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Parasitoids Modify Their Oviposition Behavior According to the Sexual Origin of Conspecific Cuticular Hydrocarbon Traces

Eric Darrouzet · Sébastien Lebreton · Nicolas Gouix · Aurore Wipf · Anne-Geneviève Bagnères

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Abstract Hydrocarbons play a crucial role in insect behavior in general and in sexual recognition in particular. Parasitoids often modify their oviposition behavior according to hydrocarbons left by conspecifics on the reproductive patch, such as oviposition markers left by females after oviposition, or cuticular hydrocarbon (CHC) traces left by individuals by walking or rubbing. This study determined whether *Eupelmus vuilleti* females are able to distinguish CHCs left by male or female conspecifics on seeds. The results show that the cuticular profile of *E. vuilleti* differs according to its gender, and that females are able to detect the sexual origin of these CHCs. Moreover, they adjust their oviposition behavior according to the nature of these traces. Although females lay fewer eggs on hosts when confronted with female CHCs, they lay more daughters when confronted with male CHCs, thus changing the sex ratio.

Key Words Offspring sex ratio · Artificial seeds · *Eupelmus vuilleti* · Hymenoptera · Eupelmidae

Introduction

Cuticular hydrocarbons (CHCs) are constituents present on the exoskeleton of most insects (Blomquist and Bagnères, 2010). These molecules play a key function in providing water-proofing and protection against the environment as well as a central role in insect communication (Gibbs, 1998; Blomquist and Bagnères, 2010). In social insects, CHCs are involved in the recognition of species, castes, and nestmates (Howard and Blomquist, 2005). They also are involved in the sexual communication of insects, for example, enabling the recognition of sexual mates and acting as courtship inhibitors that reduce the attractiveness of mated females (Ferveur, 2005). Parasitoids can use CHC residues left by their hosts to distinguish between males and females and thus to locate suitable hosts (Colazza et al., 2007). For example, females of two *Trissolcus* species discriminate between chemical residues left by the females or males of their pentatomid hosts (Conti et al., 2004). Host discrimination, i.e., parasitized vs. unparasitized hosts, also is often mediated by a chemical marker, such as hydrocarbons, deposited on the substrate (environment of the host like a seed for example) or the host by ovipositing parasitoid females (van Alphen and Visser, 1990). By marking hosts that they have already parasitized, parasitoid females prevent a second female from ovipositing on the same host (Godfray, 1994). These compounds may originate from the Dufour's gland, an accessory gland in the female genital apparatus, and are deposited after oviposition (Marris et al., 1996; Jaloux et al., 2005). This gland produces an oily secretion containing hydrocarbons, similar to the cuticular hydrocarbon profiles of the female (Howard and Baker, 2003).

The recognition and avoidance of hosts that have already been parasitized is an important issue in parasitoid behavioral ecology. It has been shown that female parasitoids can discriminate between unparasitized and parasitized hosts in several species (Gauthier et al., 1996; Santolamazza-Carbone et al., 2004; Darrouzet et al., 2007, 2008). When a female encounters a parasitized host, she can either reject it and continue to search for other unparasitized hosts, or accept and superparasitize it (i.e., lay an egg on an already parasitized host). In the latter case,

E. Darrouzet (✉) · S. Lebreton · N. Gouix · A. Wipf · A.-G. Bagnères
I.R.B.I., UMR CNRS 6035 Université de Tours,
Faculté des Sciences, parc de Grandmont,
37200 Tours, France
e-mail: eric.darrouzet@univ-tours.fr

the sex ratio of the progeny becomes important (Godfray, 1994). Both gregarious and solitary species can superparasitize hosts, but, in solitary parasitoids, the presence of supernumerary juveniles results in competition for survival and the death of all but one. This competition has been defined as a lethal larval combat (Ueno, 1997), physiological suppression (Vinson and Hegazi, 1998), or scramble competition (Mayhew and Hardy, 1998). Solitary parasitoids must, therefore, lay their eggs on unparasitized hosts to increase the chances of survival of their progeny. However, while the expected fitness gain per host is lower when females superparasitize, superparasitism by solitary species can be an adaptive behavior in certain circumstances (van Alphen and Nell, 1982), e.g., when the number of unparasitized hosts is small or when travel time between patches is long (van Alphen and Visser, 1990). Such behavior is adaptive only if the second egg laid on an already parasitized host can "win" the competition with the first immature (Lebreton et al., 2009).

