

## Pheromones affecting flying beetles colonizing the polypores *Fomes fomentarius* and *Fomitopsis pinicola*

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The existence of long-range pheromones in five beetle species breeding in polypore fruiting bodies was studied in a field experiment. Species included were: *Dorcatoma robusta* Strand (Anobiidae), *Cis Jacquemarti* Mellie (Cisidae), and *Bolitophagus reticulatus* (L.) (Tenebrionidae) breeding in *Fomes fomentarius* (L. ex Fr.), as well as *Dorcatoma punctulata* Mulsant & Rey and *Cis glabratus* Mellie breeding in *Fomitopsis pinicola* Fr. (Karst). Catches of these species in window traps baited with pieces of polypores, together with females or males of the same species, were compared with catches in traps baited with only polypores. Catches of *D. punctulata* and *C. Jacquemarti* were low, and no *B. reticulatus* were caught. Males of *D. robusta* were strongly attracted by conspecific females. In this species, only the females were attracted to host odour. No pheromone attraction was demonstrated in *C. glabratus*, in which males and females were about equally attracted to the odour of their host.

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### 1. Introduction

Insects which breed in substrate that is available only temporarily and is patchily distributed must be able to colonize new sites at a distance. The effective dispersal capacity of such an insect is determined by its flight capacity and its ability to locate suitable breeding substrate (Jonsell & Nordlander 1995). Breeding site location may first involve general responses to habitat types followed by olfactory orientation towards a particular substrate (Vinson 1981). In addition, in many species, insects already present at the substrate can attract or repel conspecifics depending on their

sex and on the density and stage of substrate colonization (Byers 1995).

Host odours are important cues for phytophagous insects searching for new hosts (Bernays & Chapman 1994), and this is probably also true for many fungivores. For example, the monophagous cisid beetles *Cis glabratus* Mellie and *C. quadridens* Mellie are attracted to the odour of chopped fruiting bodies of their polypore host, *Fomitopsis pinicola* Fr. (Karst.), but not to similar pieces of the non-host *Fomes fomentarius* (L. ex Fr.) (Jonsell & Nordlander 1995).

Attraction to breeding substrate odours may be restricted to the females if mating only takes

place near the site of emergence, before dispersal to distant breeding sites. In species mating at the breeding substrate, attraction to host odours may be a sufficiently effective way to find mates for both sexes. However, sex pheromones can also be used to attract individuals of the opposite sex after a suitable breeding substrate has been found. Usually it is the female that emits a pheromone attracting males (Cardé & Baker 1984). It seems reasonable to assume that pheromone attraction is more common among insects living on substrates that are relatively difficult to locate based on their odour.

In the present study, we searched for the existence of long-range pheromones in five beetle species, all breeding in fruiting bodies of the polypores *Fomes fomentarius* or *Fomitopsis pinicola* (Nadvornaya & Nadvornyi 1991, Kaila *et al.* 1994, Jonsell & Nordlander 1995). In particular, we wanted to determine whether beetles not attracted to host odour rely instead on intraspecific communication for the localization of breeding substrates and mates. Although a response to acoustic signals cannot be completely excluded in beetles (Hoy & Robert 1996), we interpret the attraction observed as being odour-mediated.

## 2. Materials and methods

### 2.1. The beetle species

The presence of long-range pheromones were tested in three beetle species specialized on *F. fomentarius* and two on *F. pinicola* (Table 1). Based on a study of host-odour attraction (Jonsell & Nordlander 1995) and a further examination of the sex ratio of captured beetles (Table 2), these species represent three categories of host attraction. The first category includes *Cis glabratus* (Cisidae), in which

both sexes are attracted to the odour of their host; the second includes *Dorcatoma robusta* Strand and *D. punctulata* Mulsant & Rey (Anobiidae), in which only females appear attracted; and the third includes *Cis jacquemarti* Mellie (Cisidae) and *Bolitophagus reticulatus* (L.) (Tenebrionidae), in which neither sex seems to be attracted to host odour.

### 2.2. Pheromone attraction experiment

To test for the existence of long-range pheromones, a field experiment was carried out in the summer of 1995. Window traps were baited with caged polypore pieces alone or in combination with individuals of either sex of the studied beetle species (Table 1). The field experiment was carried out at two localities in a semi-natural forest, Lunsen, located 10 km SSE of Uppsala, Sweden. One of the localities is dominated by birch and the other by spruce. Both localities contain large quantities of dead trees and polypores. The dominating polypore is *F. fomentarius* at the birch locality and *F. pinicola* at the spruce locality. The beetle species specialized on *F. fomentarius* were therefore studied at the birch locality, and those specialized on *F. pinicola* were studied at the spruce locality.

