

ORIGINAL ARTICLE

# Correlates of parasite load in bumblebees in an Alpine habitat

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## Abstract

Essentially, all animals face parasites, but little data are available on the rate of parasitism in wild animals, particularly in insects. Here, we report observations of more than 400 bumblebee workers collected at an Alpine site, including the parasites observed (*Crithidia bombi*, *Nosema bombi*, conopid parasitoid fly larvae and tracheal mites), as well as date of collection, bumblebee species and body variables (size, fat content, egg development and antibacterial activity). Among the 14 bumblebee species collected, *C. bombi* and tracheal mites reached a prevalence of approximately 10 and 6%, respectively, while conopids and *N. bombi* were almost absent. Correlations among the measured parameters suggest that larger workers are more likely to develop eggs and contain more tracheal mites. Across the season, we found a decrease in fat content but an increase in *C. bombi* and mite prevalence. Mites' fitness was higher in fatter bees and lower in bees with more tracheal mites. Antibacterial activity was found in approximately 10% of the workers, suggesting at least sporadic infection with bacteria.

**Key words:** Apidae, *Bombus*, *Crithidia*, egg development, fat content, Hymenoptera.

## INTRODUCTION

Data on the parasitization and immune parameters of insect hosts from field populations are not widely available. Such heuristic data are nevertheless extremely valuable, because observed correlations among parasites, immune measures and other body variables can help to understand the natural patterns of insect–parasite interactions and generate hypotheses that subsequently can be explored experimentally.

In this study, we investigated correlates of parasite load and body parameters in bumblebees in the central Swiss Alps. In this system, many parameters can easily be measured in large samples that serve as indicators of an individual's condition; for example, parasite load, egg development, fat content and antibacterial activity. In a bumblebee colony, the queen monopolizes most of the reproduction and workers never mate, although workers can sometimes develop eggs, which turn into males. Worker egg production is especially favored if the queen dies. Also, workers often start egg produc-

tion in advanced queen-right colonies. Fat content reflects energy stores, but the fat body also has other functions; for example, fat body cells are involved in the synthesis of antibacterial proteins in immune defense (Hetru *et al.* 1998). In uninfected animals that have never encountered a parasite, antibacterial activity is usually at near-zero titers, because antimicrobial peptides are produced *de novo* upon infection (Hetru *et al.* 1998). Thereafter, the activity may last for several weeks (Korner & Schmid-Hempel 2004). Hence, an elevated titer in field-caught individuals indicates not only current infection but also the trace of previous infection.

In central Europe, all bumblebee species are annual (Alford 1975). Queens emerge in spring from their hibernation and search for a nest site. By summer, workers are produced and the colony grows to variable size, depending, among other factors, on species. In summer and autumn, sexuals are produced, which leave the nest and mate. All males, workers and old queens die while the mated gynes go into hibernation. A number of parasites are known to infect bumblebees. In the present study, we specifically searched for conopid (a parasitoid fly; Diptera: Conopidae) eggs and larvae (Schmid-Hempel & Schmid-Hempel 1996), tracheal mites (species not identified), the flagellate gut parasite *Crithidia*

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*bombi* (Trypanosomatidae), and the microsporidian *Nosema bombi*.

Conopids are known from lowland (Schmid-Hempel *et al.* 1990; Durrer & Schmid-Hempel 1995) as well as alpine (Stupf 1992) habitats. Their abundance seems to vary among sites and years, but generally, 10–60% of bumblebee workers may be infected with conopids at any one time and site. Tracheal mites are usually uncommon in the lowland parts of Switzerland studied in our group (areas around Zurich and Basel, samples since 1985; P. Schmid-Hempel, unpubl. data, 2004). Both conopids as well as tracheal mites may exert strong negative effects on the infected bee worker.

In contrast, the gut parasite *C. bombi* may be tolerated under good conditions (Imhoof & Schmid-Hempel 1999), but can be harmful if the host is stressed (Brown *et al.* 2000). Furthermore, infection by *C. bombi* reduces the colony founding success and fitness of infected queens (Brown *et al.* 2003). *Nosema bombi* infection seems to be more severe and may lead to the decay of entire colonies (Schmid-Hempel & Loosli 1998; but see Fisher & Pomeroy 1989). Our previous observations show that *C. bombi* is a widespread parasite while *N. bombi* is less common (areas around Zurich and Basel, samples since 1985; P. Korner & P. Schmid-Hempel, unpubl. data, 2004).

## MATERIALS AND METHODS

In summer of 2001, we collected worker bumblebees at Alp Piora, an area of flower-rich alpine meadows on the southern side of the Swiss Alps (46°32'N, 8°42'E), 2000 m above sea level (a.s.l.). A total of 425 workers was collected randomly on 32 days between 25 June and 9 September, with 90% being collected between 17 July and 26 August. The bees were killed by freezing and then they were kept at –20°C. Later, the bees were identified to species according to Amiet (1996).