Eupelmus vuilleti (Crw.) (Hymenoptera: Eupelmidae) is a solitary ectoparasitoid of the larvae of *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) in the tropical zones of West Africa. Females perceive various types of information from seeds that contain their hosts, thus enabling them to change their oviposition behavior. They can discriminate between unparasitized and parasitized hosts, preferring to lay eggs on unparasitized hosts, thus changing their offspring sex ratio (Darrouzet et al., 2007, 2008). They also can discriminate unparasitized hosts from hosts parasitized by a different wasp species, *Dinarmus basalis*: this discriminative capacity seems to be due to chemical cues that probably originate from the Dufour's gland, and that were deposited by previous ovipositing females (Jaloux et al., 2005). After oviposition by *E. vuilleti* females, hydrocarbons also are found on seeds (Darrouzet, personal observation) and can be perceived by other conspecific females, which could, in turn, alter their responses. However, in this species, the nature and origin of cues perceived by conspecifics are still unknown.

This study determined whether cuticular hydrocarbons deposited artificially on seeds are perceived by *E. vuilleti* females, and whether these females change their oviposition accordingly. Specifically, (1) we tested whether the CHC profiles from males and females are different, and (2) we presented seeds labeled with CHCs from males or females to ovipositing females.

Methods and Materials

Rearing Conditions Bruchid *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae) and wasp *Eupelmus vuilleti* samples were taken from populations that have been established in the laboratory since 1997, and which originated from Burkina Faso (West Africa). They were mass-reared in a climate-controlled chamber under conditions close to those at their place of origin: 12 hr light at 33°C, 12 hr dark at 23°C and 70% r.h.

Preparation of Reproductive Patches A circle of six equidistant gelatine capsules, each containing one *C. maculatus* L_4 larva, was fixed in an arena (diam=8 cm, height=2.5 cm; henceforth called a reproductive patch, Fig. 1). Each bruchid fourth instar (L_4) was placed inside the capsule after being removed from its seed by dissection and selected by weight (9.12±0.20 mg). The gelatine capsules mimic the bruchid pupal chamber in the seed and enable oviposition by *E. vuilleti* females (Darrouzet et al., 2003). Two different areas within the patch were prepared to provide aggregates of hosts with different properties (Darrouzet et al., 2007). Three adjacent capsules were labeled with cuticular hydrocarbons (CHCs) from males or females (L capsules), and the other three adjacent capsules remained unlabeled (unL capsules). CHCs from the abdomen of parasitoids were transferred onto the cap of the capsules by rubbing the individuals for 10 sec. Each capsule was labeled by using a single individual that was put to sleep at 4°C before rubbing. Control patches were prepared with six unlabeled capsules.

Sample Preparation and Chromatographic Procedures Cuticular hydrocarbons were extracted in conical vials with 1 ml dichloromethane (Sigma Aldrich) from males (3 replicates of 10 males), females (6 replicates of 10 females), CHC labeled capsules (50 upper part of capsules; i.e., the part in contact with the ovipositing parasitoid females), and from unlabeled capsules (50). After mixing for 1 min, the insects or capsules were removed, and the extracts were dried to 2 µl and analyzed by gas chromatography. Samples first were analyzed using a Perkin-Elmer Autosystem XL GC (Perkin-Elmer, Wellesley, MA, USA) with a flame ionization detector (FID)

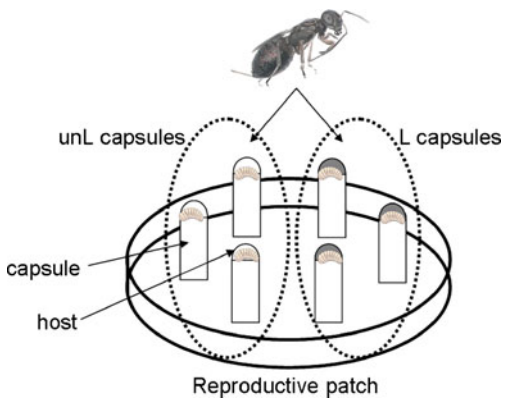

Fig. 1 Diagram of a reproductive patch

and Turbochrom software. Samples were injected into the GC-FID injector at 220°C in splitless mode and analyzed by using a BP1 capillary column (25 m, ID 0.32 mm), which was temperature programmed from 150°C (2 min) to 300°C (10 min) at 5°C/min.

