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Female-derived sex pheromone mediates courtship behaviour in the parasitoid *Lariophagus distinguendus*

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Abstract

Courtship behaviour of the polyphagous ectoparasitoid *Lariophagus distinguendus* Först. (Hymenoptera, Pteromalidae) was studied. The initial behavioural element of the courtship sequence is a characteristic wing fanning shown by the males at encounters with females. Wing fanning and arrestment of the males was elicited by paper discs treated with dichloromethane extracts from virgin females showing the existence of a female-derived sex pheromone. The pheromone is only active at a distance between 0–5 mm suggesting low volatility of the active compound(s). Females mate only once, and the pheromone is still perceived by males at least 5 days after female mating. Males exposed to dissected female heads, thoraces and abdomens, showed wing fanning towards all segments. However, extracts from female abdomens were significantly more active than those from heads or thoraces suggesting the pheromone source to be located in the abdomen.

Introduction

For successful reproduction, parasitoids have to be able to locate their mates and hosts. Whereas various aspects of host finding in parasitoids have been studied, there are few studies dealing with behaviour and cues involved in mate finding and courtship behaviour. Available studies revealed that, as in many other insects, mate finding in parasitic Hymenoptera is mediated by female-derived sex pheromones (reviewed in Quicke, 1997). Sex pheromones can have two main functions in parasitoids. First, they enable mate finding by attracting males over long distances to the females and second, they mediate close-range courtship behaviour. In pteromalids, courtship sequences have been shown to be elicited by female-derived sex pheromones in some species (King et al., 1969; van den Assem & Povel, 1973; Yoshida, 1978). However, the chemicals involved have not yet been identified and even many basic aspects of this pheromonal communication are still unclear, including volatility, range of activity, timing of production, and production site of the involved chemicals. Knowledge of these issues is essential for potential efforts on the identification of these pheromones. *Lariophagus distinguendus* Först. (Hymenoptera, Pteromalidae) is a polyphagous ectoparasitoid parasitising larvae and prepupae of several stored product infesting beetles (Steidle & Schöller, 1997). Larvae of *L. distinguendus* develop within infested seeds and feed on the paralysed host larvae. Males of *L. distinguendus* that encounter an unmated female, perform a characteristic courtship behaviour that has been described in detail (Hase, 1919; van den Assem, 1970) but has not yet been analysed quantitatively. Furthermore, it is still unclear if female sex pheromones, male sex pheromones, or both are involved. The present paper is part of a study that investigates the chemicals mediating courtship behaviour in pteromalid parasitoids. First, we analysed courtship behaviour in *L. distinguendus* quantitatively by constructing a transition matrix. Then we investigated if females of *L. distinguendus* produce a sex pheromone eliciting male courtship behaviour. Finally we investigated the range of activity, the possible production site, and the in-

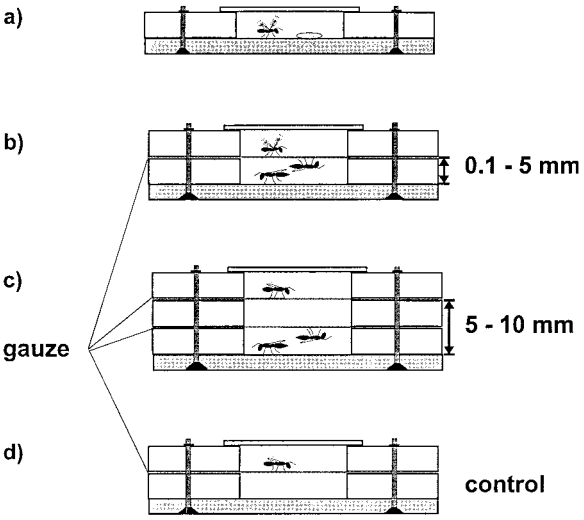

Figure 1. Bioassay chamber used for video observations and for testing paper discs and female tagmata (a) and chambers used to investigate the range of activity of the pheromone (b–d).

fluence of previous female mating on male courtship behaviour.

Materials and methods

Insects. *Sitophilus granarius* L. (Coleoptera, Curculionidae) was reared on 250 g wheat grains of about 14% moisture content in 2 l glass jars at a constant temperature of 25 °C and 75% r.h.. To obtain weevil larvae of known age, 30 ml of adult weevils were kept on 250 g grain and removed 7 days later. To rear *L. distinguendus*, newly emerged parasitoids were placed in Petri dishes with grains containing 15- to 30-day old weevil larvae. The next generation emerged 18–20 days later from the grains. Unmated females were collected immediately after hatching and kept isolated from males until used for extraction or behavioural observations. Males (age 0–24 h) were collected daily in the morning from the Petri dishes and kept individually in 1.5 ml polyethylene tubes (Sarstedt, Nürnbrecht, Germany) until used in the experiments.

