval increased steadily over time and did not peak before 15:00 h when the observation session was terminated. A similar tendency was also observed on day 2,

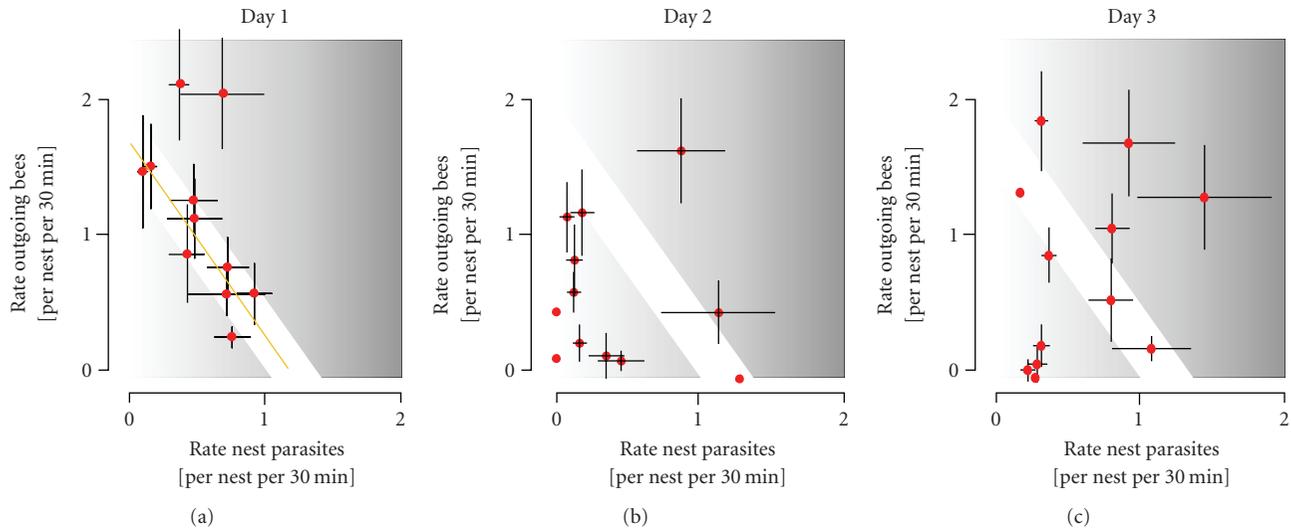


FIGURE 8: Correlation between the foraging rates and the impact rates of nest parasites on three successive days with different weather conditions. Values were normalized to nest and 30 minutes of observation time. On day 1, the bees obeyed the simple rule that foraging activity decreases with increasing parasite impact (regression function: $F_{out} = -0.4903 * I_p + 1.6834$; $n = 9$; $r = 0.892$; $P < .001$; shown by a white stripe in the correlations graphs of all experimental days). The graphs indicate that averaging of the daily rates of bee and parasite activities is crucial, in particular due to the massive influence of weather conditions.

but at lower level, whereas on day 3 the activity of parasites, after having developed in a similar way as on the previous days, was reduced due the worsened weather conditions in the early afternoon. Conforming to previous reports [29] the impact of parasites showed a general trend to increase over daytime ($P = .008$) and with ambient temperature ($P < .001$; Table 2, lines 7–10); this was assessed on each of the experimental days (Figure 4(f); Table 2: lines 1–5) and averaged over the whole session (Figure 5(b); Table 2: line 6).

If it was true that the infestation of halictine nests by nest parasites is controlled by daytime rather than ambient temperature, then it can be expected that the aspects of morning temperature are not essential for the parasites, which would contrast with the foraging activity of their host. Indeed, the match factor fm (Figure 6(b)) for the impact of nest parasites showed only a weak correlation in two short time windows. The first match with $P_m > .05$ occurred before noon at the same time when the bees were maximally active in foraging. A second peak with $P_m > .05$ occurred in the early afternoon, which corresponded also to a small peak in the P_m -values of their hosts. This finding let us assume that the parasites' activities match the foraging activity of their host, either by specific adaptation to the morning temperature conditions on their own or by pursuing their hosts. In any case, the diurnal activity cycles of nest parasites of *H. scabiosae* differ strongly from that of their host.

