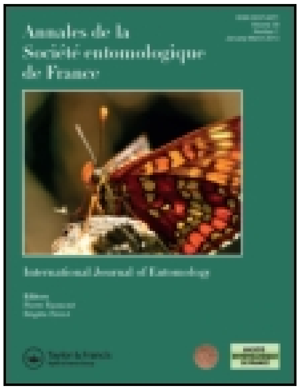

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A method for year-round rearing of cuckoo bumblebees (Hymenoptera: Apoidea: Bombus subgenus Psithyrus)

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A method for year-round rearing of cuckoo bumblebees (Hymenoptera: Apoidea: *Bombus* subgenus *Psithyrus*)

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Abstract. The study of the social interactions between host bumblebees and their inquiline species requires rearing them. Here we provide a simple method for rearing of cuckoo bumblebees (*Bombus* subgenus *Psithyrus*) in laboratory. In this study, two bumblebee cuckoo-host systems were used : *Bombus (Psithyrus) vestalis* hosted by *B. (Bombus) terrestris* and *B. (Psithyrus) sylvestris* hosted by *B. (Pyrobombus) pratorum*. First, the invasion of the *Psithyrus* female should be performed in species-specific host colonies containing approximately 10 young workers. On average, a parasitized colony of *B. terrestris* produced 90 ± 9 young males and 21 ± 3 young females of *B. vestalis*, whereas a parasitized colony of *B. pratorum* produced 16 ± 2 young males and 5 ± 1 young females of *B. sylvestris*. One week after emergence, *Psithyrus* virgin females are placed in a flight cage exposed to natural light, with *Psithyrus* males of other colonies. After successful mating, the females are isolated and overwintered in a cold room at 4°C for 2-4 months. This method enables a year-round and mass breeding of *Psithyrus* species to facilitate studies of these rare species.

Résumé. Une méthode pour élever des bourdons parasites en continu toute l'année ((Hymenoptera: Apoidea: *Bombus* subgenus *Psithyrus*). L'étude des interactions sociales entre les bourdons hôtes et leurs espèces inquilines réclame de les élever. Nous proposons ici une méthode simple pour élever et reproduire les bourdons parasites (*Bombus* subgenus *Psithyrus*) en laboratoire. Dans cette étude, deux-systèmes hôtes-parasites ont été utilisés : *Bombus (Psithyrus) vestalis* hébergé par *B. (Bombus) terrestris* et *B. (Psithyrus) sylvestris* hébergé par *B. (Pyrobombus) pratorum*. D'abord, l'invasion de la femelle *Psithyrus* doit être initiée dans des colonies-hôtes spécifiques qui comprennent environ 10 jeunes ouvrières. En moyenne, une colonie parasitée de *B. terrestris* a produit 90 ± 9 jeunes mâles et 21 ± 3 jeunes femelles de *B. vestalis*. Une colonie parasitée de *B. pratorum* a produit 16 ± 2 jeunes mâles et 5 ± 1 jeunes femelles de *B. sylvestris*. Une semaine après l'émergence, les femelles vierges de *Psithyrus* sont placées dans une cage de vol exposée à la lumière naturelle, avec des *Psithyrus* mâles d'autres colonies. Après un accouplement réussi, la femelle est isolée et mise en hibernation à 4°C pour 2-4 mois. Cette méthode permet de produire toute l'année et en quantité ces espèces de *Psithyrus*, ce qui facilite ainsi l'étude de ces espèces rares.

Keywords : *Bombus*, social parasitism, laboratory rearing, bumblebee breeding

Social parasitism has long been recognized as one of the most intriguing phenomenon and its natural history is still an immensely fascinating topic for evolutionary biologists. Social parasites live at the expense of their social host, exploiting not only their brood care behavior but also their whole social system (Wilson 1971; Nash & Boomsma 2008; Kreuter *et al.* 2012). As a result social parasitism (or inquilinism) causes a fitness reduction in the host. Because of this asymmetric relationship, the processes and dynamics of host-social parasite interactions have been investigated in numerous theoretical and empirical studies. Social parasitism has been used to explore aspects of coevolution, games theory, sympatric speciation and also recognition systems (Hamilton & Dill 2002; Lorenzi 2003; Savolainen & Vepsäläinen 2003; Brandt *et al.* 2005; Lecocq *et al.* 2011; Martin *et al.* 2010; Kreuter *et al.* 2012).