Bioassays: Quantitative analysis of courtship behaviour. For observations, bioassay chambers were used consisting of 2 sheets of acrylic glass (60 × 20 × 5 mm) connected by screws (Figure 1a). A hole (diameter 10 mm) was drilled in the upper sheet and was covered with a glass plate. The resulting cavity served as observation chamber. In order to analyse the mating behaviour of *L. distinguendus* quantitatively, mating of 25 couples was video taped (Hitachi KP-C551 CCD camera, Rodenstock 50 mm Roganar lens 1: 2.8, and NV-HS1000 EGC S-VHS recorder, Panasonic TC-1470Y monitor) and analysed using the behavioural observation software The Observer 3.0 (Noldus, Wageningen, The Netherlands). Preliminary observations of the male courtship sequence led to the definition of nine behavioural key elements. Thus, the following behavioural steps were distinguished: (1) male is running; (2) male shows wing fanning; (3) male shows wing fanning and raises abdomen simultaneously; (4) male mounts female; (5) male starts antennal stroking; (6) female lowers its head and folds down its antennae; (7) female exposes genitalia; (8) male takes up copulation position; (9) copulation. The resulting data files were analysed using the lag sequential analysis option of the software package. The resulting frequency data were transferred to a two-way contingency table and tested for independence using a collection of public domain software (Java Applets for the analysis of behavioural data, available at http://caspar.bgsu.edu/ software/Java/1Contingency.html). Expected frequencies were calculated for each cell under the null scenario. If the difference between observed and expected frequencies was large, then a link (i.e., lack of independence) between the 2 behavioural elements was concluded. Chi-square or G-tests were used to determine whether these differences were large enough to warrant rejection of the null hypothesis. Freeman-Tukey deviates were calculated allowing a cell-wise examination of the table to identify which cell values were particularly ‘large’ or ‘small’ compared to the null hypothesis, i.e. which behaviours are likely/unlikely to be followed by which other behaviours.

Female-derived sex pheromone

Experiment 1: Presence of an extractable pheromone. Three unmated females or males were homogenised in 25 μl dichloromethane and treated for 15 min in an ultrasonic bath. The resulting extracts were applied in three batches on a filter paper disc (diameter 5 mm) and the solvent was allowed to evaporate for 5 min. The discs were transferred to the observation chamber containing one male and its behaviour was observed for 5 min under a stereo microscope. The number of wing fannings and the time the male spent on the paper disc were recorded with the computer software The Observer 3.0. In control experiments discs were treated only with the solvent. Twenty repli-

cates were done for each type of extract. For each experiment new paper discs and new males were used. Males that did not respond to the paper discs were transferred to another bioassay chamber containing an unmated female. Males that did not respond to unmated females in this control experiment were assumed to be unmotivated and were excluded from the results. Statistical analysis was done by use of the Kruskall–Wallis H-test. Means were separated by the Bonferroni-corrected Mann–Whitney U-test (Sachs, 1992). Analyses were done using Statistica 4.5 scientific software (StatSoft Inc. Hamburg, Germany).

Experiment 2: Range of activity. For this experiment the volume of the bioassay chamber was enlarged by adding successive sheets of acrylic glass separated with fine polyamide gauze (mesh 120 μm, thickness 1 mm) (Figure 1b–d). By this means, it was possible to expose the males to the females at defined distances, allowing them to respond to volatile chemicals but preventing visual and physical contact. Five unmated females were presented to a male ($n = 20$) at distances of 0.1–5 mm or 5–10 mm. Male behaviour in empty bioassay chambers was observed in control experiments. The number of wing fannings and the time males spent on the gauze were recorded. The other experimental details were as described in experiment 1.

Experiment 3: Production site. In order to locate the production site of the pheromone, ten unmated females were dissected into head, thorax, and abdomen. The segments of each female were presented one after another to 1 single male in a bioassay chamber (Figure 1a) and the number of fannings within an observation time of 2 min was recorded. Ten males were tested on each female, i.e. 100 males per segment type. Additionally, segments of females were homogenised and extracted separately with dichloromethane, and extracts were used to prepare pheromone discs as described in experiment 1. Again, number of fannings within an observation period of 2 min was recorded ($n = 25$). Male responses were analysed by one way ANOVA. Means were separated by the Scheffé-test for multiple comparisons using Statistica 4.5 scientific software.