We measured the length of the forewing radial cell as a measure of body size (Müller *et al.* 1996; Schmid-Hempel & Schmid-Hempel 1996). The metasoma was dissected, and the hindgut content and a sample of the Malpighian tubules was microscopically inspected for *N. bombi*. The hindgut content was also inspected for *C. bombi*. If tracheal mites were present in the metasoma, we counted the number of adult females of the mite and the number of mite eggs. We also noted the presence of conopid larvae in the metasoma. The ovaries were checked for oocyte development. Also, with a subsample, we measured the metasomal fat content. For this, after dissection, the abdomen was dried at 70°C

for 3 days, then it was weighed, kept in 2 mL ether for 24 h, washed twice with ether and dried for another 7 days at 70°C before weighing again. The difference in weight was taken as the fat content (Ellers 1996).

For a subsample of the bees we measured the antibacterial activity against *Arthrobacter globiformis*. The defrosted mesosoma was homogenized in 200 µL ice-cold buffer (sodium cacodylate: 0.01 mol/L Na-Cac, 0.005 mol/L CaCl<sub>2</sub>). After centrifugation (10 min at 3700 g, 4°C), the supernatant was used for the antibacterial assay. We placed 2 µL of supernatant into a hole (diameter 2 mm) punched into a thin layer of agar (6 mL in Petri dishes, diameter 90 mm; agar recipe: 10 g bacto-tryptone, 5 g yeast extract, 10 g NaCl, 1000 mL distilled water, pH 7.5) containing the test bacterium *A. globiformis* (no. 81.84 T; Institut Pasteur Paris) adjusted to 10<sup>5</sup> bacteria/mL. The agar plates were incubated at 37°C for 24 h. If the bee had antibacterial activity for our test bacterium, a clear zone of inhibition was seen around the hole with the bee homogenate. The diameter of the zone of inhibition was taken as a measure of antibacterial activity.

In our explorative data analysis, we performed backwards model selection based on the likelihood ratio criterion in logistic regression (models with a binary response variable) or the *F*-value in general linear models (models with a continuous response variable). Species, date of collection and body size were always entered as explanatory variables, while one of the four variables, egg development, fat content, *C. bombi* prevalence (see below) and tracheal mite prevalence (see below), was, in turn, used as the response variable, while the other three were entered as explanatory variables. The antibacterial activity was not included in these models because only a subsample of the dissected bees was measured for antibacterial activity. This parameter was analyzed in a separate step, with the parameters mentioned above as explanatory variables. All variables used are defined in Table 1. The analyses were done using SPSS Version 11 for Macintosh (SPSS, Chicago, IL, USA).

## RESULTS

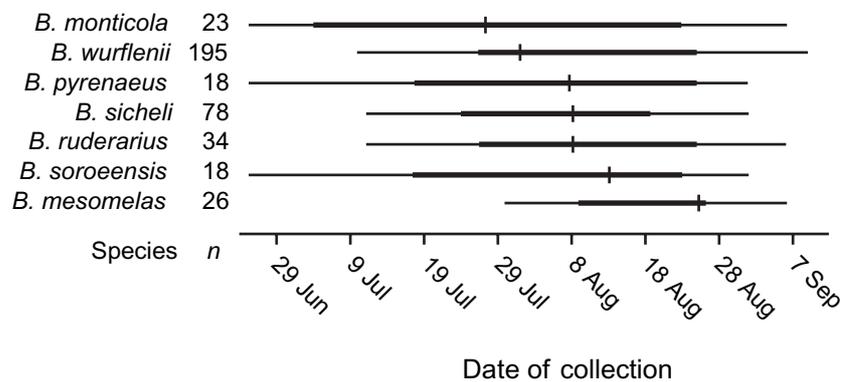
### Bumblebee species

The 425 collected workers belonged to 14 bumblebee species (Table 2), all of which have been known from the region (Amiet 1996). No attempts were made to separate the three morphologically similar species *Bombus lucorum*, *B. terrestris* and *B. cryptarum*. For all

