# Suppression of ovarian development of Bombus terrestris workers by B. terrestris queens, Psithyrus vestalis and Psithyrus bohemicus females

Carlos Vergara, Stefan Schröder, Maria Almanza, Dieter Wittmann

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**Original article**

# Suppression of ovarian development of *Bombus terrestris* workers by *B. terrestris* queens, *Psithyrus vestalis* and *Psithyrus bohemicus* females

Carlos H. VERGARA[a*], Stefan SCHRÖDER[b], Maria Teresa ALMANZA[c], Dieter WITTMANN[b]

[a] Departamento de Química y Biología, Universidad de las Américas-Puebla, Santa Catarina Mártir, Puebla, 72820, Mexico
[b] Institut für Landwirtshaftliche Zoologie und Bienenkunde, Rheinische Friedrich-Wilhelms-Universität Bonn, Melbweg 42, 53127 Bonn, Germany
[c] Facultad de Ciencias Biología Aplicada, Universidad Militar Nueva Granada, cra 11 N° 101-80, Bogotá, Colombia

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**Abstract** – Ovarian development was studied in workers of *Bombus terrestris* under four different treatments, which produced different levels of ovarian development. *Psythirus* females of both specific (*P. vestalis*) and non-specific (*P. bohemicus*, which is the specific parasite of *B. lucorum*) species were able to inhibit worker ovarian development and to reproduce in *B. terrestris* colonies. The effect of *P. vestalis* females on ovarian development of workers was statistically not different from the effect of *B. terrestris* queens (positive control) and significantly different from ovarian development inhibition measured in groups of workers alone (negative control). Ovarian development inhibition by *P. bohemicus* was not statistically different both from the positive and the negative control, indicating that this non-specific parasite is able to affect the ovarian development of workers, but not to the same extent as the queens of the same species or the females of the specific parasite.

***Bombus terrestris* / *Psithyrus* spp. / cuckoo bumblebee / ovarian development / social parasitism**

## 1. INTRODUCTION

Suppression of worker ovarian development in the presence of a dominant fecund female is characteristic of social insects (Wilson, 1971; Michener, 1974). In primitive social insects overt aggressive dominance interactions are the main mechanisms involved in ovarian suppression (Fletcher and Ross, 1985). In advanced eusocial insects ovarian development inhibition is accomplished by a combination of behavioral and chemical mechanisms. Colony development in *Bombus terrestris* (L.) is characterized by two social phases. During the initial phase the queen is the only reproductive female, and ovarian development in young workers and oviposition in older ones with mature eggs are inhibited by behavioral and pheromonal mechanisms (Honk et al., 1980). The second, or "competition" phase is characterized by reproduction of workers and by antagonistic interactions among workers and between workers and the queen. During this phase workers are the major factor inhibiting the reproductive development of their nestmates (Doorn and Heringa, 1986; Ratnieks and Visscher, 1989; Bloch and Hefetz, 1999; Bloch and Hefetz,

* Corresponding author: cvergara@mail.udlap.mx

1999a; Bloch et al., 2000). When groups of newly emerged workers are maintained in a queenless state, one or two of them start to develop their ovaries after the third day and ovarian development is significantly higher by day 6 (Bloch et al., 1996).

Cuckoo bumblebees, *Psithyrus*, are obligate social parasites in the nests of many *Bombus* species. *Psythirus vestalis* (Geoffroy) exclusively parasitizes colonies of *B. terrestris* and *Psythirus bohemicus* (Seidl) is the parasite of *Bombus lucorum* (L.) colonies. All the *Psythirus* species lack pollen collecting structures and rely on the host workers for the rearing of their own offspring. Ovarian suppression of host workers by cuckoo bumblebees has only been studied in two American species. *Psithyrus ashtoni* Cresson females invade colonies of *Bombus affinis* Cresson and *Bombus terricola* (Kirby), coexist with the foundress queen and seem unable to control the ovarian development of workers (Fisher, 1983). Females of *Psithyrus citrinus* Sm. invade *Bombus impatiens* Cresson and *Bombus vagans* Cresson colonies, displace or kill the queen, and may be able to suppress worker ovarian development (Fisher, 1984).