A second, much simpler, but very gross way to assess the interrelation between bees and their nest parasites is to directly correlate the activities of both (Figure 8). As would be expected, the correlation graphs differ strongly due to the different weather conditions. Again, the first experimental day was representative of the diurnal pattern of the foraging activity of bees and of the impact of parasites: the bees obeyed

the rule that they decreased their foraging activity with increasing parasite impact ($P < .001$), with the exception of the time when foraging activity peaked. At this point, the bees were more active than predicted by the regression function. This regression line has been marked as a white stripe for comparison with the graphs of day 2 and 3. On day 2, the basic activity of the parasites was low; only a single individual of *Sphecodes albilabris* visited a limited number of nests directly at their entrances for at least two times and caused two of the singular activity peaks (cf. Figure 4(f_2)). On day 3, the activities of both host and parasites were high throughout the first half of the day; afterwards the activities strongly decreased due to the cloudy and windy weather in the early afternoon. Thus, the graphs in Figure 8 show that averaging of the daily rates of bee and parasite activities for correlation could be crucial, in particular due to the strong influence of the weather.

4. Discussion

4.1. Diurnal Foraging Activity Patterns of *H. scabiosae*. The daily pattern of the pollen- and nectar-collecting activities of *H. scabiosae* is very similar to those of other halictine species. In *Halictus ligatus* [19, 23, 28], the first foraging flights were observed before 9:00 h. Within one nest, each worker may start independently or may be influenced by her nest mates. In the latter case, the rate of activation is a linear function of the number of workers waiting in the nest [30]. It is an open question [5, 22] whether bees nesting in aggregations in close proximity to each other, such as at our study site, stimulate each other to forage. The foraging activities in *Halictus ligatus* showed a diurnal peak activity around 10:30 h for queens and 10:30–13:00 h for workers

TABLE 2: Regression functions for the activities of parasites infesting *Halictus scabiosae* nests. ^[b][r]: black and red curves (see text). For other details see legend of Table 1.

Number	Parasites reference	Regression	Day	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>r</i>	<i>n</i>	<i>P</i>
1	Figure 4(<i>f</i> ₁)	$Ip = a * t + b$	1	0.120	-0.928			0.744	10	.014
2	Figure 4(<i>f</i> ₂) ^[b]	$Ip = a * t + b$	2	0.0393	-0.757			0.549	11	.081
3	Figure 4(<i>f</i> ₂) ^[r]	$Ip = a * t + b$	2	0.060	-0.592			0.904	9	<.001
4	Figure 4(<i>f</i> ₃) ^[b]	$Ip = a.t^3 + b.t^2 + c.t + d$	3	-0.008	0.150	-0.124	-5.326	0.730	11	.065
5	Figure 4(<i>f</i> ₃) ^[r]	$Ip = a * t + b$	3	0.279	-2.458			0.633	8	.092
6	Figure 5(b)	$Ip = a.t^3 + b.t^2 + c.t + d$	1-3	0.001	-0.023	0.238	-0.207	0.445	34	.008
7		$Ip = a * T_{amb} + b$	1	0.006	11.606			0.543	13	.055
8		$Ip = a * T_{amb} + b$	2	0.039	-0.756			0.513	13	.073
9		$Ip = a * Ln(T_{amb}) + b$	3	2.343	-7.248			0.503	13	.080
10		$Ip = a * T_{amb} + b$	1-3	0.058	-1.174			0.550	34	<.001

[19, 31]; some of the workers continued foraging as late as 18:30 h. In July, the worker foraging period of *H. ligatus* started and peaked after two weeks. It is known [19, 31] that foragers undertake fewer, longer foraging bouts or more numerous, shorter ones. Average handling time, flight time, and round trip time were consistently negatively correlated with the number of bouts per day. Foragers that have a higher foraging rate tended to take shorter bouts [19, 31].

In the pleometrotic halictine species such as *H. scabiosae* foraging is achieved by the smaller females, the auxiliaries, while the bigger egg-laying female (Figure 1(b)), the queen, generally guards the nest at the entrance [10, 12]; this may occur in up to 75% of the nests, even in the matrilineal phase. We frequently observed at *H. scabiosae* nests that the guard bee blocked the entrance against homing individuals which potentially could have been own nest members [10, 12]. Such aggressiveness displayed by queens is supposed to increase with parasite pressure and leads to nest founding by the smaller females which may successively usurp nests of unrelated halictine species in close vicinity [10].

4.2. The Trade-off between Foraging and Becoming Infested. It is a common view that insects use temperature to determine the “time of season” [32]. Temperature is an important factor for bees to control activity rates, but it does not control all aspects of life; different species, of both foragers and food sources, are geared differently to rising temperatures [33]. Regarding the decision of the bees to emerge in the morning for the first foraging flight, it has been demonstrated [17] for halictide (*H. rubicundus*) and anthophorid bees (*Anthophora plumipes*) that they depend on a certain nest tunnel temperature just inside the entrance. Similarly, the foragers of other halictine bees (e.g., *H. confusus* and *H. ligatus*) opened their burrows soon after sunrise, when the soil temperature 2.5 cm below the surface was about 18°C [34]. In this paper we investigated how temperature controls activity rates of *H. scabiosae*. We tested whether foraging rates over daytime obeyed one of the following two rules: Rule A defines that the current ambient temperature