**Table 1** Variables examined in the present study

Variable	Description (unit)	Type of variable
Species	The bumblebee species	Random factor
Date	Date of collection	Covariable
Size	Length of radial wing cell (mm)	Covariable, ln-transformed
Egg prevalence	Proportion of workers that had developing eggs	Binary
Fat content	Total abdominal fat content ( $\mu\text{g}$ )	Covariable
<i>Crithidia</i> prevalence	Proportion of workers infected with <i>Crithidia bombi</i>	Binary
<i>Crithidia</i> intensity	Average number of <i>C. bombi</i> cells per bee, including bees without <i>C. bombi</i> infection	Covariable
Tracheal mite prevalence	Proportion of workers containing at least one adult tracheal mite in the abdomen	Binary
Tracheal mite intensity	Average number of adult tracheal mites in the abdomen, including bees without tracheal mites	Covariable
Antibacterial activity prevalence	Proportion of workers that showed any antibacterial activity in our assay	Binary
Antibacterial activity	Mean diameter (mm) of clear zone of inhibition of bacterial growth, including bees without visible antibacterial activity	Covariable

**Figure 1** Phenology of the *Bombus* (bumblebee) species caught in summer 2001 on Alp Piora, Switzerland (2000 m above sea level). The thin line depicts the range, the thick line includes 90% of the individuals, the vertical bar indicates the median date of collection and  $n$  is the number of workers caught. The order of the species equals the order of the medians. Only species with  $n > 10$  are depicted.



statistical analyses, we used the seven common species for which more than 10 specimens were sampled (given in bold in Table 2). These included 395 dissected bees. For 307 workers, we also measured the fat content.

Date of collection (Fig. 1) differed significantly among the common species (Kruskal–Wallis test:  $\chi^2 = 30.7$ , d.f. = 6,  $P < 0.001$ ). The effect was mainly due to *B. mesomelas*. When this species was excluded, date of collection did not differ significantly among the remaining six common species (Kruskal–Wallis test:  $\chi^2 = 4.4$ , d.f. = 5,  $P = 0.49$ ). Table 2 summarizes measurements on size, egg development, fat content, infection by *C. bombi*, tracheal mite numbers, and the observed antibacterial activity.

Body size differed significantly among species (ANOVA:  $F_{6,369} = 26.3$ ,  $P < 0.001$ ), an effect mainly

attributable to *B. wurflenii*, which is larger than all other common species in this study.

### Egg development and fat content

Table 3A and B presents the results of the model selection with egg development and fat content as the response variables, respectively. Our data suggest that egg development correlates with the presence of *C. bombi* and with body size. We found that workers infected with *C. bombi* had a significantly higher probability of having developing eggs (Table 4;  $\chi^2$ -test:  $P = 0.04$ ). Larger workers were more likely to have developing eggs, as suggested by the model selection (Table 3A; Fig. 2).

Species, date of collection and size remained as highly significant variables when fat was chosen as the

Table 2 Overview of the measures taken from 416 bumblebee (genus *Bombus*) workers

Species	Size			Egg prevalence	Fat content (µg) (mean ± SD)	<i>Critidia</i>		Tracheal mites		Antibacterial activity	
	(mean ± SD)	(n)	(n)			Prevalence	Intensity (×10 <sup>4</sup> ) (mean ± SD)	Prevalence	Intensity (mean ± SD)	Prevalence	Mean ± SD
<i>B. hortorum</i> (Linnaeus)	2.92 ± 0.11 (3)	0 (2)	138 ± 35 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0.67 (3)	0.7 ± 0.8 (3)
<i>B. jonellus</i> (Kirby)	2.22 ± 0.08 (2)	0 (2)	121 (1)	0 (2)	0 (2)	0 (2)	0.5 (2)	2.5 ± 3.5 (2)	0 (2)	0 (2)	0 (2)
<i>B. lapidarius</i> (Linnaeus)	2.66 ± 0.31 (7)	0 (7)	137 ± 63 (6)	0 (7)	69.9 ± 184 (7)	0 (7)	0 (7)	0 (7)	0 (7)	0.14 (7)	1.4 ± 3.8 (7)
<i>B. lucorum</i> <sup>†</sup>	3.00 ± 0.14 (5)	0 (5)	142 ± 17 (4)	0 (5)	2.8 ± 6.3 (5)	0.20 (5)	0 (5)	0 (5)	0 (5)	0.40 (5)	0.5 ± 0.7 (5)
<i>B. mendax</i> Gerstäcker	2.56 ± 0.15 (4)	0 (4)	125 ± 99 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0.25 (4)	0.6 ± 1.3 (4)
<i>B. mesomelas</i> Gerstäcker	2.44 ± 0.21 (25)	0 (26)	111 ± 64 (21)	0 (26)	8.0 ± 17.4 (26)	0.23 (26)	0 (26)	0 (26)	0 (26)	0.12 (25)	0.3 ± 0.9 (25)
<i>B. monticola</i> Smith	2.42 ± 0.28 (23)	0.09 (23)	101 ± 47 (21)	0.09 (23)	0.09 ± 0.4 (23)	0.04 (23)	0.09 (23)	0.7 ± 2.4 (23)	0.09 (23)	0.35 (23)	1.0 ± 1.7 (23)
<i>B. mucidus</i> Gerstäcker	2.20 (1)	0 (1)	85 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	1 (1)	3.0 (1)
<i>B. pratorum</i> Linnaeus	2.76 (1)	0 (2)	32 (1)	0 (2)	27.0 ± 38.2 (2)	0.5 (2)	0 (2)	0 (2)	0 (2)	0 (1)	0 (1)
<i>B. pyrenaeus</i> Pérez	2.43 ± 0.26 (15)	0 (18)	110 ± 85 (14)	0 (18)	5.6 ± 22.1 (18)	0.11 (18)	0.33 (18)	1.7 ± 2.9 (18)	0.33 (18)	0.13 (15)	0.5 ± 1.8 (15)
<i>B. ruderarius</i> (Müller)	2.43 ± 0.22 (36)	0.17 (36)	112 ± 74 (28)	0.17 (36)	13.4 ± 55.9 (36)	0.14 (36)	0 (36)	0 (36)	0 (36)	0.31 (36)	1.8 ± 3.6 (36)
<i>B. sichelii</i> Radoszkowski	2.46 ± 0.17 (76)	0.05 (79)	120 ± 53 (67)	0.05 (79)	0.28 ± 1.9 (79)	0.03 (79)	0.06 (79)	0.1 ± 0.8 (79)	0.06 (79)	0.03 (76)	0.1 ± 0.7 (76)
<i>B. soroensis</i> (Fabricius)	2.54 ± 0.23 (17)	0 (19)	119 ± 51 (16)	0 (19)	0 (19)	0 (19)	0.11 (19)	0.6 ± 1.8 (19)	0.11 (19)	0.24 (17)	0.4 ± 0.7 (17)
<i>B. wurflenii</i> Radoszkowski	2.74 ± 0.24 (184)	0.03 (194)	123 ± 57 (148)	0.03 (194)	9.8 ± 38.0 (194)	0.11 (194)	0.05 (194)	0.4 ± 1.7 (194)	0.05 (194)	0.09 (184)	0.4 ± 1.4 (184)