The present study examines the effectiveness of *B. terrestris* queens and *P. vestalis* and *P. bohemicus* females in influencing ovarian development in *B. terrestris* workers and compares such ability between specific (*P. vestalis* on *B. terrestris* workers) and non-specific (*P. bohemicus* on *B. terrestris* workers) social parasites. To obtain the information presented here, groups of six workers were subjected to different treatments, which produced different levels of ovarian development. The cuckoo bumblebees were able to suppress ovarian development in the workers studied, and were able to reproduce in the experimental colonies used.

## 2. MATERIALS AND METHODS

### 2.1. Hosts and parasites

Colonies of *B. terrestris* were reared in the laboratory (Gretenkord, 1996) from queens captured in the field between February and April 2001, while they were searching for nesting places. Females of *Psithyrus vestalis* and *Psithyrus bohemicus* were also captured in the field during the same period. For each experiment, newly built wooden boxes were used to rear the colonies and to keep the *Psithyrus* females or the queenless groups of workers. At the end of each experiment the box was discarded.

Prior to the experiments, the *Psythirus* females were kept in boxes with brood from a *B. terrestris* colony chosen at random and groups of newly emerged *B. terrestris* workers, until they clearly showed dominant behavior (i.e. overt aggression towards the introduced *B. terrestris* workers, positioning themselves on top of the workers and holding them with the legs for a variable period of time) and the possibility of disruption of reproduction by nematodes or other parasites could be ruled out. For the same reasons, only *Bombus* queens that had successfully started a colony were used for the experiments.

The taxonomic identity of all *Bombus* queens and *Psithyrus* females was established on the living individuals prior to the experiments and was confirmed after the queens or the parasites had died naturally. Also, male offspring from all females were obtained and used to confirm the identity of the queens and parasites used for the experiments.

### 2.2. Experimental design

Groups of six newly emerged or "callow" (less than 12 hours old) *B. terrestris* workers were obtained at random from different colonies. The workers were individually marked with numbered plastic tags (Opalith® Plättchen) and introduced in wooden boxes where they were fed API-Invert® (72.7% inverted sugar solution) ad libitum and a mixture of pollen and API-Invert® as solid food every 48–72 hours.

Each group of workers was subjected to one of the four different treatments described below. Each treatment was replicated six times, except for treatment 2, of which five replications were performed.

Treatment 1. *B. terrestris* queen (positive control)

Four queens were used (two once, two twice). Prior to the experiments all adult workers (8–10 workers) were removed and the six newly emerged workers were put into the box, with the queen and her brood. This treatment is considered as a positive control, since maximal ovarian activity suppression is occurring under these conditions (Duchateau and Velthuis, 1989).

Treatment 2. *P. vestalis* female

Four different females were used (three once, one twice). The six workers were put with the

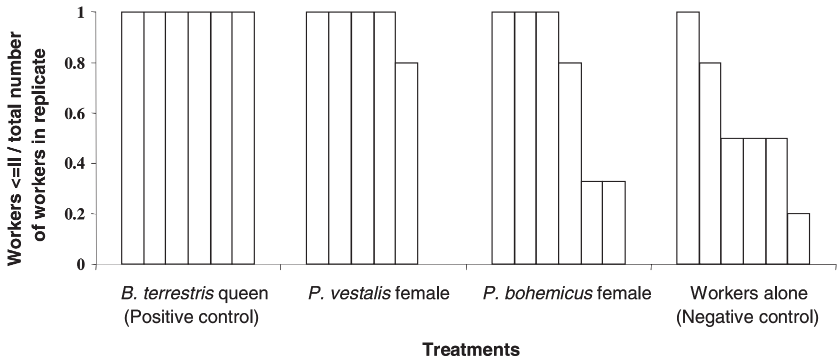


**Figure 1.** Proportion of workers with ovarian development measurement <=II (stages 0-II) for all replicates in each treatment.

*P. vestalis* female and brood chosen at random from *B. terrestris* colonies.

Treatment 3. *P. bohemicus* female

Four females were used (two once, two twice). The six newly emerged workers were put with the *P. bohemicus* female and brood chosen at random from *B. terrestris* colonies.

Treatment 4. Group of workers alone

Each group of six newly emerged workers was put in a box with brood of different ages, chosen at random from one *B. terrestris* colony. This treatment is considered as a negative control, because workers kept queenless can have mature eggs in their ovaries 5 days after emergence, and ovarian development suppression may not occur on all the workers in the queenless group.