The components then were identified by GC-MS analysis by using a Hewlett-Packard 5890 GC system coupled to a 5989A MS, controlled by HP ChemStation software. Pools of males (30 individuals) and females (60 individuals) were injected into the GC-MS injector using the procedure described above. Hydrocarbons were identified by their mass spectra corroborated by their ECL indices (for review see Lockey, 1988; Blomquist, 2010), using the M^+ of diagnostic ions and the $M\text{-}15^+$ when visible to confirm their methylbranch numbers (Nelson, 1993).

Oviposition of Mated Females To obtain mated females, 2-h-old virgin females were mated with a 24-h-old virgin male. Two patches containing unlabeled (unL) and labeled (L) capsules were offered daily (2 exposure periods of 4 hr) to each mated female (24 individuals, 13 females with L capsules labeled with CHCs from males, and 11 females with L capsules labeled with CHCs from females). Oviposition was performed in the climate-controlled chamber. At the end of each period, the positions of each egg laid in each capsule were noted. After each period, old capsules were replaced by new ones. To determine the sex of eggs laid by mated females, at the end of each period, each egg laid on a capsule was transferred to a different host and deposited in a different cell on a Plexiglas sheet closed by a Plexiglas cover-slide until the emergence of the adult parasitoids (Darrouzet et al., 2003). On emergence, the sex ratio was measured as the proportion of females emerging from the cells on the Plexiglas sheets (i.e., the secondary sex ratio).

Results were compared with mated (19) females ovipositing in the same experimental conditions in reproductive patches containing a circle of six unlabeled capsules.

Statistical Analyses Data were tested for normal distribution and homogeneity of variance (*Levene test*) and then were analyzed by using a *Mann-Whitney's U-test* for unpaired samples, a *Wilcoxon test* for paired samples, and a *Student's t-test*. The sex ratios of eggs laid in parasitism and superparasitism were compared by using a standard χ^2-*test*. All statistical analyses were performed with Statistica 6.0 software (Statsoft, Inc.).

Results

Analyses of CHCs from Males and Females The GC-FID analyses distinguished 38 peaks in females and 24 in males, ranging in chain length from 26 to 37 carbon atoms (Fig. 2). These peaks were found also on labeled capsules (results not shown). The GC-MS analyses identified 95 different molecules in the CHC profiles from females but only 64 in males (Table 1). CHCs profiles from males contained fewer methylated compounds than those from females (see Table 1).

Oviposition of Females Within Control Patches (Unlabeled Capsules) Comparison of two random areas of three adjacent capsules showed that females had the same oviposition activity for the number of parasitized hosts (*Student t test*: t=0.44, df=18, P=0.67) (Fig. 3a), eggs laid (*Student t test*: t=0.30, df=18, P=0.77) (Fig. 3b), and self-superparasitized hosts (*Wilcoxon test*: Z=1.32, P=0.19) (Fig. 3c).

Oviposition of Females Within Patches when Capsules L Were Labeled with Female CHCs Ovipositing females parasitized more hosts and laid more eggs in unL capsules than in L capsules (*Wilcoxon test*: Z=2.28, P=0.023 and Z=2.03, P=0.043, respectively) (Figs. 3a and b). However, they self-superparasitized the same number of hosts in unL and L capsules (*Student t test*: t=1.63, df=22, P=0.12) (Fig. 3c). These females had the same oviposition activity in unL capsules as females ovipositing in three random adjacent capsules in control patches (number of eggs laid, *Student t test*: t=0.023, df=29, P=0.98; number of parasitized hosts: t=0.72, df=27, P=0.47; number of self-superparasitized hosts: t=0.54, df=29, P=0.59) (Figs. 3b, a, and c, respectively). However, they laid fewer eggs in L capsules than females ovipositing in three random adjacent capsules in control patches (*Mann-Whitney U test*: U=47.5, P=0.05) (Fig. 3b), and parasitized fewer hosts (*Mann-Whitney U test*: U=49.5, P=0.05) (Fig. 3a). But they self-superparasitized the same number of hosts (*Student t test*: t=0.21, df=29, P=0.83) (Fig. 3c).