Social insects have evolved highly sophisticated recognition systems which enable them to reject any foreign individuals. A common colony odour resulting from shared cuticular hydrocarbons among nestmates is at the basis of recognition mechanisms (van Zweden & d'Ettorre 2010). Social parasites have evolved several morphological, behavioral and chemical adaptations to evade host front-line defenses and to integrate its social system (Lenoir *et al.* 2001; Zimma *et al.* 2003; Lorenzi 2006; Nash & Boomsma 2008; Sramkova & Ayasse 2009; Bunk *et al.* 2010; Kreuter *et al.* 2010, 2012)

Social parasites are common in social hymenopterans, particularly among ants, social wasps and social bees (Buschinger 1986; Cervo 2006; Michener 2007). In this last group, social parasitism is especially important in the bumblebee subgenus *Psithyrus* that is exclusively composed of workerless inquilines. Nearly 30 species of cuckoo bumblebees are described worldwide (Williams 1998). However, very little is known about the evolutionary ecology of their interactions with their host of the same genus *Bombus*. This lack of knowledge is due to several reasons, mostly because of the relative rarity of cuckoo bumblebees but also because continuous observations of wild nests are impossible. It is therefore necessary to rear cuckoo bumblebees in laboratory to have a better understanding of their ecology. Rearing cuckoo bumblebees has already been undertaken in the last decades (van Honk *et al.* 1981; Fisher 1988; Küpper & Schwamberger 1995; Sramkova & Ayasse 2009; Bunk *et al.* 2010; Kreuter *et al.* 2010, 2012; Lhomme *et al.* 2012) but so far no precise rearing method has been published. Here we describe a simple method that enables a year-round and mass production for rearing of cuckoo bumblebees in laboratory. It was tested on two *Psithyrus* species: *Bombus (Psithyrus) vestalis* (Fourcroy) and *B. (Psithyrus) sylvestris* (Lepeletier).

Description of Procedure

Preparation of the host colonies

Nest searching *Bombus* queens are collected in the field during the spring and used for founding colonies in the laboratory. Young *Bombus* host colonies reared industrially can also be used to enhance production of new

Psithyrus females but it could be preferable to rear them in wild colonies for behavioral experiments. In our studies we used two bumblebee cuckoo-host systems, *B. (Psithyrus) vestalis* hosted by *B. (Bombus) terrestris* and *B. (Psithyrus) sylvestris* hosted by *B. (Pyrobombus) pratorum*. These host bumblebees are both pollen-storers (Sladen 1912).

Bombus host queens are reared in a controlled climate dark room at 28-30°C and 60-65% humidity and fed with unlimited sugar syrup or a 50 % sugar solution of API-Invert® (72.7%; Südzucker AG, Germany; 1g citric acid and 3g potassium sorbate were added per liter API-Invert solution) and fresh pollen (*Salix sp.*; Ruchers de Lorraine). In early spring, willows (i.e. *Salix sp*) are almost the only food supply for the emerging bumblebee species. It is also a protein-rich pollen that is very effective for bumblebee ovarian maturation (Regali 1996). It is thus preferable to use willow pollen for bumblebee colony initiation, but diversifying the pollen resources is also possible. Only fresh pollen immediately deep-frozen should be used. The pollen is moistened with sugar-water (ratio 2:1) to make pollen lumps. Sugar syrup is provided in pierced plastic tubes. Sugar syrup and pollen are offered under red light to not disturb colonies. The host queens are put singly or in pairs in small plastic-covered wooden nest boxes (30 x 13 x 12 cm) separated in two equal parts (Figure 1). We used several different materials to build nest boxes (plastic or wood), making no differences in the colony life. The inner part contains a lump of pollen (place of the future nest), and the outer part contains the sugar syrup feeder. Once five workers had emerged, colonies are moved to plastic dual chamber boxes (32 x 23 x 12 cm) (Figure 2).

INSERT HERE FIG. 1, 12cm BW centered

INSERT HERE FIG.2 12cm BW centered

There are already numerous studies about how to establish and rear year-round *Bombus* colonies in laboratory (Plowright & Jay 1966; Pomeroy & Plowright 1980; Röseler 1985; Eijnde *et al.* 1991; Ptácek 1991; Pouvreau 1993; Tasei 1994; Tasei & Aupinel 1994; Gretenkord & Drescher 1997; Kwon et al. 2003, 2006; Velthuis & van Doorn 2006). We refer you to these authors for a complete description of the laboratory rearing methods of the *Bombus* hosts.

Preparation of *Psithyrus* females

Host nest searching *Psithyrus* females are collected in the spring to be reared in initiated *Bombus* host nests. *Psithyrus* females are kept individually in small wooden nest boxes (10 x 10 x 10 cm) under darkness and controlled temperature and humidity conditions (28-30°C and 60-65% humidity) to rear the *Bombus* hosts. They are fed *ad libitum* with pollen and sugar-water until host colonies are ready to be infested. They can be maintained until 3 weeks waiting the availability of host colonies. In this study we used two species of cuckoo bumblebees, *B. vestalis* hosted by *B. terrestris* and *B. sylvestris* hosted by *B. pratorum*.