The experiments were terminated when the workers were six days old. Workers that died before the end of the experiment (two for Treatment 1, in two different replicates, one for Treatment 2, and one for treatment 3) were not used for data analysis. The workers were sacrificed by freezing them at –18 °C and their ovaries were dissected afterwards in a bumblebee saline solution (Huang et al., 1991; Bloch et al., 1996). The length of the terminal oocyte in each ovariole was measured with an ocular ruler under a dissecting microscope (X6.3-X40).

The length of the largest of the eight terminal oocytes was used as the ovarian development measurement for each individual.

## 2.3. Data analysis

Ovarian development measurements were grouped into the following six categories, based on the classification of Duchateau and Velthuis (1989) (given is the mean length of the terminal oocyte and the range for each category): Stage 0 – 0 mm; stage I – 0.12 mm (0.06–0.18 mm); stage II – 0.56 mm (0.21–1.18 mm); stage III – 1.71 mm (1.27–2.22 mm); stage III$^{+}$ – 2.31 mm (2.10–2.66 mm); stage IV – 2.91 mm (2.43–3.46 mm).

For every replication within each treatment, data were grouped according to the stage of ovarian development, separating individuals with ovaries in stages 0, I, and II (group "<= II") from individuals with ovaries in stages III, III+, and IV (group "> II"), and the proportion "workers in group <=II / total number of workers in replication" was calculated. Group "<=II" was considered to be the condition occurring in the positive control group. Treatments 2 (*P. vestalis* females) and 3 (*P. bohemicus* females) were compared against the positive and the negative controls, by using Mann-Whitney tests.

## 2.4. Reproduction of *Psithyrus* females

Production of male and female reproductives was recorded for all *P. vestalis* and for one of the *P. bohemicus* colonies used in the experiments.

# 3. RESULTS

## 3.1. Ovarian development of workers under different treatments

The proportions of workers with ovarian development measurements <=II under the different treatments are presented in Figure 1.

### 3.2. Comparisons between treatments with *Psithyrus* females and controls

There was no statistically significant difference between the proportions of workers in group <=II in the positive control [*B. terrestris* queens] and in treatment 2 [*P. vestalis* females] ($P = 0.64$; two tailed Mann-Whitney U test). A statistically significant difference was found between the proportions of workers in group <=II in the negative control (workers alone) and in treatment 2 (*P. vestalis* females) ($P = 0.04$; two tailed Mann-Whitney U test).

No statistically significant difference was found between the proportions of workers in group <=II in the positive control [*B. terrestris* queens] and in treatment 3 [*P. bohemicus* females] ($P = 0.17$; two tailed Mann-Whitney U test). Also, there was no statistically significant difference between the proportions of workers in group <=II in the negative control (workers alone) and in treatment 3 (*P. bohemicus* females) ($P = 0.52$; two tailed Mann-Whitney U test).

### 3.3. Reproduction of *Psithyrus* females

All four *P. vestalis* females, as well as the only *P. bohemicus* observed for this trait, produced offspring. The sex ratio observed for *P. vestalis* females was strongly male biased, while the number of males was equal to the number of females produced by the only *P. bohemicus* female for which this trait was recorded (Tab. I).

**Table I.** Offspring produced by *Psithyrus* females.

| Colony | Males | Females |
|---|---|---|
| *P. vestalis* | | |
| 1 | 11 | 0 |
| 2 | 16 | 2 |
| 3 | 0 | 2 |
| 4 | 42 | 1 |
| *P. bohemicus* | | |
| 1 | 13 | 13 |

## 4. DISCUSSION

The information presented here demonstrates that *Psythirus* females are able to effectively inhibit ovarian development in *B. terrestris* workers. In the case of the specific parasite, *P. vestalis*, ovarian suppression is not statistically different from the suppression caused by a *B. terrestris* queen, under the same experimental conditions.

The non-specific parasite, *P. bohemicus*, seems to have an effect on ovarian development of *B. terrestris* workers, but clear cut differences between this treatment and the negative control may be difficult to detect under the experimental design used. Due to the fact that one or a few workers in a group will develop ovaries, while the ovaries of the other workers in the group remain inactive, it is not possible to obtain negative control groups where all the workers show a uniform stage of ovarian development, which would make it possible to detect fine differences with the treatments.