Oviposition of Females Within Patches When Capsules L Were Labeled with Male CHCs Females had the same oviposition activity in unL and L capsules (parasitized hosts: *Student t test*: t=0.16, df=12, P=0.88; eggs laid: t=0.12, df=12, P=0.90; self-superparasitized hosts: *Wilcoxon test*: Z=0.25, P=0.80) (Figs. 3a, b, and c, respectively). Females parasitized the same number of hosts in unL or L capsules as females ovipositing in three random adjacent capsules in control patches (unL capsules, *Student t test*: t=−1.85, df=30, P=0.074; L capsules: t=−1.88, df=30, P=0.069), laid the same number of eggs (unL capsules: t=−1.12, df=30, P=0.268; L capsules: t=−1.38, df=30, P=0.176), and self-superparasitized the same number of hosts (unL capsules, *Mann-Whitney U test*: U=168.5, P=0.074; L capsules: U=121.5, P=0.95).

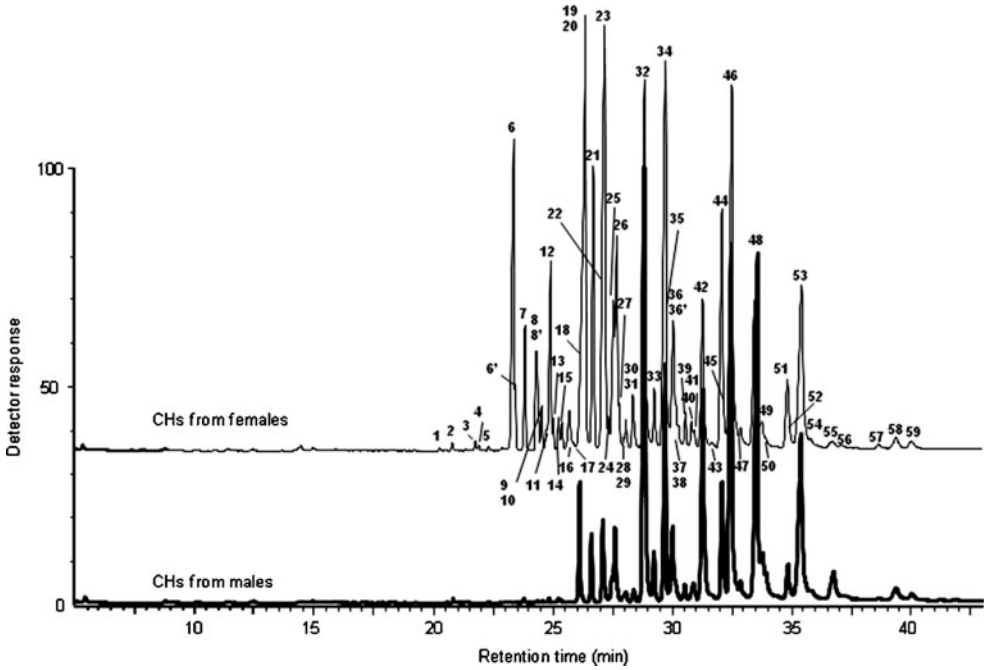

Fig. 2 GC-FID chromatogram of cuticular hydrocarbon profiles from *Eupelmus vuilleti* males and females

Oviposition of Females Between Separate Patches Females had the same oviposition behavior for patches containing L capsules labeled with male CHCs as females ovipositing in control patches (mean number ± SE of eggs laid: 33.23±1.42 vs. 29.84±1.58; *Student t test*: t=2.19, df=31, P=0.15; self-superparasitized hosts: 1.9±0.31 vs. 2.71±0.48; *Mann-Whitney U test*: U=160, P=0.157). When patches containing L capsules were labeled with female CHCs, females also had the same oviposition behavior in those patch as females in control patches (mean number ± SE of eggs laid: 25.83±1.17; *Student t test*: t=3.17, df=30, P=0.08; self-superparasitized hosts: 3.44±0.6; *Student t test*: t=0.86, df=25, P=0.36). However, females laid fewer eggs in the patch when L capsules were labeled with female rather than male CHCs (*Student t test*: t=15.90, df=24, P<0.001). Also, they self-superparasitized more hosts (*Student t test*: t=6.05, df=19, P=0.02).