Invasion procedure

Newly emerged workers are fully dominated by their queen, poorly aggressive and are not in competition with the parasite for reproduction which starts only at the competition point (Röseler and Röseler 1977; Röseler *et al.* 1981; Duchateau 1989). It is thus really important to introduce *Psithyrus* females in young colonies containing only the first batch of workers or at least only young workers. The number of workers in host colonies before *Psithyrus* female invasion should be about 5 to 10 workers, depending on the size of the first batch (Sramkova & Ayasse 2009). The *Psithyrus* female is introduced in the outer compartment of the nest box, outside of the host colony. If the cuckoo female is not accepted in the host colony after one day or if she is still trying to escape, she is removed from the nest box and killed. The *Psithyrus* female is considered as accepted by the host members when no aggressive interaction is observed between the *Psithyrus* female and the hosts.

Invaded colonies

After successful invasion, the cuckoo females face the challenge of integrating host social system to dominate the host workers. During the initial phase of the colony life cycle, dominant fecund queens suppress workers' ovarian development by behavioral and pheromonal mechanisms (Röseler and Röseler 1977; van Honk *et al.* 1980; Röseler *et al.* 1981). It has been shown that *Psithyrus* species have the same ability to suppress host workers ovarian development as the real queen (Vergara *et al.* 2003; Kreuter *et al.* 2012). Fully dominated workers are less disposed to be controlled by the usurper so it is preferable (in the case of queen-intolerant inquilines like *B. vestalis*) to remove the host queen to facilitate *Psithyrus* female domination on host workers. This technique reduces competition for domination between both reproductives and thus facilitates the cuckoo female social integration. It allows mass producing of *Psithyrus* offspring. In the case of behavioral studies dealing with *Psithyrus* female infiltration and social integration, it is better to keep the host queen present in the colony to model natural conditions. *Psithyrus* offspring start to emerge approximately one month after nest infiltration (see results).

Mating and hibernation

After one week, newly emerged *Psithyrus* females are placed in a flight cage (50 x 50 x 50 cm) exposed to natural light, with *Psithyrus* males of other colonies (approximately one female for five males). Generally with other bumblebees, the mating pair is removed from the flight cage during the copulation to avoid the harassment by the other males. This is not necessary with the *Psithyrus* as the copulation spent at most 3 minutes (see results). Mating duration is determined by observing the mating pairs in the flight cage. After successful mating, the females are isolated and fed with pollen and sugar syrup for one week. They are then placed in small plastic boxes (10 x 10 x 10 cm), with a moist paper towel to keep up humidity, and overwintered in a cold room at 4°C for 2-4 months.

Use of the CO_2 narcosis

Queens that overwintered in captivity are often difficult to activate. An increased temperature alone does not always break diapause. For *Bombus terrestris*, using CO_2 narcosis helps to activate hibernated queens and induces egg-formation so that they start nest-building after a few days (Röseler 1985). A similar technique can be used with the *Psithyrus*. After hibernation, *Psithyrus* females are put in a glass jar and treated with a CO_2 stream until they are immobilized. They remain 30 min in CO_2 saturated atmosphere in the jar closed with parafilm. If the *Psithyrus* queens have spent less than five weeks in hibernation, the narcosis is repeated (24h and 48h after emerging). It has been shown with *B. terrestris* that CO_2-narcosis of mated females makes it possible to rear the species continuously by preventing the females from going into diapause (Röseler 1985). However, this approach should be avoided if colonies are required for behavioral or developmental studies because the narcosis has both immediate and delayed physiological and behavioral effects on insects (Pomeroy & Plowright 1979; Kukuk *et al.* 1997). After narcotic treatment, the females are isolated and fed with unlimited pollen and sugar syrup for one week before new invasion. In this study, females of *B. sylvestris* and *B. vestalis* were not treated with CO_2, except the *B. vestalis* female used in the sample colony 1.

Results and discussion

Psithyrus invasion success

The cuckoo females (n=14) were completely accepted in the host nest within a few hours. Both cuckoo species *B. vestalis* and *B. sylvestris* firstly reacted in the same way by trying to avoid any contact with the members of the host colony and hiding themselves into the nest comb. *B. sylvestris* females rarely induced aggressive behaviors from the queen and workers and never tried to sting anyone. In all cases they were accepted quite fast by the host members, within an hour approximately. Host queen and workers never attacked a *B. sylvestris* female but the workers (not the queen) reacted to the intrusion of the cuckoo by getting excited, buzzing and walking very quickly over the nest.