Experiment 4: Influence of previous mating. The influence of the female virginity on male courtship behaviour was investigated in this experiment. For this purpose unmated females ($n = 25$) were allowed to mate in an observation chamber under controlled conditions using a stereo microscope. After mating had occurred, females were kept individually without hosts in plastic tubes with perforated lids containing pieces of moist filter paper. The behaviour of males (age 0–24 h) towards these mated females was observed after 1, 2, and 5 days. Four behavioural key elements of the male courtship behaviour, i.e., (1) wing fanning/abdomen raising, (2) mounting, (3) antennal stroking, and (4) copulation were recorded for each time. In control experiments male courtship behaviour towards unmated females of the same age ($n = 25$ for each age) was studied. The response of the males was compared by the Bonferroni corrected χ^2-test (Sachs, 1992).

Results

Quantitative analysis of courtship behaviour. The results of the quantitative analysis of the courtship behaviour are summarised in Table 1. Statistical analysis revealed that behavioural elements were not distributed randomly ($\chi^2 = 1466$, df $= 64$, P< 0.001). In our analysis 'running' was used as first behavioural element. Male courtship behaviour started with the initial key element 'wing fanning' normally shown immediately at encounters with unmated females (Figure 2). This behaviour was often accompanied by raising of the abdomen. Mounting occurred on average 57 ± 61 s after the first encounter. Immediately after mounting, males started to move their antennae in a characteristic manner performing cyclic stroking movements along the female antennae. In response to the stroking behaviour, which lasted on average 28 ± 32 s, females lowered their head, folded down their antennae and almost simultaneously exposed their genitalia. Immediately following this behaviour, males took up the copulating position and mating occurred (mean duration 18 ± 18 s). In 19 out of 25 observations, mating occurred within the observation time of 5 min. The courtship behaviour from the first encounter until copulation lasted 98 ± 79 s on average. After copulation, males either started running and/or entered a sequence of post-copulatory behaviour (Table 2). Because courtship behaviour by definition cannot be preceded by post-copulatory behaviour, in Table 1 elements of post-copulatory behaviour were listed in the last row, but were not included in the χ^2-analysis for Table 2. Post-copulatory behaviour, including the elements running and the preceding copulation, is