conditions trigger the current foraging activities. The other, nonalternative, rule B defines that the foraging activities over daytime are determined by the temperature conditions in the morning. The correlation between ambient temperature and foraging activity in the course of the experiment with different weather conditions resulted in a weak correlation (Table 1: line 11). This clearly demonstrates that foraging activity is apparently too complex to propose a mere dependency of ambient temperature according to rule A. However, the pre-noon aspect of foraging, when considered independently of the foraging activity later on the day, correlated significantly with ambient temperature (Table 1: line 12). Pre-noon foraging activity was strongly correlated with the initial morning temperatures (Figure 6(a)), thus obeying rule B. However, this is restricted particularly to a single time window before noon in which the foraging rate complied with the morning temperature aspect (Figure 6), in the same time period when foraging activity was the greatest. The important point here is that in *H. scabiosae* this match between the aspect of morning temperature and foraging was even stronger than the match between the aspect of morning temperature and the ambient temperature at any time of the day. With other words, the morning temperature aspect was a rather weak predictor of the temperature over the day, because in the model the correlation did not exceed in the model the critical level of $P_m = .05$ after 10:00 h (Figure 6(d)), at least not for nest temperatures above 15°C. This finding let us assume that the bees control their daily activity cycle by the temperature relations in the morning, in particular in the main foraging period in the morning and not later. Interestingly, the model was rather robust to changes of the virtually defined reference, which was the temperature inside the nest, and delivered similar results for the range of nest temperatures between 12°C up to 19°C (Figures 6(a) and 6(b)).

The question arises of why *H. scabiosae* reduces foraging in the early afternoon. We observed that the match between foraging activity and the aspect of morning temperature became less significant just after the peak of foraging before

noon. This means that the bees control their afternoon activities by other principles. One of the proximate aspects of reducing foraging is the fact that food sources may degrade in the course of the day. The pollen offer could diminish over daytime [35] although it hardly changes in its residual attractiveness throughout the day. It is more likely that the foraging bees become conditioned to terminating their foraging bouts by a possible shortage of nectar secretion in the afternoon. The second proximate cause for the pre-noon foraging peak activity could be linked with the fact that the bees have to cope with their nest parasites. Following this argument, this temporal activity pattern would constitute a further line of defence [21] against parasite pressure. This surmise is strongly supported by the fact that the bees not only guard their nest as good as they can throughout the day (Figure 1) but start to close up their entrances just in the period after their midday foraging peak (Figures 4(e) and 7(a)). Therefore, it is plausible to consider the decrease in the foraging rate in the early afternoon (Figures 4(c), 4(d), and 6(a)) as a response not only to degrading nectar sources but also to increasing parasite pressure.

4.3. The Parasite View: When Is the Best Time for Infesting the Host? In this paper the activities of nest parasites are compared with those of their hosts, *H. scabiosae*. Their tendency to infest bee nests was estimated by their impact per nest and 30 minutes observation interval. The activity patterns of the nest parasites showed significant correlations with daytime and with the current ambient temperature (Table 2, Figure 5(b)), which corresponds to rule A that the current ambient temperature conditions trigger the current activities. Additionally, they exhibit two short peaks with a probability of matching of $P_m > .05$ (Figure 6(b)). The first peak occurred before noon, in the same period when the bees were maximally active in foraging, and the second peak was observed in the early afternoon when the bees had already terminated their foraging, and some of the nests of the experimental batches had already closed their entrance holes. Thus, it seems that the nest parasites also correspond with rule B, although much weaker than *H. scabiosae* did.

4.4. Anticyclicity between Host and Parasite Activity. In summary, the diurnal activity patterns of bees and their nest parasites display different strategies. The bees control their foraging activity by aspects of the morning temperature, while they decrease their activities outside of the nest in the early afternoon, independent of the rules A or B. The nest parasites' activities are primarily controlled by daytime and ambient temperature, which confirms to rule A. They additionally displayed two short-time windows in which they acted as if guided by rule B; in the first, shorter, activity window they obviously synchronized themselves with the activities of their hosts; the second window in the noon is seemingly broader. However, the data cannot indicate whether the parasites follow rule B on their own or through pursuing the pre-noon activity patterns of the bees. In any case, the findings confirm that the strategies of nest parasites differ from those of their hosts. The nest parasites strive

to increase the infestation rate over the day. In response to parasite pressure, the bees mainly tend to decrease their activities outside of the nest and to close the nests in the early afternoon. The nest parasites would still have the chance to visit a few open and unguarded nests of *H. scabiosae* in the afternoon, but they also experience that even in one and the same habitat the formation of host chains changes during daytime [36].

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