Unlike *B. pratorum* workers, *B. terrestris* workers reacted more aggressively towards the intruder. Some host workers tried to bite and sting the *B. vestalis* female and the latter responded by killing the aggressive workers. Except in two colonies (1 and 7), few host workers were generally killed within a few hours after *B. vestalis* female introduction and in the colony 3, even all the 5 workers present in the colony were killed (Table 1).

INSERT HERE TAB. 1

Our results show that 5-10 is the optimal number of host workers to maximize *Psithyrus* female invasion success but also to allow the cuckoo females to produce a great number of sexuals. In the field, *Psithyrus* females are often seen searching for host nests more or less a few weeks after the emergence of their *Bombus* host. At this time the host queen has generally only produced the first batch of workers, which consists only of a few small and young workers. The first batch of workers is less aggressive than the second batch (Alford 1975). Van Honk *et al.* (1981) and later Sramkova & Ayasse (2009) showed that only the young workers of *B. terrestris* (≤ 8 days old) survived after *B. vestalis* female invasion. *Bombus vestalis* females should only kill the workers with the highest likelihood of trying to reproduce themselves. These workers will be the first to compete with her for reproduction, as they eat non-self-laid eggs (Fisher 1987). Sramkova & Ayasse (2009) showed that *B. vestalis* females are able to discriminate and kill the oldest workers according to the presence/absence of fertility signals (Sramkova *et al.* 2008). This worker discrimination ability helps the parasitic females to maximize their own reproductive success. The same authors also demonstrated that the size of a host colony largely determines the invasion success and survival rate of a *Psithyrus* female (Sramkova & Ayasse 2009). Increasing the host colony size will increase the potential reproductive success of the inquiline but also the chances of being killed by the host workers (Fisher 1984, 1987; Sramkova & Ayasse 2009). The usurper needs therefore to find the right compromise. Moreover, it is essential for the *Psithyrus* female to be able to fully dominate the host for as long as possible to maximize her reproductive success which becomes difficult with larger numbers of workers (van Honk *et al.* 1981). Sramkova & Ayasse (2009) have shown that colonies of about 5-10 workers provided the best invasion rate for *B. vestalis* females. This colony size maximizes reproductive success and minimizes the chances of the *Psithyrus* female to be ejected, which is confirmed by our results. This method was always successfull (n=14).

The interactions between *B. vestalis* and *B. terrestris* were clearly more aggressive than the interactions between *B. pratorum* and *B. sylvestris* despite the fact that *B. pratorum* often presented more workers than *B. terrestris* colonies. The type of interaction between cuckoo bumblebee and their hosts are not always clear cut (Fisher 1988), however, our behavioral observations confirmed the previous ethological investigations made on both systems (Sladen 1915; van Honk *et al.* 1981; Küpper & Schwammberger 1995). Küpper and Schwammberger (1995) noted that *B. pratorum/B. sylvestris* interactions can also be quite aggressive. They hypothesized that the physiological (i.e. sexual maturity) state of the cuckoo females may also explain the type of interactions they exhibit with their hosts.

As previously described by different authors (van Honk *et al.* 1981; Fisher 1988; Sramkova & Ayasse 2009), our results showed that the invasion of *B. vestalis* females often leads to the death of the workers who tried to attack them but also to the death or ejection of the host queen within a few days. This was never the case with *B. sylvestris* females which confirms the observations of Hoffer (1889) and Küpper & Schwammberger (1995).