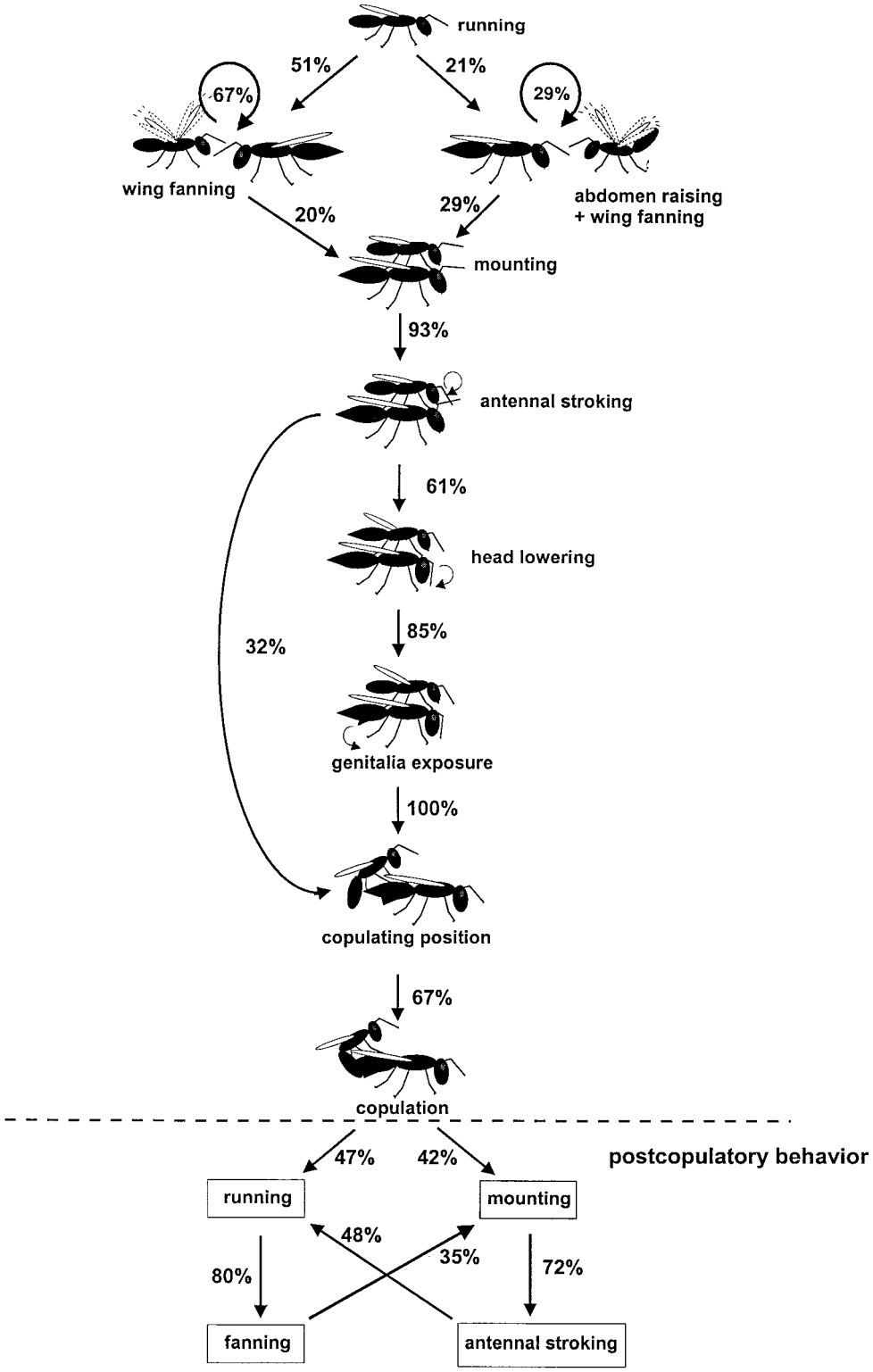

Figure 2. Flow diagram of courtship behaviour sequence of *L. distinguendus*.

Table 1. Transition probabilities between particular behavioural elements of the courtship sequence in *L. distinguendus*. Preceding behaviours are listed in the first column, following behavioural elements are arranged horizontally. Cells representing transitions which have been found to be likely (+)/unlikely (−) during statistical analysis are marked in bold (pc = post-copulatory)

	Total freqency	Running	Wing fanning	Abdomen raising	Mounting	Antennal stroking	Head lowering	Genitalia exposure	Copulating position	Copulation	Pc-behaviour
Running	38	**0.00** −	**0.51** +	**0.21** +	**0.00** −	**0.00** −	**0.00** −	**0.00** −	**0.00** −	**0.00** −	
Wing fanning	135	0.07	**0.67** +	0.07	**0.20** +	**0.00** −	**0.00** −	**0.00** −	**0.00** −	**0.00** −	
Abdomen raising	28	**0.00** −	0.43	**0.29** +	**0.29** +	**0.00** −	0.00	0.00	**0.00** −	0.00	
Mounting	42	0.05	**0.00** −	**0.00** −	**0.00** −	**0.93** +	0.02	**0.00** −	**0.00** −	**0.00** −	
Antennal Stroking	32	0.06	**0.00** −	**0.00** −	0.03	**0.00** −	**0.61** +	0.00	**0.32** +	**0.00** −	
Head lowering	20	0.00	**0.00** −	0.00	**0.00** −	**0.00** −	0.00	**0.85** +	0.15	0.00	
Genitalia exposure	17	0.00	**0.00** −	0.00	**0.00** −	**0.00** −	0.00	0.00	**1.00** +	0.00	
Copulating position	30	0.13	**0.00** −	**0.00** −	0.20	**0.00** −	**0.00** −	0.00	**0.00** −	**0.67** +	
Copulation	19	**0.47** +	**0.00** −	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53

presented in Table 2. Again, behavioural elements were not distributed randomly ($\chi^2 = 1466$, df = 25, P<0.001). Up to the onset of the actual copulation, post-copulatory behaviour consists of the same elements as courtship behaviour, ordered in the same way. After antennal stroking it ends and males continue with running. No additional matings occurred.

Female-derived sex-pheromone

Experiment 1: Presence of an extractable pheromone. Paper discs treated with three female equivalents of a dichloromethane extract elicited wing fanning behaviour (Figure 3a) and caused arrestment (Figure 3b) of the males. Control discs treated with male extracts or with solvent only were behaviourally inactive. Thus, male courtship behaviour is elicited by a female-derived sex pheromone.

Experiment 2: Range of activity. Males exposed to chemicals emitted by unmated females responded with courtship behaviour and arrestment when the females were presented at distances between 0.1 and 5 mm (Figure 4a-b). At a distance beyond 5 mm no wing fanning was observed and males did not stay longer on the gauze of the observation chamber than in the control experiment. Thus, the activity of the sex pheromone is restricted to the direct vicinity of the females, suggesting low volatility of the involved compound(s).

Experiment 3: Production site. Wing fanning of the males was observed in the presence of all types of female segments (Figure 5a). However, when thoraces or abdomens were presented, mean number of fannings was significantly higher compared with heads. Extracts from female abdomens elicited significantly stronger wing fanning responses in males than extracts from heads or thoraces (Figure 5b).