Sex Ratio of Progeny When two capsule areas were compared within patches, we showed that females produced the same offspring sex ratio (proportion of females) in unL and L capsules, whatever the sexual origin of the CHCs deposited on L capsules (female CHCs: SR=0.68 in unL capsules vs. 0.62 in L capsules χ^2=1.07, df=1, P=0.30; male CHCs: SR=0.79 vs. 0.71, χ^2=2.38, df=1, P=0.12). Females within control patches also produced the same offspring sex ratio in two random areas (SR=0.65 vs. 0.62, χ^2=0.24, df=1, P=0.62).

Offspring sex ratios (L + unL capsules) were female biased, i.e., SR=0.66 for females ovipositing in patches containing L capsules labeled with female CHCs (χ^2=21.74, df=1, P< 0.001), 0.75 for females ovipositing in patches containing L capsules labeled with male CHCs (χ^2=68.00, df=1, P< 0.001), and 0.64 for females ovipositing in control patches (χ^2=28.31, df=1, P<0.001). However, females laid more daughters in patches containing L capsules labeled with male CHCs than in control patches or patches containing L capsules labeled with female CHCs (χ^2=9.52, df=1, P= 0.002 and χ^2=5.02, df=1, P=0.025, respectively) (Fig. 4).

Discussion

Eupelmus vuilleti females had different ovipositing activities depending on whether or not the seeds were labeled previously with CHCs. This adaptive oviposition shows that *E. vuilleti* females can detect these compounds present on seeds, and that their oviposition behavior is modified by the sexual origin of these CHCs. The presence of CHCs from other females modified egg distribution on hosts, whereas the male CHC label affected only the sex ratio of the progeny. These results demonstrate that parasitoid females can use (i) chemical residues present in the environment (such as CHCs deposited on seeds) and (ii) the sexual origin of such compounds to change their oviposition behavior.

In *E. vuilleti* species, males and females have different CHC profiles. CHCs from females contain certain mono- and dimethyl-branched compounds, which are absent from males. This shows a sexual dimorphism in the profiles. Although the functional significance of this sexual dimorphism in *E. vuilleti* remains to be tested, it may be significant, as is the case in some insects such as the cricket *Teleogryllus oceanicus* (Thomas and Simmons,

Table 1 Identification (using GC-MS) and relative amounts (% of total, using GC-FID) of the cuticular hydrocarbons from *Eupelmus vuilleti* males and females. A "/" separates carbon atom numbers when compounds have a methyl group that may be placed at either of these positions and cannot be discriminated using GC-MS

				Presence in			
				females		males	
Peak number	CHC identity	ECL	CN	mean	se	mean	se
1	4/2Me-C24	24.65	25	traces		–	
2	*n*-C25	25.00	25	traces		–	
3	4/2Me-C25	25.65	26	traces		–	
4	3Me-C25	25.70	26	traces		–	
5	*n*-C26	26.00	26	traces		–	
6 + 6′	4/2+3Me-C26 + C27:1	26.70	27	2.09	0.5	traces	
7	*n*-C27	27.00	27	1.83	0.23	0.37	0.14
8 + 8′ + 9	13+11+9+7Me-C27	27.35	28	0.57	0.07	–	
10	5Me-C27	27.49	28	0.31	0.07	traces	
11 + 12	4/2+3Me-C27	27.71	28	1.56	0.23	0.25	0.08
13	5,9diMe-C27	27.76	29	traces		–	
14	*n*-C28	28.00	28	0.52	0.03	0.22	0.05
15	3,7diMe-C27	28.06	29	0.21	0.07	0.15	0.07
16	14+13+12+11+10Me-C27	28.33	28	0.38	0.02	0.05	0.05
17	6Me-C28	28.41	29	0.04	0.03	–	
18	4/2Me-C28	28.69	29	3.53	0.4	3.1	0.12
19 + 20	C29:1 + 3Me-C28	28.75	29	1.84	0.23	traces	
21	*n*-C29	29.00	29	5.93	0.71	2.3	0.32
22	15+13+11Me-C29	29.33	30	6.92	0.69	2.47	0.59
23	9+7Me-C29	29.39	30	traces		–	
24	5Me-C29	29.46	30	0.32	0.04	0.18	0.04
25	4/2Me-C29 + 9,19+9,17+9,15+9,13diMe-C29	29.65	30 + 31	2.54	0.52	–	
26	3Me-C29	29.69	30	1.95	0.26	1.09	0.24
27	5,11+5,9diMe-C29	29.73	31	1.71	0.44	1.55	0.19
28 + 29	3,13+3,11+3,7diMe-C29	30.10	31	0.77	0.04	traces	
30 + 31	16+15+14+13+12Me-C30	30.32	31	0.98	0.08	traces	
32	4/2Me-C30	30.68	31	7.03	0.76	14.15	1.22
33	*n*-C31	31.00	31	1.43	0.09	1.38	0.22
34 + 35	15+13+11Me-C31	31.36	32	10.38	0.6	9.09	0.29
36 + 36′	11,21+11,19+11,17+11,15+9,13diMe-C31	31.53	33	3.1	0.37	2.07	0.44
37	7,13diMe-C31	31.59	33	traces		0.64	0.2
38	3Me-C31	31.65	32	0.38	0.04	–	
39	3,13diMe-C31	32.13	33	0.09	0.06	traces	
40	16+15+14+13+12+11Me-C32	32.32	33	0.58	0.02	traces	
41	C33:2	32.38	33	0.46	0.1	0.23	0.07
42	11,17+11,15diMe-C32+4Me-C32	32.72	34 + 33	4.19	0.31	5.96	0.85
43	*n*-C33	33.00	33	0.42	0.19	1.08	0.3
44 + 45	17+15+13+11MeC33	33.33	34	7.62	0.7	5.45	0.63
46	11,15diMe-C33	33.67	35	14.99	0.58	15.53	0.93
47	diMe-C33	34.16	35	traces		–	
48 + 49 + 50	C35:2+11,17diMe-C34+C35:1	34.4–34.6	35 + 36	4.18	0.69	22.05	2.68
51 + 52	17+15+13+11Me-C35	35.36	36	2.99	0.43	2.33	0.3
53	13,17+11,17diMe-C35	35.70	37	8.2	0.79	8.31	0.69
54	C37:2		37	Not	quantified		