Psithyrus **reproductive success**

We managed to produce 895 males and 212 virgin females of *B. vestalis* in 10 *B. terrestris* host nests invaded by reproductive parasite females, and 65 males and 18 females of *B. sylvestris* in 4 *B. pratorum* host nests invaded reproductive parasite females. On average, a parasitized colony of *B. terrestris* produced 90±9 young males and 21±3 young females of *B. vestalis* (Table 2), whereas a parasitized colony of *B. pratorum* produced 16±2 young males and 5±1 young females of *B. sylvestris* (Table 3). We found that *Psithyrus* reproductive success is positively correlated with the number of workers available in the host colony (correlation coefficient=0.93; Figure 3). Our results demonstrate a high reproductive success of *Psithyrus* females, especially for *B. vestalis*, with a high correlation between the number of workers produced and the number of *Psithyrus* offspring emerged. These results agree with the study of Küpper & Schwammberger (1995) on *B. sylvestris* reproduction but are not in accordance with the previous study of van Honk *et al.* (1981) on *B. vestalis* reproduction. In the two parasitized colonies of *B. terrestris* studied by van Honk *et al.* (1981), only 6 males and 3 females of cuckoos emerged in the first colony and 26 males and 57 females of *B. vestalis* emerged in the second one, despite the fact that both colonies produced respectively 150 and 217 host workers. It seems that the correlation we found is only valuable when the *Psithyrus* female invades small colonies of few young workers. Van Honk et al. (1981) introduced the cuckoos in bigger colonies, with larger brood and consisting of 40 workers in the first one and 27 workers in the second one. These authors noticed high rates of aggressive interactions between workers and the cuckoo females but also high rates of cuckoo egg and larvae rejections. Even if both *Psithyrus* females succeed in invading *B. terrestris* colonies, it seems that they were not able to fully dominate the host workers, mostly because of the age and size of both colonies. Kreuter *et al.* (2012) showed that *Psithyrus* females are only able to dominate host workers and to inhibit their ovary development when they had direct contact to the workers. Therefore, the larger the host colonies are the more difficult it is for parasite females to inhibit worker reproduction. It is also confirmed by the presence of worker produced males in both colonies studied by van Honk *et al.* (1981) while our colonies parasitized by *B. vestalis* never produced host males and brood rejection came from the cuckoo females and not from the host workers. These results confirm that our method enables full domination of *Psithyrus* females over host workers.

INSERT HERE FIG.3, 8cm BW one column

In parasitized colonies of *B. pratorum*, we also noted host sexual offspring (male and female) emergence but only before parasite offspring emergence. These results confirm the conclusions of Küpper & Schwammberger (1995) that the queen-tolerant inquiline *B. sylvestris* allows host reproduction but only before the first parasitic eggs are laid.

INSERT HERE TAB. 22

The differences in the reproductive success of *B. sylvestris* and *B. vestalis* can be explained by two main reasons. Firstly, *B. sylvestris* females partly tolerate host reproduction (Table 3) so host workers do not only care for the parasite offspring. Whereas colonies parasitized by *B. sylvestris* females all produced both host and parasite sexuals, no sexuals of *B. terrestris* hatched in parasitized colonies. Secondly, *B. pratorum* colonies don't have the same reproductive potential as *B. terrestris* colonies. Nest sizes of *B. pratorum* rarely reach more than 40 workers (Løken 1973) whereas *B. terrestris* nests can contain hundreds of workers (Goulson *et al.* 2001). The fact that host worker resources are less important and not totally allocated to parasite reproduction may explain why *B. sylvestris* females had a lower reproductive success than *B. vestalis* females.

INSERT HERE TAB. 3

Our data on *Psithyrus* reproductive success also show that sex ratios in both cuckoo species are male biased with a tendency to protandry. Females of *B. vestalis* produced a median of 81% of males (range 79-85%) and 79% of males (range 74-85%) for *B. sylvestris*. These results are not always in accordance with literature data. In fact, Bourke (1997) concluded in his review of bumblebee sex ratios that *Psithyrus* sex ratio was consistently female biased at the opposite of non-parasitic *Bombus* species. The sex ratios observed could also be artefactual due to laboratory-reared conditions (De Jonghe Roland, *pers. com.*).

INSERT HERE FIG.4, 12cm BW centered

We further noticed that *B. vestalis* male brood has shorter development time (31±2 days) than the female brood (35±2 days) (Figure 4). If we compare these results to the times of cuckoo male and female emergence (Table 2), we can approximately say that *B. vestalis* females start to lay male and female eggs respectively 4 and 10 days after invasion on average. However we observed that the first *Psithyrus* egg cells were often composed of a mix of cuckoo male and female eggs (Figure 5). Using the development times of *B. sylvestris* (males: 14-19 days, females: 22-27 days) measured by Küpper & Schwammberger (1995) we can also confirm that *B. sylvestris* females start to lay male and female eggs approximately at the same time but with a higher proportion of males at the beginning of the egg laying period. Several studies have previously demonstrated that CO_2 narcosis can sometimes affect reproduction depending on CO_2 concentration and narcosis timing (Röseler 1985, Tasei 1994). However the observed colonies with narcosed *B. vestalis* females (colony 1) and non-narcosed *B. vestalis* females (colony 2-10) showed similar results. As far as we have observed, our results cannot be explained by the

CO_2 treatment.

INSERT HERE FIG. 5, 14cm BW centered

Psithyrus mating and hibernation success

Our results show a striking difference in the copulation durations between the cuckoo bumblebees and their hosts (Figure 6). We found significant differences in the mating duration between the different species (Kruskall Wallis, df=3, p<0.001). However we found no difference among the *Psithyrus* species or among the non parasitic *Bombus* species. Whereas *Psithyrus* species took on average nearly 3 min to copulate the other *Bombus* needed on average more than 26 min.