Experiment 4: Influence of previous mating. Courtship activity of males towards unmated females did not decrease within the observation period of 5 days (Figure 6a-d, black columns). On the other hand, males exhibited particular behavioural elements of the courtship sequence less frequently towards mated females than towards virgin females of comparable age. The degree of wing fanning/abdomen raising towards virgin females was significantly reduced 2 days after mating, but not 1 and 5 days after mating (Figure 6a). Mounting and antennal stroking was reduced 1, 2 and

Table 2. Transition probabilities between particular behavioural elements of the post-copulatory behaviour in *L. distinguendus*. For explanations see Table 1

	Total freqency	Running	Pc-fanning	Pc-abdomen raising	Pc-mounting	Pc-antennal stroking
Running	15	**0,00** −	**0,80** +	**0,13** +	0,07	**0,00** −
Copulation	19	**0,47** +	**0,056** −	0,00	**0,42** +	**0,00** −
Pc-fanning	37	0,27	0,27	0,00	**0,35** +	0,11
Pc-abdomen raising	2	0,00	0,50	0,00	0,50	0,00
Pc-mounting	25	**0,06** −	0,19	0,00	**0,00** −	**0,72** +
Pc-antennal stroking	21	**0,48** +	0,52	0,00	**0,00** −	**0,00** −

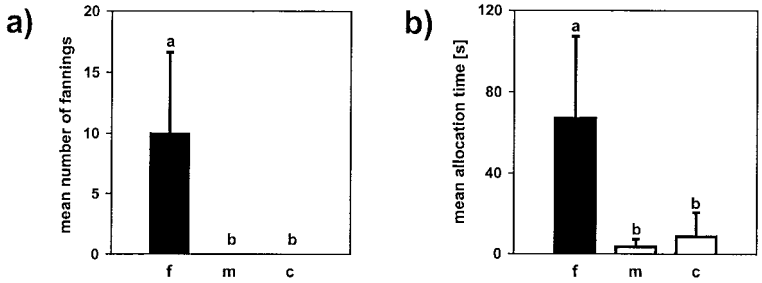

Figure 3. Response of males towards differently treated paper discs (treatments: f = female extract, m = male extract, c = solvent control). Mean number of fannings (± s.d.) (a) and mean allocation time (± s.d.) on the paper disc (b) during a 5 min observation period. Means with different lower case letters are significantly different at $P<0.0001$ (Kruskall–Wallis H-test/Bonferroni corrected U-test, $n = 20$).

5 days after mating (Figure 6b, c). However, several males showed the full courtship sequence even towards females that were mated 5 days ago. In no case were mated females observed to lower their heads, withdraw their antennae and expose their genitalia during antennal stroking of the males. Thus, no second matings were observed (Figure 6d).

Discussion

The results presented reveal that courtship behaviour in *L. distinguendus* consists of a sequence of behavioural elements, the main components being wing fanning, mounting and antennal stroking of the male, head lowering and genital exposure of the female and the actual copulation. This agrees with descriptions from Hase (1919) and van den Assem (1970) for the same species and the descriptions for several other species of Pteromalidae (Barras, 1960, 1976; van den Assem, 1989, 1996; van den Assem & Werren, 1994; Yoshida, 1978). Thus, courtship behaviour seems to be very similar within the Pteromalidae. The role of post-copulatory behaviour, that has been described for a number of parasitoids is not yet fully understood. The most common explanation for this behaviour is mate guarding, i.e. the prevention of other males from copulation with the same female (Gordh & DeBach, 1978; Quicke, 1997). For females of the pteromalid *Nasonia vitripennis* Walker it has been found that after post-copulatory behaviour the probability for second matings with other males is decreased (van den Assem & Feuth-de Bruijn, 1977). This might be true as well for *L. distinguendus*, as in our experiments, females were found to be receptive only once. However, van den Assem et al. (1989) provided evidence, that under certain (still unclear) circumstances second matings may occur in *L. distinguendus*.

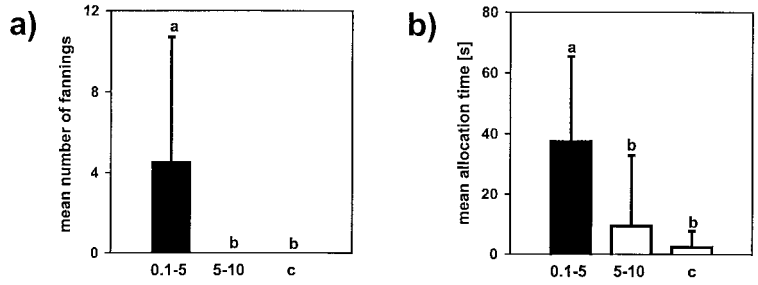

Figure 4. Response of males towards females presented at various distances (0.1–5 mm, 5–10 mm, c = control). Mean number of fannings (± s.d.) (a) and mean allocation time (± s.d.) on the gauze of the bioassay chamber (b) during a 5 min observation period. Means with different lower case letters are significantly different at $P<0.001$ (Kruskall-Wallis H-test/Bonferroni corrected U-test, $n = 20$).