Variables as defined in Table 1. Sample size is given in parentheses. Species with more than 10 sampled workers are given in bold.

<sup>†</sup>Worker of the species complex *B. cryptarum* (Fabricius), *B. lucorum* (Linnaeus), *B. terrestris* (Linnaeus).

**Table 3** Results of the explorative model selection analyses

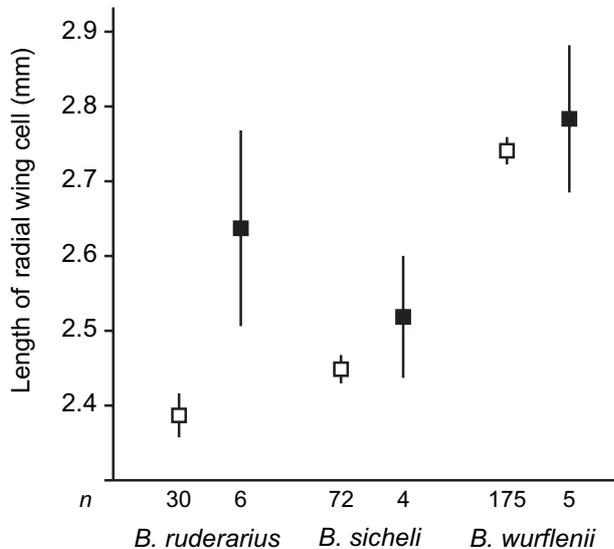
<b>(A) Egg development (response variable)</b>				
<i>Full model:</i> Egg prevalence as a function of species + date + size + fat content + <i>Crithidia</i> prevalence + tracheal mite prevalence				
Step	Variable removed	Wald statistic	d.f.	P-value
1	Tracheal mite prevalence	0.018	1	0.895
2	Fat content	0.304	1	0.581
3	Date	1.994	1	0.163
<i>Reduced model</i>				
	Variable retained	Wald statistic	d.f.	P-value
	Species	10.872	6	0.092
	<i>Crithidia</i> prevalence	5.356	1	0.021
	Size	4.535	1	0.033
<b>(B) Fat content (response variable)</b>				
<i>Full model:</i> Fat content as a function of species + date + size + egg prevalence + <i>Crithidia</i> prevalence + tracheal mite prevalence				
Step	Variable removed	F statistic	d.f.	P-value
1	<i>Crithidia</i> prevalence	0.011	1, 295	0.915
2	Tracheal mite prevalence	0.024	1, 296	0.878
3	Egg prevalence	0.537	1, 297	0.464
<i>Reduced model</i>				
	Variable retained	F statistic	d.f.	P-value
	Species	4.464	6, 298	<0.001
	Date	15.638	1, 298	<0.001
	Size	117.370	1, 298	<0.001
<b>(C) <i>Crithidia</i> prevalence (response variable)</b>				
<i>Full model:</i> <i>Crithidia</i> prevalence as a function of species + date + size + egg prevalence + fat content + tracheal mite prevalence				
Step	Variable removed	Wald statistic	d.f.	P-value
1	Size	0.068	1	0.794
2	Tracheal mite prevalence	0.145	1	0.703
3	Fat content	0.480	1	0.489
4	Species	6.241	6	0.397
<i>Reduced model</i>				
	Variable retained	Wald statistic	d.f.	P-value
	Date	8.149	1	0.004
	Egg prevalence	5.882	1	0.015
<b>(D) Tracheal mite prevalence</b>				
<i>Full model:</i> Tracheal mite prevalence as a function of species + date + size + egg prevalence + fat content + <i>Crithidia</i> prevalence				
Step	Variable removed	Wald statistic	d.f.	P-value
1	Fat content	0.163	1	0.686
2	Egg prevalence	0.459	1	0.498
3	<i>Crithidia</i> prevalence	0.659	1	0.417
4	Size	1.206	1	0.272
<i>Reduced model</i>				
	Variable retained	Wald statistic	d.f.	P-value
	Species	11.663	6	0.070
	Date	2.673	1	0.102