A randomized block design, with eight blocks per studied beetle species, was used in the experiment. Traps baited with the fungus alone served as controls for all species tested simultaneously at the same locality. The blocks of traps were separated by at least 10 m, and the traps within a block by about 5 m. All traps were baited with a piece of polypore, with an approximate diameter of 7 cm. The polypore pieces were taken from fresh fruiting bodies that had been kept at  $-70^{\circ}\text{C}$  for two days before starting the experiment. The freezing was carried out to ensure that any insects present in the polypores were killed. Each trap consisted of a transparent PVC window (width 35 cm, height 50 cm). The window was nailed to two wooden poles fixed in the ground. The top of the window was located between 1 and 1.5 m over the ground. Immediately beneath the window, a plastic jar with an  $18 \times 18$  cm opening was attached to catch insects hitting the window. The jar contained water with some detergent and had a drain hole covered with metal mesh on one side. The bait was sealed in a metal tube with a fine metal mesh covering both ends. Each bait was wedged

Table 1. Experimental design (fo = piece of *Fomes fomentarius*, pi = piece of *Fomitopsis pinicola*).

Studied species	Locality	Test period	Treatments		
			Females + fungus	Males + fungus	Fungus
<i>Dorcatoma robusta</i>	Birch	13.6–11.7	fo + 2 ♀	fo + 2 ♂	fo
<i>Cis jacquemarti</i>	Birch	13.6–08.8	fo + 5 ♀	fo + 5 ♂	fo
<i>Bolitophagus reticulatus</i>	Birch	18.7–08.8	fo + 2 ♀	fo + 2 ♂	fo
<i>Dorcatoma punctulata</i>	Spruce	13.6–11.7	pi + 2 ♀	pi + 2 ♂	pi
<i>Cis glabratus</i>	Spruce	13.6–08.8	pi + 5 ♀	pi + 5 ♂	pi

inside the tube so that it was not in contact with the metal mesh. The tube was suspended in an opening of the window with the bottom located about 5 cm over the water surface. Once each week, the contents of the plastic jars were emptied, and new water with detergent was added.

The bait insects originated from rearings of fruiting bodies in the laboratory. To determine whether females used as baits had copulated prior to the experiment, rearings from the bait polypores were done after the test. After a couple of months we found many larvae in five of eight *B. reticulatus* female baits, few larvae in two *C. jacquemarti* female baits, a few larvae in one *C. glabratus* female bait, but no *Dorcatoma* larvae.

Cisid males were distinguished from females based on the processes on the head of the males. However, this proved to be difficult for *C. jacquemarti*, in which the horns often are obsolete. Rearings from the baits revealed that a few females of *C. jacquemarti* had mistakenly been identified as males. In the *Dorcatoma* species, sex was determined by examining the last sternal abdominal segment which is more rounded in males than in females. Sex determination in *B. reticulatus* was carried out by looking for the apical torn of the meso- and metatarsi, which is present only in males (Hansen 1973).

During the experiment we regularly checked the status (alive vs dead) of the *Dorcatoma* and *Bolitophagus* individuals used for baits. Many of the *D. robusta* remained alive for the duration of the experiment. The bait individuals of *D. punctulata* died faster. After two weeks only a few of them were still alive, and all were dead by the end of the experiment. Almost all of the bait individuals of *B. reticulatus* remained alive throughout the experiment. The cisids could not be counted in the same way because they were hidden inside the polypore baits. Numerous entrance holes were found in all the cisid baits after the experiment, and some of the bait individuals were found alive when the baits were cut into pieces a couple of months later.

All individuals of the target species caught in the traps were identified and sexed. These trap catch data were log ( $x + 1$ ) transformed, and differences between treatments were tested with an ANOVA followed by Tukey's test for the multiple comparisons.

Table 2. Catches of females and males of some species in window traps baited with chopped fruiting bodies between 28 April and 29 September 1993 (see Jonsell & Nordlander 1995).

	Females	Males
<i>Dorcatoma robusta</i>	18	0
<i>Dorcatoma punctulata</i>	10	0
<i>Cis glabratus</i> <sup>1</sup>	72	59

<sup>1</sup>Seven additional specimens not available for sex determination.

### 3. Results

Only females of *D. robusta* and *D. punctulata* appeared to be attracted by host odour in flight (Table 2). Males of *D. robusta* were evidently attracted to a female sex pheromone, since they were caught in significantly higher numbers in traps baited with conspecific females than in traps baited with males or in traps with only the fungus (Table 3). When the material was subdivided into weekly catches, three out of four weeks showed the same attraction pattern. During the third week, between 4 and 11 July, only two males were caught, one in a trap baited with females and the other in a trap baited with males. On average, the number of females was 1.5 times higher in the traps baited with the fungus alone than in traps baited also with males or females. However, the difference was not significant. Both males and females were caught during each of the four weeks that the experiment was run. Males were caught in six of the eight blocks and females in all eight blocks. Only two female *D. punctulata* were caught, one in a female-baited trap and one in a male-baited trap.

Both sexes of *C. glabratus* were about equally attracted to host odour (Table 2). No long-range pheromones were apparent as there was no significant attraction to either females or males (Table 4). Sixty of the 70 individuals were caught between 20 June and 4 July. Males were caught in six of the blocks and females in all eight. Only three males and two females of *C. jacquemarti* were caught. One male was caught in each treatment,

Table 3. Numbers of *Dorcatoma robusta* females and males caught in differently baited window traps between 13 June and 11 July 1995. All baits contained a piece of *Fomes fomentarius*. Asterisks denote a significant difference in comparison with the two other treatments (\*\* =  $p < 0.01$ , Tukey test). There were no other significant differences between treatments at the 5% level (ANOVA).