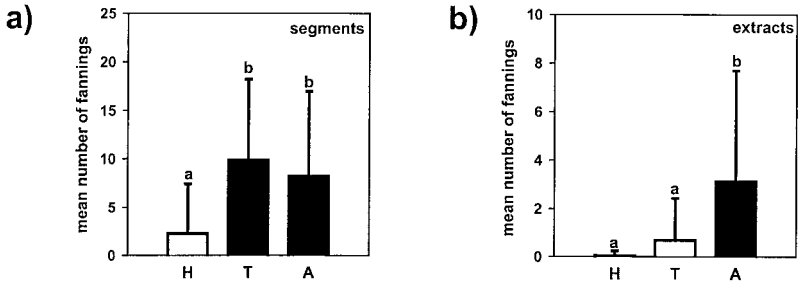

Figure 5. Mean number of fannings (± s.d.) shown by males towards different female segments (a) ($n = 10$) and paper discs treated with extracts from different female segments (b) ($n = 25$) during a 2 min observation period (H = head, T = thorax, A = abdomen). Means with different lower case letters are significantly different at $P<0.05$ (one way ANOVA/Scheffé-test).

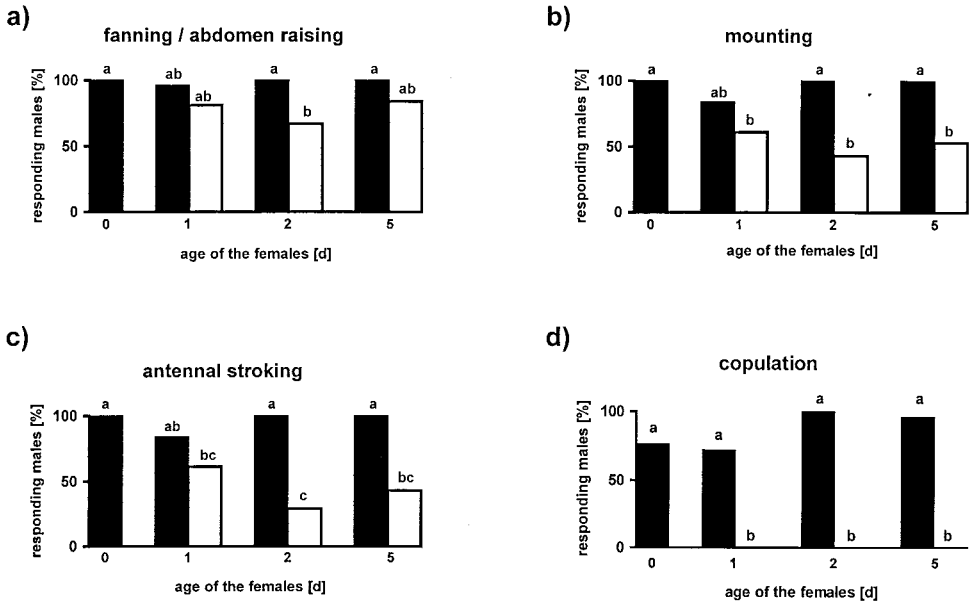

Figure 6. Behavioural key elements of the courtship sequence shown by males towards unmated females of different age (black columns) and towards mated females 1, 2 and 5 days after mating. (a) wing fanning/abdomen raising, (b) mounting, (c) antennal stroking, and (d) copulation. Different lower case letters show significant differences at $P<0.05$ (Bonferroni-corrected χ^2-test, $n = 25$).

The first visible behaviour of the courtship sequence in all species is wing fanning by the males. Because males of *L. distinguendus* exhibit this behaviour towards solvent extracts of females only, it must be induced by a female-produced sex pheromone and is not mediated by physical cues such as vibrations. The existence of a female-derived sex pheromone eliciting male wing fanning in Pteromalidae has been previously demonstrated in *N. vitripennis* (King et al., 1969) and *Anisopteromalus calandrae* Howard (Yoshida, 1978). However, no chemicals have been identified so far. Because *L. distinguendus* is a haplodiploid species, in which local mate competition occurs, females gain extra fitness from daughters and depend on mating to produce diploid female eggs. Thus, selection should be aimed at the production of female sex pheromones to stimulate male courtship (Godfray, 1994).

Our results demonstrate that in *L. distinguendus* the pheromone has a close-range arresting effect on males. Because the activity of the pheromone is restricted to the direct vicinity (0–5 mm) of the females, the involved compound(s) probably are only slightly volatile. This finding is supported by the fact that paper discs treated with female extracts were still active after several weeks (unpublished data). Thus, this sex pheromone only stimulates courtship behaviour and arrestment, but cannot be used for mate finding over long distances. In *L. distinguendus*, long-range orientation of the males is mediated by volatiles emitted by larval host faeces or host-associated mites living in the faeces (Ruther & Steidle, 2000).