Only the common species (more than 10 individuals collected) are included. Variables as in Table 1. Size was ln-transformed. Total  $n = 307$ .

**Table 4** Cross-tabulation of egg development and the presence of *Crithidia bombi* in 395 wild caught bumblebee workers belonging to six species

		Egg prevalence		Total
		No	Yes	
Crithidia prevalence	No	345 (96.4%)	13 (3.6%)	358 (100%)
	Yes	33 (89.2%)	4 (10.8%)	
Total		378	17	395

For variable declaration see Table 1.



**Figure 2** Correlation between size (measured as the length of the wing radial cell; mean  $\pm$  SE) and the probability of developing eggs in workers of three bumblebee species: *Bombus ruderarius*, *B. sichelii* and *B. wurflenii*.  $\square$ , Workers without developing eggs;  $\blacksquare$ , workers with developing eggs. Only species with  $n > 3$  are depicted.

response variable (Table 3B). The  $\beta$  parameters, which indicate the quantitative relationship, were  $-0.918 \pm 0.232 \mu\text{g fat/day}$  (parameter estimate  $\pm$  SE) for date of collection and  $139.4 \pm 12.9 \mu\text{g fat/mm}$  for body size (represented by forewing radial cell length). Total fat content declined with season (Fig. 3) and was larger in larger workers.

### Parasites

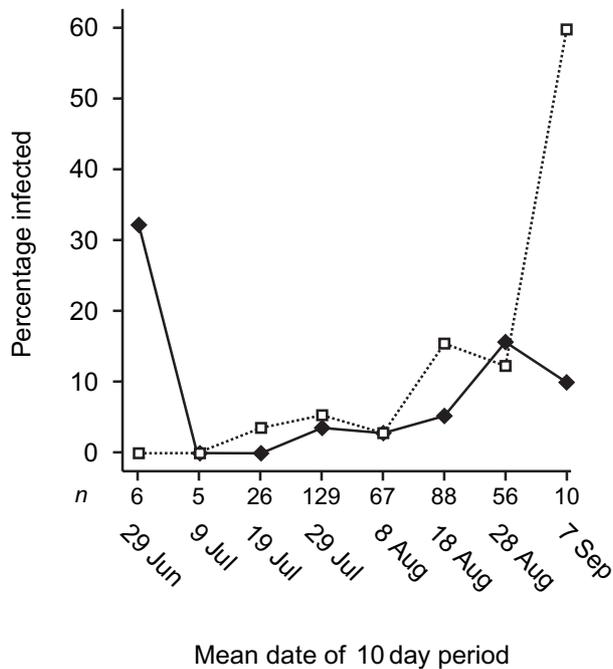
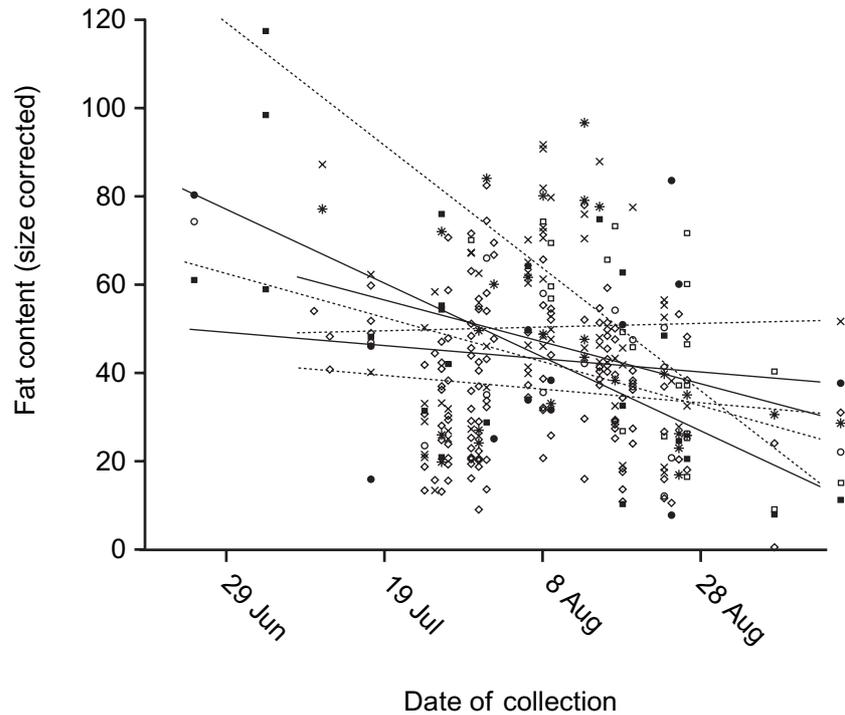
*Nosema bombi* was never encountered in 420 dissected workers. During the present study, we found one conopid larva, in a *B. lapidarius* worker collected on 31 July. Across all species, only 9.6% of workers were infected with *C. bombi*, and 6% were found to have tracheal mites in their abdomen. Due to the low fre-