Table 1 (continued)

Peak number	CHC identity	ECL	CN	Presence in females mean	se	males mean	se
55	C37:1		37				
56	11,17diMe-C36		38				
57	19+17+15+13+11Me-C37		38				
58	13,17+11,17diMe-C37		39				

ECL equivalent chain length, *CN* carbon number

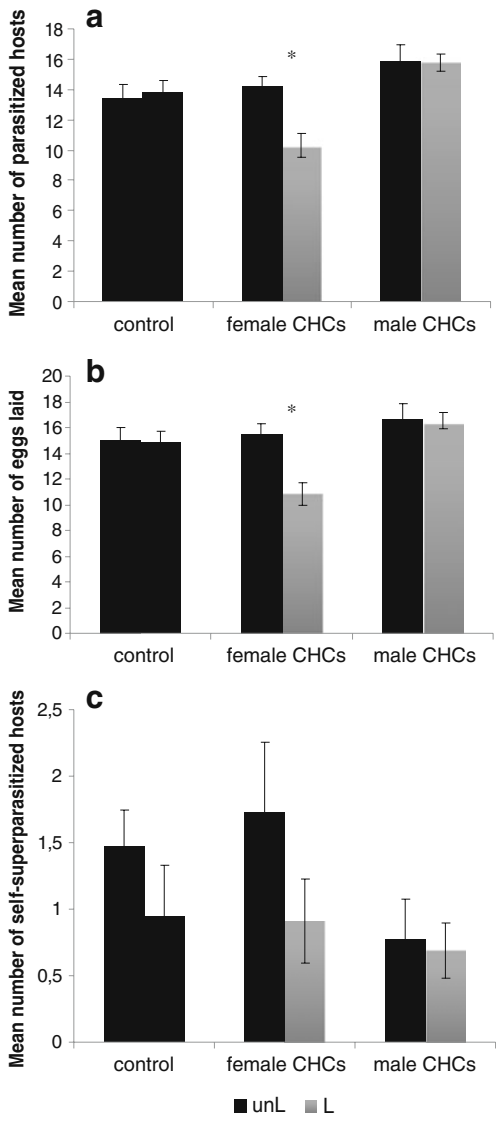

Fig. 3 Mean number and standard error of parasitized hosts (**a**), eggs laid (**b**) and self-superparasitized hosts (**c**) by *Eupelmus vuilleti* females ovipositing in unlabeled (unL) or labeled (L) capsules. *$P<0.05$