Data on the end of the pheromone production are not unequivocal. The presence of the pheromone even 5 days after mating of the females as shown by the male wing fanning behaviour was demonstrated in experiment 4. However, a reduction of mounting and antennal stroking within 1 or 2 days after mating was observed. It is unclear, if this reduction of courtship activity is due to the females' behaviour or to a complete cessation or a temporary reduction of pheromone production. Possibly, residues of the low volatile pheromone remain on the female cuticle even after the pheromone production has ceased. These considerations are supported by the behaviour of the females. Females did not become receptive again, neither directly after first mating in the course of post-copulatory courtship activities of the males nor during courtship sequences 1, 2, or 5 days after the first mating. Therefore, it is clear that in our experiments only virgin females of *L. distinguendus* were receptive. As pheromone release in unreceptive females would involve costs in terms of energy and time due to fend off courting males, it is reasonable to assume that pheromones are released by receptive females only. It cannot be excluded though, that females become receptive again and restart pheromone production when sperm is depleted due to continuous ovipositions. This was not tested in our experiments since parasitoids had no access to hosts.

Experiments on the production site of the pheromone revealed that thoraces and abdomens elicited stronger responses in males than heads. However, pheromonal activity was present in all segments of virgin females. This might be due to pheromone glands that are widely distributed over the insect's cuticle. Alternatively, the fact that extracts from abdomens were superior to extracts from heads and thoraces may suggest that the pheromone source is located in the abdomen. From this site the pheromone may be distributed over the whole body surface by diffusion or cleaning behaviour. However, differences in activities of the segment extracts might as well be due to differing masses or surface areas of the extracted segments.

Descriptions of courtship behaviour in the present paper and in previous studies with other parasitoids suggest that a number of other cues, physical and chemical, may be involved in the courtship behaviour of pteromalids. The function of the male wing fanning remains unclear. As demonstrated in *L. distinguendus* and some other pteromalids, species-specific vibrations are produced during wing fanning that can be interpreted as male signals towards the females. However, it has been demonstrated that these vibrations are also produced after the wings have been removed, suggesting that only wing muscles are involved (van den Assem & Putters, 1980). For example honeybees are able to produce vibrations without moving the wings (Kirchner, 1997). An additional function for wing fanning can be assumed as this behaviour could help to spread a male pheromone. Such pheromone could mediate mate selection as reported for *N. vitripennis* (White & Grant, 1977). However, so far no obvious reaction of female parasitoids towards male fanning behaviour has been described in the literature nor observed by us.

The fact that males start to stroke female antennae with their own antennae after mounting was also observed in several other parasitic Hymenoptera (e.g., van den Assem, 1996; Barras, 1960, 1976; Bin et al., 1999; Isidoro et al., 1996). Morphological studies in-

vestigating both male and female antennae revealed not only the presence of several types of sensillae but also of glands. It has been suggested that during stroking behaviour males transfer sex pheromones on the female antennae (Isidoro & Bin, 1995; Isidoro et al., 1996). Furthermore, *N. vitripennis* males have been shown to release a chemical from their mouth parts during antennal stroking to stimulate receptiveness in females (van den Assem et al., 1980). In *L. distinguendus* antennal stroking of the males stops when females lower their heads and withdraw their antennae. As the females expose their genitalia simultaneously, and males take up the copulation position immediately after this step, head lowering of the female obviously signals receptiveness to the male. Van den Assem & Vernel (1979) were able to imitate this receptiveness signal (head lowering and withdrawing of the antennae) in unreceptive *N. vitripennis* females by mechanical manipulation. This induced courting males to take up the copulation position.

In conclusion, courtship behaviour in *L. distinguendus* and in other pteromalids consists of a sequence of behavioural elements that are mediated by chemical and physical cues. However, various aspects are still unclear demonstrating the lack of knowledge on mating and host finding in parasitoids. Future studies on the courtship behaviour of *L. distinguendus* will not only include identification of the female sex pheromone but will also investigate the possible role and identity of male-derived chemicals in this species.

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References

Assem, J. van den, 1970. Courtship and mating in *Lariophagus distinguendus* (Först.) Kurdj. (Hymenoptera, Pteromalidae). Netherlands Journal of Zoology 20: 329–352.

Assem, J. van den, 1989. Mating behaviour in parasitic wasps. In: J. Waage & D. Greathead (eds.), Insect Parasitoids. Academic Press, London, pp. 137–167.