Although the distribution of the proportion of "<=II" in the *P. bohemicus* treatment is not statistically different from either the positive or the negative control groups, all the workers in two of the six replications of this treatment had ovaries in stage II (Fig. 1). These two replications were performed with different *P. bohemicus* females, and individual differences between females may account for the high variability of ovarian development inhibition found for this treatment. In agreement with the results obtained by Fisher (1983), who worked with *P. ashtoni* and its specific host, *B. affinis*, the females of *P. bohemicus* in our study were able to suppress the development of ovaries in *B. terrestris* workers, but there were a number of workers that escaped the suppression.

Mechanisms other than pheromonal specificity of primer pheromones acting on the suppression of ovarian development of workers also contribute to determine host-parasite specificity. One of these mechanisms is the recognition by the parasite of chemical cues given off by the host's nest (Fisher, 1983a) or by the host's body (Fisher et al., 1993).

Other factors, such as sympatry of host and parasite species, synchronism in the emergency of overwintering adults, and appropriate behavior could also be important in determining the development of social parasitism.

Male-biased investment ratios have been observed in *B. terrestris*, although female biased investment ratios also occur (Bourke, 1997; Beekman and Stratum, 1998). Colonies that show male-biased investment ratios, which are also protandrous and female-biased colonies, which are also protogynous, can coexist depending on the frequency of both colony types. This subject has not been studied in cuckoo bumble bees and our scarce data preclude making generalizations about the phenomenon in *Psythirus*.

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**Résumé – Inhibition du développement ovarien des ouvrières de *Bombus terrestris* par les reines de *B. terrestris* et les femelles de *Psithyrus vestalis* et *P. bohemicus*.** L'inhibition du développement ovarien chez les ouvrières est caractéristique des insectes sociaux. Chez les insectes eusociaux elle est due à des mécanismes comportementaux et chimiques (phéromones). Les ouvrières dominantes empêchent aussi le développement ovarien des autres ouvrières. Les parasites sociaux obligés présents dans les nids de nombreuses espèces de *Bombus*, comme les femelles de psithyres (*Psithyrus*) pourraient exercer eux aussi une influence sur le développement ovarien.

Ce travail compare l'influence des reines de *B. terrestris* (*B.t.)* avec celle des femelles de *P. vestalis* (parasite spécifique de *B.t.*) et de *P. bohemicus* (parasite non spécifique de *B.t.*) sur le développement ovarien des ouvrières de *B.t.* Des groupes de 6 ouvrières récemment écloses ont été formés à partir de différentes colonies de *B.t.* et maintenus dans les conditions suivantes : (i) avec une reine de *B.t.* (témoin positif), (ii) avec une femelle de *P. vestalis*, (iii) avec une femelle de *P. bohemicus*, (iv) avec uniquement des ouvrières (témoin négatif). La longueur de l'ovocyte terminal de chaque ovariole a été mesuré et les mesures ont été réparties selon les stades décrits par Duchateau et Velthuis (1989). Il en a résulté 2 groupes : le groupe A avait des ovocytes plus petits ou égaux au stade II et le groupe B des ovocytes plus gros que le stade II. Le rapport ouvrières du groupe A au nombre total d'ouvrières a été calculé par le test de Mann-Whitney.

Il n'y avait aucune différence statistique entre les reines de *B.t.* et les femelles de *P. vestalis* ($P$ = 0,64), alors que la différence entre les ouvrières seules et les femelles de *P. vestalis* était significative ($P$ = 0,04). Aucune différence statistiquement significative n'a été trouvée entre les reine *B.t.* et les femelles de *P. bohemicus*, ni entre les ouvrières seules et les femelles de *P. bohemicus* ($P$ = 0,17 et $P$ = 0,52, respectivement). Les 4 femelles de *P. vestalis* et une femelle de *P. bohemicus* ont donné des descendants (Tab. II). L'inhibition du développement ovarien par *P. vestalis* était comparable à celle exercée par les reines de *B.t. P. bohemicus* a inhibé le développement ovarien partiellement, mais le pourcentage d'ouvrières ayant des ovaires développés n'était pas différent de celui du groupe sans reine. Une inhibition totale du développement ovarien n'était donc pas indispensable à la reproduction du parasite.