2008) or various species of Drosophila (Cobb and Jallon, 1990). If the chemical composition of CHCs is used as a contact pheromonal signal for mate choice, sexual selection may drive differentiation of these compounds. Various studies of several species of insects have demonstrated such differentiation (Howard and Blomquist, 2005; see appendix in Thomas and Simmons, 2008; Ferveur and Cobb, 2010). Particular compounds produced by only one sex can stimulate the sexual behavior of the other, or a specific sex ratio among hydrocarbons that are present in the two genders can influence mate choice (Ginzel et al., 2003). It also has been shown that some selected traits related to mate choice need maturation delay to be effective. This delay also may involve CHC profiles, as the differences among these compounds are more marked between sexes when insects are older (Chenoweth and Blows, 2005). The CHCs profiles of *E. vuilleti* were not analyzed according to age in this study. This trait may not be of great importance in this species, however, as adults are sexually mature when they emerge (Darrouzet, personal observation).

Many insects deposit chemical residues on different substrates, and these traces can provide intraspecific and/or

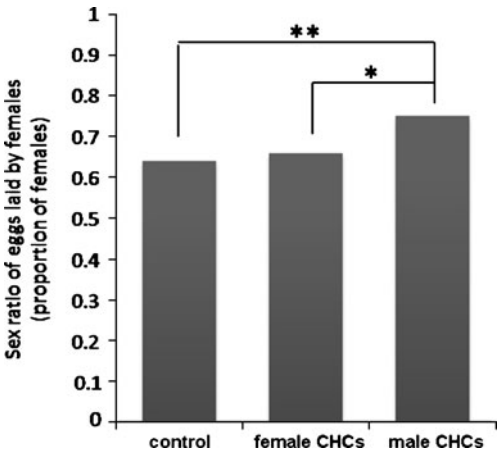

Fig. 4 Sex ratio of eggs laid by *Eupelmus vuilleti* females confronted with only unlabeled capsules (control), capsules labeled by female CHCs or male CHCs. *$P<0.05$; **$P<0.01$

interspecific cues that modify the behavior of receiving individuals. Marking pheromones are used by many parasitoid species, thus avoiding the laying of their eggs in hosts that have already been parasitized and that are less useful for the larval development of their offspring (Godfray, 1994; Nufio and Papaj, 2001). In a previous study, Darrouzet et al. (2007) showed that *E. vuilleti* females discriminated parasitized from unparasitized hosts. Under the same experimental conditions, the parasitized status of hosts was mimicked by labeling seeds containing unparasitized hosts with female CHCs. Females showed the same differences in oviposition strategy (in the location of eggs laid) when they were in the presence of either unlabeled or female CHC labeled seeds (in this study) as in the presence of both unparasitized and parasitized hosts (see Darrouzet et al., 2007). They laid more eggs on unparasitized hosts (unlabeled seeds in this study) than on parasitized hosts (labeled seeds). This mimicry was not obtained with seeds labeled with male CHCs. Hydrocarbons, thus, may be the chemical information used by ovipositing females to discriminate unparasitized from parasitized hosts. Jaloux et al. (2005) observed that *E. vuilleti* females used such compounds, deposited by *Dinarmus basalis* females after laying eggs, to superparasitize their hosts. In this interspecific competition, *E. vuilleti* females laid more eggs in superparasitism than in parasitism. The glandular secretion deposited by *D. basalis* females originates from the Dufours' gland.

Cuticular and Dufour's gland secretions may result in identical activity because of the similarity in hydrocarbon composition. However, there are differences in the relative concentrations between cuticular and Dufour's gland hydrocarbons, as is the case in *E. vuilleti* (Darrouzet, personal observation). This similarity seems to be a widespread phenomenon in insects as described, for example, in bumblebees (Oldham et al., 1994), polistes wasps (Dani et al., 1996), honeybees (*Apis mellifera*: Gozansky et al., 1997), and some parasitoid wasps (*Cephalonomia tarsalis*, *C. waterstoni*, *Anisopteromalus calandrae* and *Pteromalus cerealellae*: Howard and Baker, 2003; *Dinarmus basalis*: Jaloux et al., 2005).