	Baits		
	<i>D. robusta</i> females + fungus	<i>D. robusta</i> males + fungus	Fungus
Females	10	10	15
Males	26 **	1	0

while both of the females were caught in traps baited with females. No *B. reticulatus* were captured.

#### 4. Discussion

This study shows that a long-range sex pheromone is used during the host colonization process by *D. robusta*. Apparently females locate a new breeding substrate by orienting to host odours, whereupon they attract the males. The existence of a sex pheromone attracting males of *D. robusta* was expected because they are not attracted to the odour of their host. Functionally similar pheromones have also been described in other anobiids (Kuwahara et al. 1975, Levinson & Levinson 1987, White & Birch 1987, Birch & White 1988).

At the end of the experiment, no feeding scars were visible on the polypore baits with the *D. robusta* females. This indicates that the females do not need to feed on host tissues to initiate pheromone production, as is the case in many bark beetles (Wood 1982, Byers 1995). It also excludes the possibility that host volatiles released by feeding were responsible for attracting conspecifics, as is the case with the pine weevil (Tilles et al. 1986).

No conclusions about long-range pheromones in *D. punctulata* can be drawn from the present study because so few individuals were caught and because the bait individuals died so soon. However, of the *D. punctulata* caught in window traps baited with chopped polypores in the 1993 odour experiment (Jonsell & Nordlander 1995), all were females (Table 2). We therefore suggest that males

of *D. punctulata* are attracted to a female sex-pheromone rather than to host odour. Another possibility, however, is that mating occurs prior to dispersal in this species, in which case males would not have to locate new substrate.

Many individuals of *D. robusta* and *D. punctulata* were caught in window traps placed beneath living fruiting bodies in an experiment in 1992 (Jonsell & Nordlander 1995). Two different explanations for these high catches were presented in that paper: First, the *Dorcatoma* species could have been more attracted to living polypores than to dead ones, and second, pioneer individuals already present at the living polypores could have been attracting conspecifics with pheromones. Further analysis of the catches in 1992 revealed that very few males were caught, which excludes the possibility that female sex-pheromones or aggregation pheromones were responsible for the catches. It thus appears that living fruiting bodies are more attractive to *D. robusta* and *D. punctulata* than dead ones. Other studies also indicate that species of *Dorcatoma* utilize living polypores (Matthewman & Pielou 1971, Kaila et al. 1994).

Both males and females of *C. glabratus* are attracted to the odour of their host, *F. pinicola* (Table 2; Jonsell & Nordlander 1995), but neither males nor females were attracted to individuals of either sex in the present study. Long-range pheromones may not be necessary for *C. glabratus* since both sexes appear equally able to locate a new host by its odour. Similarly, long-range pheromones could also be expected to be absent in *C. quadridens*, in which both sexes are also about equally attracted to the odour of *F. pinicola* (Jonsell & Nordlander unpubl. data). These results contradict the suggestion by Lawrence (1973) that cisids probably use aggregating pheromones because too little of any attractant volatiles would be emitted from dead fruiting bodies.

In the present study *C. jacquemarti* was caught in very low numbers, and *B. reticulatus* was not caught at all. Whether they use long-range pheromones, therefore, can not be concluded from the present study. Similarly, catches of the two species were both very low in the 1993 odour experiment (Jonsell & Nordlander 1995). However, both species are abundant and widely distributed in the region (pers. obs.), and *B. reticulatus* have been caught in window traps in stands of Scots pine

Table 4. Numbers of *Cis glabratus* females and males caught in the differently baited window traps between 13 June and 8 August 1995. All baits contained a piece of *Fomitopsis pinicola*. There were no significant differences between treatments at the 5% level (ANOVA).

	Baits		
	<i>C. glabratus</i> females + fungus	<i>C. glabratus</i> males + fungus	Fungus
Females	10	7	14
Males	10	12	17

where polypores are scarce (M. Schroeder pers. comm.). How these species, with their apparent low incidence of flight, locate new substrates is still unknown. It is possible that occasionally they effectively disperse by flight and show a high degree of colonization success on such occasions.

Although this study provided insufficient data regarding some of the studied species, it indicates that among the beetle species living in polypores a variety of different strategies are used for finding breeding substrates and mates. In some species, both sexes rely entirely on orientation to host odours, whereas in others the males respond to female sex pheromones but not to odours from the host substrate. No evidence was found for the existence of any long-range aggregation or repellent pheromones. Knowledge about how insects dependent on dead wood and wood-inhabiting fungi disperse and locate new breeding sites is needed for evaluating their chances of long-term survival in managed boreal forests, where such substrates have become increasingly scattered (Linder & Östlund 1992, Bader *et al.* 1995).

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