La spécificité hôte-parasite peut aussi s'établir par des mécanismes autres qu'une spécificité phéromonale agissant avant que le parasite ne pénètre dans le nid de l'hôte. Elle pourrait dépendre de la capacité du parasite à reconnaître les signaux chimiques produits par son hôte ou être liée à des caractéristiques comportementales lors de la recherche du nid de l'hôte. La co-existence de l'hôte et du parasite, la compatibilité de la période d'émergence des adultes à la sortie de l'hivernage et un comportement adapté pourraient aussi être importants pour le développement du parasitisme social.

***Bombus terrestris* spp. / *Psithyrus* spp. / développement ovarien / parasitisme social**

**Zusammenfassung – Unterdrückung der Ovarentwicklung von Arbeiterinnen bei *Bombus terrestris* durch *B. terrestris* Königinnen und durch Weibchen von *Psithyrus vestalis* und *P. bohemicus*.** Die Unterdrückung der Ovarentwicklung bei Arbeiterinnen ist für soziale Insekten charakteristisch. Bei eusozialen Insekten erfolgt sie durch Verhaltensweisen und durch Pheromone. Auch dominante Arbeiterinnen können die Ovarentwicklung behindern.

Obligate soziale Parasiten in den Nestern vieler *Bombus* Arten wie Weibchen der Kuckuckshummeln (*Psithyrus)* könnten ebenfalls einen Einfluss auf die Ovarentwicklung haben.

In dieser Arbeit wurde der Einfluss der spezifischen *P. vestalis* und der nicht-spezifischen *P. bohemicus* auf *B. terrestris* Arbeiterinnen untersucht und mit *B. terrestris* Königinnen verglichen. Es wurden Gruppen aus 6 frisch geschlüpften *B. terrestris* Arbeiterinnen aus verschiedenen Völkern gebildet und unter 4 Bedingungen gehalten: 1. mit einer

*B. terrestris* Königin (positive Kontrolle); 2. mit einem *P. vestalis* Weibchen; 3. mit einem *P. bohemicus* Weibchen; 4. nur Arbeiterinnen (negative Kontrolle). Die Länge der terminalen Oocyte der Ovarien der Arbeiterinnen wurde gemessen und in Stadien nach Duchateau and Velthuis (1989) eingeteilt. Es wurden 2 Gruppen gebildet: eine Gruppe hatte Oocyten kleiner als Stadium II (<= II) und eine größer als Stadium II (> II). Das Verhältnis der Arbeiterinnen der Gruppe <=II zur Gesamtzahl der Arbeiterinnen wurde mit dem Mann-Whitney Test berechnet.

Es gab keine statistisch signifikanten Unterschiede zwischen *B. terrestris* Königinnen und *P. vestalis* Weibchen ($P$ = 0,64), während der Unterschied zwischen nur Arbeiterinnen und *P. vestalis* Weibchen signifikant war ($P$ = 0,04).

Weder zwischen *B. terrestris* Königinnen und *P. bohemicus* Weibchen noch zwischen der Arbeiterinnengruppe und *P. bohemicus* Weibchen ergaben sich signifikante Unterschiede ($P$ = 0,17 bzw $P$ = 0,52). Sowohl die 4 *P. vestalis* als auch das einzige *P. bohemicus* Weibchen erzeugten Nachkommen (Tab. II). Die Unterdrückung der Ovarentwicklung durch *P. vestalis* war vergleichbar zur Unterdrückung durch *B. terrestris* Königinnen. *P. bohemicus* unterdrückten die Ovarentwicklung zwar zum Teil, aber der Anteil der Bienen mit entwickelten Ovarien unterschied sich nicht von dem Anteil, die in der Arbeiterinnengruppe ohne Königinnen gefunden wurde. Eine vollständige Unterdrückung der Entwicklung war für eine Vermehrung des Parasits nicht nötig.

Die Wirt – Parasit Spezifität kann auch durch andere Mechanismen als spezielle Pheromone entstehen, die sich auswirken, bevor der Parasit in das Wirtsnest eindringt. Das hinge von der Fähigkeit des Parasiten ab, chemische Reize, die vom Wirt erzeugt werden, zu erkennen. Oder sie könnten mit der Art des Verhaltens bei der Nestsuche zusammenhängen. Die Co-Existenz von Wirt und Parasit, Kompatibilität in der Schlupfzeit nach der Überwinterung und angepasstes Verhalten könnte auch für die Entwicklung des sozialen Parasitismus wichtig sein.

***Bombus terrestris* / *Psithyrus* spp. / Kuckuckshummeln / Ovarentwicklung / sozialer Parasitismus**

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