INSERT HERE FIG. 6, 8cm BW one colum

Our results on the mating durations of hosts are in accordance with literature (Djegham *et al.* 1994; Duvoisin *et al.* 1999; Brown & Baer 2005; Amin *et al.* 2009), but to our knowledge it is the first study that investigated cuckoo bumblebee mating durations.

At the opposite of honey bees, where copulation takes more or less a few seconds (Koeniger & Koeniger 1991; Winston 1991), copulation duration in non-parasitic bumblebees is unexpectedly long, despite the high costs (energetic loss, predator exposure) that it entails (Brown & Baer 2005). Prolonged copulation behavior in bumblebees is therefore hypothesized to be under selection due to high risk of sperm competition or to allow males to manipulate females (Simmons 2001). For example, in *B. terrestris*, sperm transfer takes not more than 2 minutes and the mating plug transfer takes about 10 minutes whereas the mean copulation duration is about 36.9 minutes (Duvoisin *et al.* 1999). Both traits seem thus unlikely to explain such long copulation durations (Brown & Baer 2005). Male facilitation of sperm migration into the female spermatheca, whether physically, chemically or by mate guarding, seems the most likely reason for long copulations (Djegham *et al.* 1994), however a general adaptive explanation is still lacking (Brown & Baer 2005). It is therefore difficult to explain, without further studies, such a large difference in the mean copulation duration between parasitic and non-parasitic bumblebees. We can, however, hypothesize that *Psithyrus* males don't produce mating plugs or don't play an active role in sperm migration into the spermatheca.

Concerning the hibernation success of *Psithyrus* females with our rearing method, no precise data has been registered. However, for *B. vestalis*, we can confirm that more than 50% of the hibernated females survived from hibernation and were able to reproduce after CO_2 narcosis. We managed to produce successfully 2-3 generations per year.

The rearing method described here has also been successfully used in other studies to rear *B. norvegicus* the cuckoo of *B. hypnorum* (Zimma *et al.* 2003) and *B. bohemicus* the cuckoo of *B. lucorum* (Kreuter *et al.* 2010, 2012). This technique should thus work with other *Psithyrus* species as well.

To conclude, rearing cuckoo bumblebees (*s.g. Psithyrus*) should be successful with the next conditions: (i) The *Psithyrus* should be established in their species-specific host nest; (ii) The invasion of the *Psithyrus* queen should be performed in host colonies containing no more than 5-10 young workers; (iii) For mating, the sexuals should be exposed to natural light; (iv) The mated queens should be overwintered in a cold room (or a fridge) at 4°C for 2-4 months. If the hibernation is too short, one or two 30 min CO_2 narcoses should be used.

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Table 1: Number of host workers present in the nest before cuckoo invasion and number of host workers killed by the cuckoo after invasion in 10 colonies of *B. terrestris* parasitized by

B. vestalis and in 4 colonies of *B. pratorum* parasitized by *B. sylvestris*.

	B. vestalis		***B. sylvestris***	
Colony	Nb of workers	workers killed	Nb of workers	workers killed
1	5	0	11	0
2	9	3	9	0
3	5	5	8	0
4	10	6	13	0
5	7	2	-	-
6	9	1	-	-
7	5	0	-	-
8	10	2	-	-
9	5	3	-	-
10	8	4	-	-
m ± s.d.	**7 ± 1**	**3 ± 1**	**10 ± 1**	**0**

Table 2: Number of host workers produced, number and emergence period of cuckoo offspring in 10 colonies of *B. terrestris* parasitized by *B. vestalis*.

Colony	Number host workers	Number of *B. vestalis* emergences		First and last day of *B. vestalis* emergence			
		Males	Females	Males		Females	
1	44	92	18	33	49	40	46
2	81	117	30	38	69	44	61
3	89	133	36	38	71	48	67
4	69	90	21	39	62	55	58
5	72	111	28	35	72	44	69
6	56	52	9	28	46	42	54
7	63	74	19	31	45	46	53
8	41	88	13	35	43	49	57
9	27	37	7	37	58	49	52
10	64	101	31	34	71	36	62
m ± s.d.	**61 ± 6**	**90 ± 9**	**21 ± 3**	**35 ± 1**	**59 ± 4**	**45 ± 2**	**58 ± 2**

Table 3: Number of workers produced, number and emergence period of host and cuckoo offspring in 4 colonies of *B. pratorum* parasitized by *B. sylvestris*.