quency, we only included presence versus absence (i.e. prevalence) of *C. bombi* and tracheal mites in the model selections. Table 3C presents the results of the model selection with *C. bombi* prevalence as the response variable. Date of collection and egg prevalence (presence or absence of developed eggs) remained in the model. The latter has already been explored above. We found that workers caught later in the season had a greater chance of being infected with *C. bombi* (Fig. 4).

Although the sample size was small, we can still look at the seasonal development of *C. bombi* intensity; that is, the number of cells per bee, among the infected hosts. We included only the three species for which more than four infected individuals were available – *B. mesomelas*, *B. ruderarius* and *B. wurflenii* – and found no obvious trend in *C. bombi* intensity (Fig. 5).

Finally, the model selection with tracheal mite prevalence as response variable suggested a correlation with two variables, species and date of collection, albeit their *P*-values were greater than 0.05 (0.067 and 0.107, respectively; Table 3D). For the model selection, a number of workers had to be excluded due to the lack of fat measurement. As the final model did not include this variable, we re-calculated the final model including all available workers (387 instead of 307). In this case, both species ( $P = 0.018$ ) and date of collection ( $P = 0.024$ ) became significant, and the  $\beta$  parameter for date of collection was  $0.036 \pm 0.016$  (parameter estimate  $\pm$  SE). This suggests an increase in tracheal mite prevalence with season, which is also visible in Figure 4 (note that the high prevalence in the first period is based on six workers only). We explored the effect of date of collection, size and species on the number of tracheal mites; that is, mite intensity, including only bee species with more than four individuals infected with mites (*C. pyrenaicus*,  $n = 5$ ; *C. sichelii*,  $n = 5$ ; *C. wurflenii*,  $n = 8$ ). We found no significant effect of date of collection ( $F_{1,13} = 0.439$ ,  $P = 0.519$ ) or species ( $F_{2,13} = 2.375$ ,  $P = 0.132$ ), but size showed a positive correlation ( $F_{1,13} = 7.24$ ,  $P = 0.019$ ,  $\beta = 5.728 \pm 2.129$  SE mites/

**Figure 3** Correlation between fat content (size corrected: absolute fat content/size<sup>3</sup> × 10<sup>5</sup>) and date of collection. For each species, regression lines are drawn. The symbols to the right of the regression lines identify the species: from top to bottom *Bombus sichelii* (×), *B. pyrenaicus* (●), *B. wurflenii* (◇), *B. ruderarius* (\*), *B. soroeensis* (○), *B. mesomelas* (□) and *B. monticola* (■). Regression lines of species represented with open symbols and × are dotted for better readability.