Eupelmus vuilleti females drill the seeds with their ovipositor and oviposit on the bruchid larvae *C. maculatus,* which develop in seeds of a legume, the cowpea *Vigna unguiculata* (Walp.). The bruchid and its parasitoid emerge in granaries after the cowpea seeds have been harvested and reproduce in this storage environment. Several generations of *C. maculatus* and *E. vuilleti* develop in granaries throughout the dry season. *Eupelmus vuilleti*, therefore, lives in an environment where several types of seed are stored. Under these conditions, females exploring the surface of the seeds looking for bruchid hosts to oviposite, or males searching for females with whom to copulate, could touch seeds and thus deposit CHCs because of crowding. These chemical residues could then be perceived and used by conspecifics to identify the gender of individuals. The perception of female CHCs on a seed that contains a host could inform a conspecific female that the host may already be parasitized and the perception of male CHCs could indicate that the host is unparasitized. The lengths of the CHCs identified in this study range from 24 to 37 carbon atoms, which is similar to that found in other parasitoid species (Howard and Baker, 2003). Given their long chains, these compounds mainly are non-volatile and are, therefore, probably detectable for some time after being deposited. As female CHCs prevent other females from laying their eggs on the same hosts, they probably are perceptible for at least a period that will prevent the first individual laid from being affected adversely as a result of a second oviposition (Roitberg and Mangel, 1988). It is not known how long such a marker can last. Moreover, the way in which *E. vuilleti* deposits CHCs in a seed store still has to be determined. However, chemical residues may remain on a substrate as described in previous studies, and thus indirectly associated with the host (Borges et al., 2003; Conti et al., 2004). The footprint may consist of non-volatile lipids, secreted by specialized glands, identical to those found on the cuticle (Nakashima et al., 2004), or CHCs present in the wax layer of the cuticle (Colazza et al., 2007). The egg parasitoid species *Trissolcus basalis* uses the CHCs on the cuticle of their hosts (the stink bug *Nezara viridula*) as contact kairomones to discriminate gender (Colazza et al., 2007). Additionally, Müller and Riederer (2005) showed that compounds from a phytophagous insect could be adsorbed on a plant surface and be used as host-finding kairomones for parasitoids.

In Hymenoptera parasitoids, females have a haplodiploid genetic system for allocating the sex of their progeny, where males develop from unfertilized eggs and females from fertilized eggs (Godfray, 1994). This sex allocation can be modulated by various factors such as the number of conspecific females in the reproductive patch (Hamilton, 1967) or the presence of already parasitized hosts (Darrouzet et al., 2008). Although a single female is expected to lay the minimum number of sons able to mate with all their sisters, when the competition level increases (with more females or more already parasitized hosts), the same female would benefit by producing a higher proportion of sons in order to mate with both her own daughters and those of other females. This is predicted by Hamilton's local mate competition (LMC) theory (Hamilton, 1967). Moreover, additional information also could help the female to adjust her progeny allocation for already parasitized hosts, such as the sex of the juvenile in or on the host (Lebreton et al., 2010), its age (Lebreton et al., 2009), or its species (Gauthier et al., 1999). *Eupelmus vuilleti* females can alter their offspring sex ratio as predicted by the LMC theory. The

sex ratio changes in response to the presence of conspecific females and/or already parasitized hosts (Darrouzet et al., 2003, 2007, 2008).

Little attention has been paid to the role of males in influencing sex allocation, although males can influence female sex allocation in a number of ways in haplodiploid organisms (Henter, 2004). Males may change their fertilization ability by producing sperm unable to fertilize eggs: mated females thus would produce more unfertilized eggs, i.e., sons. A second possibility is that males could transfer sperm to females during mating, but that incompatibility between paternal and maternal genomes could lead to the death of daughters. Males also may actively search for mates and then influence female sex allocation because they only transfer their genes to the next generation when the females lay fertilized eggs, i.e., daughters.

Finally, according to the constrained model (Godfray, 1990), when the proportion of virgin females increases in the population, mated females should produce a higher proportion of daughters, thus compensating for the number of sons produced by these "constrained" females. However, mated females would benefit by detecting the proportion of virgin females present. This estimation could be performed on the basis of the encounter rate of courting males in the environment (Ode et al., 1997). Under our experimental conditions, there were no males. However, females produced more daughters when they perceived male odor on seeds. This suggests that males may influence female sex allocation by depositing their odor in the environment.

In summary, this study supports the idea that, in *E. vuilleti*, females adjust their oviposition behavior according to the sexual origin of cuticular traces left by conspecifics on seeds. When they detect traces of other females, they lay fewer eggs on these seeds but when they perceive traces of males, they produce more daughters. This result highlights the importance of the precise nature of the chemicals present in the environment on strategy adjustments.

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