Colony	**Host emergence**							***B. sylvestris* emergence**					
	Number of hosts			First and last day				Number of cuckoos		First and last day			
	Workers	Males	Females	Males		Females		Males	Females	Males		Females	
1	20	4	2	11	18	24	25	17	6	28	38	33	39
2	16	5	1	17	22	25	25	11	2	25	31	29	30
3	14	4	1	21	22	23	23	14	4	26	39	29	33
4	24	7	3	19	24	29	34	23	6	30	43	39	44
m ± s.d.	**19 ± 2**	**5 ± 0.4**	**2 ± 0.3**	**17 ± 1**	**22 ± 1**	**25 ± 1**	**27 ± 2**	**16 ± 2**	**5 ± 1**	**27 ±1**	**38 ± 2**	**33 ± 1**	**37 ± 2**

Legends of the figures :

Figure 1 : *Bombus terrestris* or *B. pratorum* colony initiation box

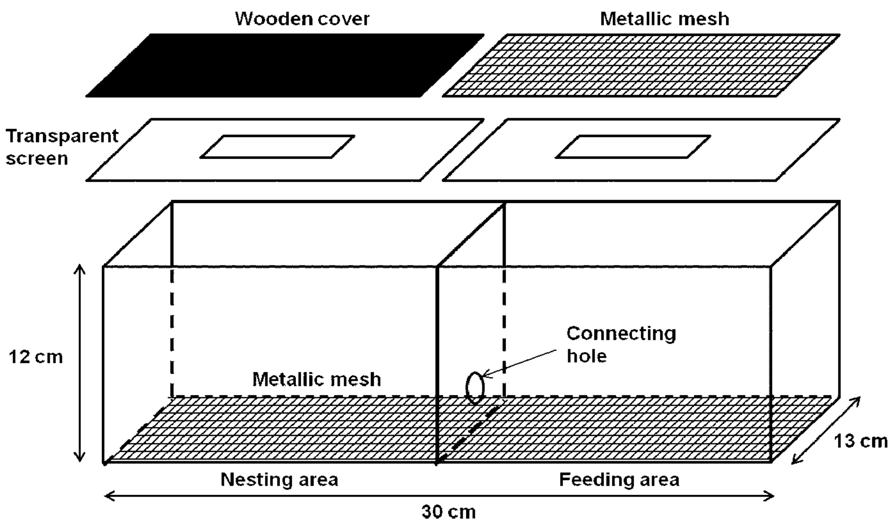

Figure 2 : *Bombus terrestris* or *B. pratorum* colony development box also used for inquiline introduction.

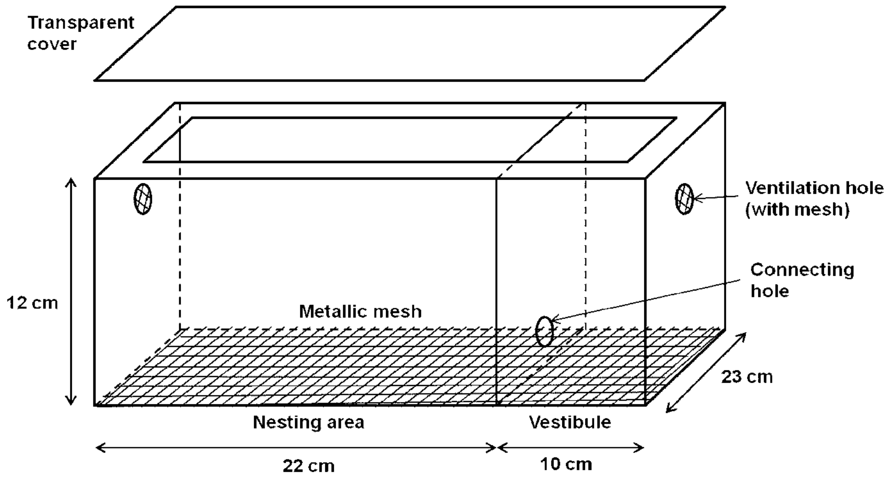

Figure 3: Number of sexuals produced by a *Psithyrus* female in function of the number of host workers produced during a colony cycle (R=0.93, *n*=14). Triangles: *B. sylvestris* hosted by *B. pratorum*; Circles : *B. vestalis* hosted by *B. terrestris*.