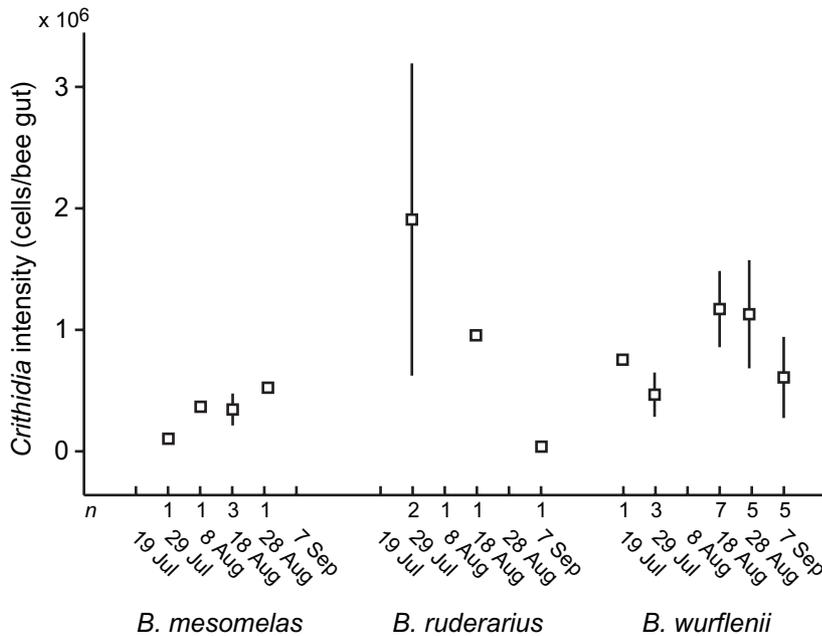


**Figure 4** Percentage of workers infected with *Crithidia bombi* (□) and tracheal mites (◆) for each 10 day catching period. The total number of workers caught per period (*n*) is given below the x-axis as well as the mean date for each period.

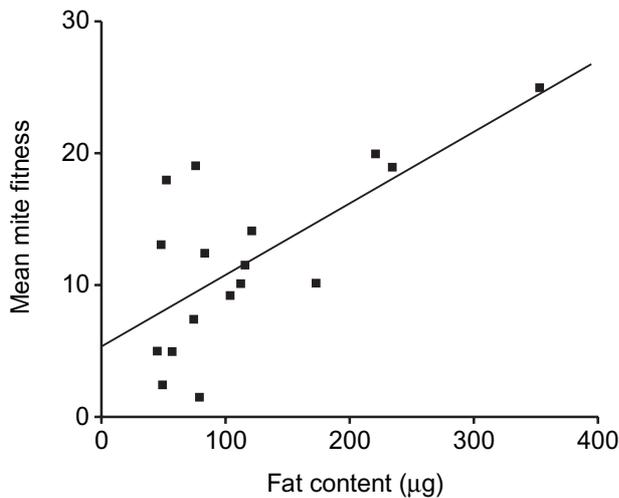
mm). For 17 workers infected with tracheal mites, we also measured the fat content. Using these workers, we looked for effects on “mite fitness”, defined as the number of mite eggs per adult mite. We started with a model including species, date, size, number of adult tracheal mites and fat content as the explanatory variables. Size was dropped first ( $P = 0.97$ ), date second ( $P = 0.54$ ) and species third ( $P = 0.39$ ). The reduced model then included number of adult tracheal mites ( $F_{1,14} = 4.01$ ,  $P = 0.062$ ) with  $\beta = -0.807 \pm 0.399$  SE (mite fitness/number of adult mites) and fat content ( $F_{1,14} = 19.68$ ,  $P = 0.001$ ) with  $\beta = 0.071 \pm 0.016$  SE (mite fitness/ $\mu\text{g}$  fat); that is, mites in bees with more mites were less fit, and fatter bees had mites with higher fitness (Fig. 6).

#### Antibacterial activity

Including all 226 measured workers, 10.6% showed antibacterial activity in our assay. One hundred and fifty workers were available for a logistic regression model selection analysis equivalent to the ones done above, now with the response variable “antibacterial activity prevalence”; that is, presence versus absence of antibacterial activity. The only variable that remained in the model, species, was not significant, however ( $P = 0.345$ ). Therefore, antibacterial activity was present – most likely indicating past infection – but



**Figure 5** *Crithidia bombi* intensity (i.e. number of cells per bee; mean  $\pm$  SE) across the season for three *Bombus* species. Only workers that were infected with *C. bombi* are included here. Below the x-axis, samples sizes ( $n$ ) and the mean date for each period are depicted.



**Figure 6** Correlation between the fat content of the host bumblebee and the mean fitness of the tracheal mites (number of eggs per adult mite) within the bee, including the regression line. Only bees infected with mites are included.

randomly distributed with respect to our putative explanatory variables.

## DISCUSSION

Model selection is a powerful tool for generating new hypotheses or for verifying known correlations in a set

of heuristic data. Our analysis suggests a number of interactions between different variables, which may be explored further in future studies. In addition, our measurements of parasite and body parameters help to understand the conditions that the bumblebees face in the field.