Assem, J. van den, 1996. Mating behaviour. In: M. Jervis & N. Kidd (eds.), Insect Natural Enemies-Practical Approaches to their Study and Evaluation. Chapman & Hall, London, pp. 163–221.

Assem, J. van den & E. Feuth-De Bruijn, 1977. Second matings and the effect on the sex ratio of the offspring in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). Entomologia Experimentalis et Applicata 21: 23–28.

Assem, J. van den & G.D.E. Povel, 1973. Courtship behaviour of some *Muscadifurax* species (Hym.: Pteromalidae): a possible example of a recently evolved ethological isolating mechanism. Netherlands Journal of Zoology 23: 465–487.

Assem, J. van den & F.A. Putters, 1980. Patterns of sound produced by courting chalcidoid males and its biological significance. Entomologia Experimentalis et Applicata 27: 293–302.

Assem, J. van den & C. Vernel, 1979. Courtship behavior of *Nasonia vitripennis* (Hym.: Pteromalidae): Observations and experiments on male readiness to assume copulatory behaviour. Behaviour 118–135.

Assem, J. van den & J.H. Werren, 1994. A comparison of the courtship and mating behavior of three species of *Nasonia* (Hymenoptera: Pteromalidae). Journal of Insect Behavior 7: 53–66.

Assem, J. van den, F. Jachmann & P. Simbolotti, 1980. Courtship behavior of *Nasonia vitripennis* (Hym., Pteromalidae): Some qualitative, experimental evidence for the role of pheromones. Behaviour 75: 301–307.

Assem, J. van den, J.J.A. van Iersel & R.L. Los-Den Hartogh, 1989. Is being large more important for female than for male parasitic wasps? Behaviour 108: 160–195.

Barras, R., 1960. The courtship behaviour of *Mormoniella vitripennis* Walk. (Hymenoptera, Pteromalidae). Behaviour 15: 185–209.

Barras, R., 1976. Courtship behaviour in *Meraporus graminicola* (Hymenoptera) and other Pteromalidae. Physiological Entomology 1: 77–81.

Bin, F., F. Wäckers, R. Romani & N. Isidoro, 1999. Tyloids in *Pimpla turionellae* (L.) are release structures of male antennal glands involved in courtship behaviour (Hymenoptera: Ichneumonidae). International Journal of Insect Morphology 28: 61–68.

Godfray, H.C.J., 1994. Parasitoids. Princeton University Press, Chichester, West Sussex, 473 pp.

Gordh, G. & P. DeBach, 1978. Courtship behaviour in the *Aphytis lingnanesis* group, its potential usefulness in taxonomy, and a review of sexual behaviour in the parasitic Hymenoptera (Chalc.; Aphelinidae). Hilgardia 46: 37–75.

Hase, A., 1919. Beiträge zur morphologischen und biologischen Kenntnis der Schlupfwespe *Lariophagus distinguendus*. Sitzungsberichte der Gesellschaft Naturforschender Freunde Berlin: 402–432.

Isidoro, N. & F. Bin, 1995. Male antennal gland of *Amitus spiniferus* (Brethes) (Hymenoptera: Platygastridae), likely involved in courtship behavior. International Journal of Insect Morphology and Embryology 24: 365–373.

Isidoro, N., F. Bin, S. Colazza & S.B. Vinson, 1996. Morphology of antennal gustatory sensilla and glands in some parasitoid Hymenoptera with hypothesis on their role in sex and host recognition. Journal of Hymenoptera Research 5: 206–239.

King, P.E., R.R. Askew & C. Sanger, 1969. The detection of parasitized hosts by male of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) and some possible implications. Proceedings of the Royal Entomological Society of London, Ser. A44: 85–90.

Kirchner, W.H., 1997. Acoustical communication in social insects. In: M. Lehrer (ed.), Orientation and Communication in Arthropods. Birkhäuser, Basel, pp. 273–300.

Quicke, D.L.J., 1997. Parasitic Wasps. Chapman & Hall, London, 470 pp.

Ruther, J. & J.L.M. Steidle, 2000. Mites as matchmakers: Semiochemicals from host associated mites act as kairomones for both

sexes of the parasitoid *Lariophagus distinguendus*. Journal of Chemical Ecology 26: 1205–1217.

Sachs, L., 1992. Angewandte Statistik. Springer Verlag, Berlin, 7th edition, 846 pp.

Steidle, J.L.M. & M. Schoeller, 1997. Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). Journal of Insect Behavior 10: 331–342.

White, H.C. & B. Grant, 1977. Olfactory cues as a factor in frequency-dependent mate selection in *Mormoniella vitripennis*. Evolution 31: 829–835.

Yoshida, S., 1978. Behaviour of males in relation to the female sex pheromone in the parasitoid wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). Entomologia Experimentalis et Applicata 23: 152–162.