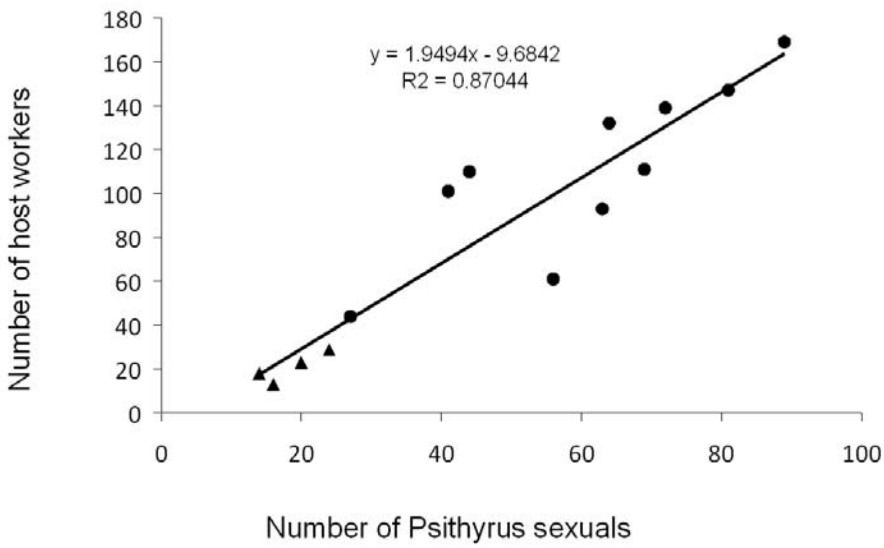

Figure 4: Development time of *B. vestalis* brood.

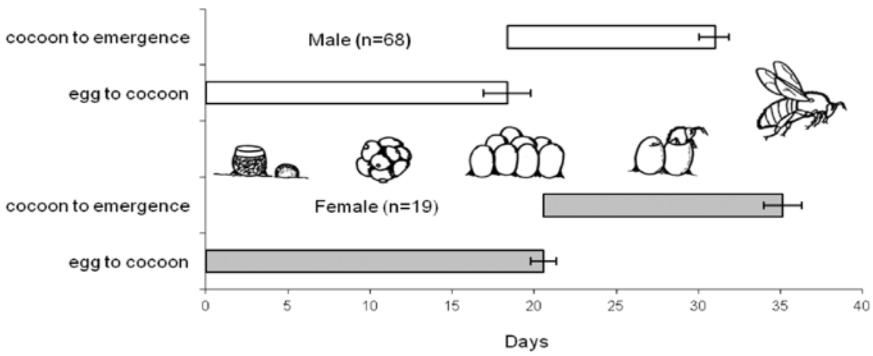

Figure 5: Development of the cuckoo brood in a sample colony (colony 1) of *Bombus terrestris* parasitized by *Bombus vestalis*. The appearance of egg cells and the emergence of adults are shown cumulatively.

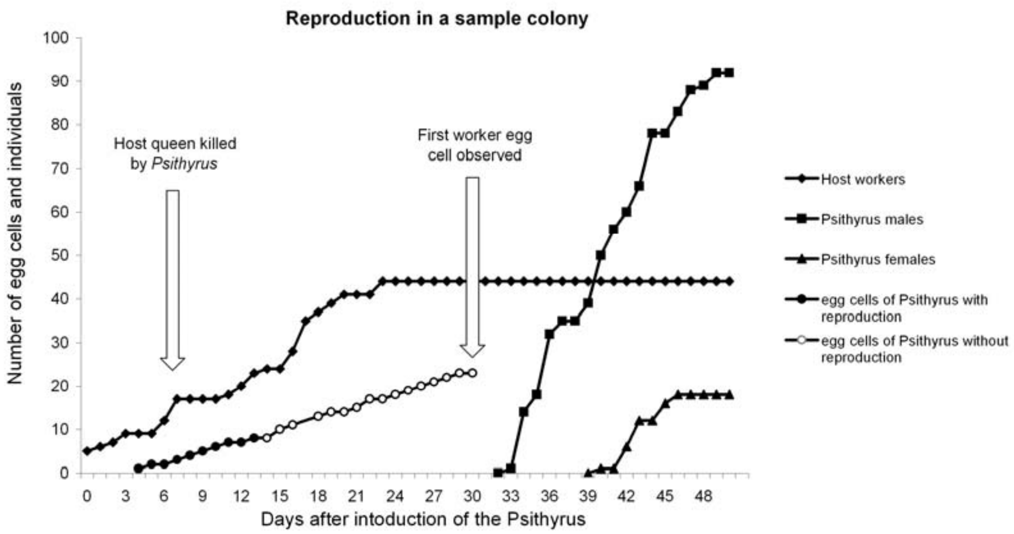

Figure 6: Copulation duration (in min.) of several species of parasitic and non-parasitic bumblebees.