The phenology (Fig. 1) shows large overlaps among most species, suggesting little temporal specialization, which may be due to the constraint of a short season at this altitude. The exception was *B. mesomelas*, which appeared very late. The proportion of workers with developed eggs was low in all species (0–17%; Table 2). We found a positive correlation between infection with *C. bombi* and the probability of developing eggs. This may be an effect of age; that is, older workers are more likely to develop eggs and are also more likely to have picked up *C. bombi* during their life compared to young bees. Larger bees were more likely to have developed eggs (Fig. 2). Larger workers are indeed known to be more dominant and/or have more resources to develop eggs (Blom 1986; Duchateau & Velthuis 1988). Furthermore, older colonies may produce larger workers and, at the same time, the queen suppression of worker egg production may decline, leading to the observed correlation.

Total fat content increased with worker size and therefore also varied among species. More interestingly, we found a negative correlation with season (Fig. 3). Possibly, workers use up their reserves when the colony

starts to produce sexuals. All bumblebee workers perish after completion of the colony cycle and therefore reserve that has not been used up by the production of sexuals is wasted.

*Crithidia bombi* prevalence was comparable to another study in the Alps (Stupf 1992), but the value was lower than that in lowland areas where prevalence of up to 50–80% has been observed (Shykoff & Schmid-Hempel 1991; Imhoof & Schmid-Hempel 1999). The increase in prevalence across season (Fig. 4) corroborates previous observations in the lowlands (Imhoof & Schmid-Hempel 1999) as well as in the Alps (Stupf 1992). Data from various species from low altitudes suggest that a relatively small fraction of spring queens is infected with *C. bombi* (samples since 1985; P. Schmid-Hempel, unpubl. data, 2004). From these, the *C. bombi* population seems to build up, gradually infecting more workers within the nest as well as via shared use of flowers (Durrer & Schmid-Hempel 1994). The intensity (i.e. number of *C. bombi* cells per bee) did not increase with season (Fig. 5). Therefore, we have no indication that the *C. bombi* strains became more virulent across season as could be expected if among-strain competition would select the most virulent strains (Van Baalen & Sabelis 1995). However, our sample size was small and the question needs further analysis.

While *C. bombi* is found regularly in bumblebees, conopids seem to be more variable in time and/or space. Stupf (1992) found 13 infected among 242 bumblebees (mostly workers) in the Alps (2000 m a.s.l.), while in the lowlands, conopids can be found more or less regularly, with a prevalence of approximately 10–60%, depending on season and species (Schmid-Hempel *et al.* 1990; Schmid-Hempel & Durrer 1991; Müller & Schmid-Hempel 1992). In our study, we only found one conopid larva in more than 400 dissected bees. Therefore, conopids may be less common at high altitudes, or show strong cyclic changes in population size.

Tracheal mites were found in 0–33% of the workers of the common species (Table 2). This is similar to a study in the lowlands (Shykoff & Schmid-Hempel 1991), although other studies in the lowlands often found very low numbers of tracheal mites (samples since 1985; P. Schmid-Hempel, unpubl. data, 2004). Our data provide some evidence that later in the season, bees are more likely to have tracheal mites (Table 3D; Fig. 4). Among the infected bees, larger individuals had more mites, although it remains unclear whether they stem from a single infection or from multiple infections. From the point of view of the mite, our data suggest that mites that have to share their individual host with fewer other

mites can produce more offspring, an observation well in line with general life history theory. Also, mites found in bees with more fat had more offspring.

Shykoff and Schmid-Hempel (1991) regularly found *N. bombi* (prevalence approximately 10%) in a study at low altitudes, while in this study as well as in Stupf (1992), *N. bombi* was almost absent in Alpine bees.

To summarize the observations on the parasites, our data suggest that conopid parasitoids and tracheal mites are not common in our study area, while *C. bombi* seems to be common at low as well as at higher altitudes, with increasing prevalence toward the end of the colony season, and *N. bombi* appears to be absent at high altitudes and scarce to common at low altitudes.

Of the 226 tested workers, 10.6% had antibacterial activity against our test bacterium *A. globiformis*. Under laboratory conditions, untreated *B. terrestris* workers almost always show zero antibacterial activity. The difference between laboratory and field conditions may be, for example, due to different chances of infection. The model selection exploring correlations between antibacterial activity and other parameters measured in this study failed to find significant correlations. However, in *Drosophila melanogaster*, Boulanger *et al.* (2001) showed that in this system, a parasite of the genus *Crithidia* triggered the production of antibacterial proteins. Similar work is under way in our laboratory.

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