

Prey Specificity and the Importance of Close-Range Chemical Cues in Prey Recognition in the Digger Wasp, *Liris niger*

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Females of the digger wasp species Liris niger paralyzed and layed eggs on six of seven tested cricket species. L. niger females with unmanipulated antennae always rejected the cricket Gryllus bimaculatus, but those without antennal flagella paralyzed and layed eggs on it. Even L. niger females from maggots raised artificially on G. bimaculatus never paralyzed it. L. niger first orients toward a prey visually and then touches the prospective prey briefly with its antennal flagella. This contact determines about the suitability of the prey. The ability to differentiate between prey species required at least one partially unmanipulated antennal flagellum. The responsible sensilla were located on the upper surface of the antennal flagella. Chemical cues which make the cricket Acheta domesticus attractive to L. niger females could be transferred to G. bimaculatus.

KEY WORDS: predator-prey interaction; *Liris niger* (Sphecidae, Insecta); crickets (Gryllidae, Insecta); prey specificity; prey recognition.

INTRODUCTION

Female wasps of the family Sphecidae prey upon other insects or spiders for provisioning their nests with food for the offspring (Bohart and Menke, 1976; Rathmayer, 1978). First descriptions of such behavior are already known from

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the last century (Fabre, 1856), but detailed studies on the role of sensory systems involved in hunting behavior are very rare.

Prey specificity varies largely within different digger wasp species. There are highly specialized species like *Ampulex bantua* which hunt only one prey species (Gess, 1984) and generalist species like *Microbembex monodonta* which hunt insects belonging to different orders (Evans and West-Eberhard, 1970).

Females of the solitary palearctic digger wasp species *Liris niger* Fabricius 1775 (Menke and Bohart, 1979) hunt exclusively crickets as food for their offspring (Steiner, 1986). The female wasp follows a clearly defined chain of behavior. It first digs a burrow. Then, searching for a prey, the female runs very fast over the ground (Gnatzy and Kämper, 1990). If a female accepts a prey item, it climbs on the prey's back, although the cricket usually fights back vigorously (Hustert and Gnatzy, 1995) and paralyzes it (as a rule) with four stings (Steiner, 1986; Gnatzy and Otto, 1996). Then the wasp drags the paralyzed cricket to a burrow, glues an egg between the fore- and the middle legs of the prey, and closes the burrow. After hatching from the egg, the wasp larva feeds on the paralyzed cricket (Steiner, 1968; Roces and Gnatzy, 1997).

The prey specificity of *L. niger*, however, is not exactly known; earlier reports mention *Nemobius sylvestris* (Grandi, 1961) and *Acheta domesticus* (Steiner, 1965; Gnatzy and Heußlein, 1986) as prey. Our previous laboratory observations have shown that the cricket, *Gryllus bimaculatus*, which is common in the natural habitat of *L. niger*, is not accepted as a prey (Gnatzy *et al.*, 1990). To investigate the "prey specificity" of *L. niger*, we tested additional cricket species as well as bushcrickets and locusts. As this study does not address the question of prey specificity in *L. niger* in general, we use the term in quotation marks. We wanted to know mainly if there was variability in "prey specificity" between females and if there were other potential prey species which were rejected, apart from *G. bimaculatus*.

It is not known how the preference for certain prey species is determined in digger wasps. In other Hymenoptera, like some Ichneumonidae, preimaginal or imaginal conditioning on certain prey species was shown (Thorpe, 1938, 1939). Since our experiments (ablation of the antennal flagella) caused *L. niger* females to lay eggs on the "naturally" rejected cricket species *G. bimaculatus*, we were able to raise larvae on the "wrong" prey species. Thus we were able to test the prey preference of adult females raised on the "correct" or "wrong" prey species.

The rejection of certain potential prey species implies a sensory mechanism to differentiate between the "correct" and the "wrong" prey. In only a few Hymenoptera species are the sensory modalities responsible for prey recognition known (e.g., Tinbergen, 1932, 1935; Baerends, 1941; Arthur, 1971). Even less information is available on the possible sensilla types involved in these mech-

animals and on the characteristics of the adequate stimuli which allow recognition (for review, see Vinson, 1976; Steiner, 1984).

Here we have studied how *L. niger* females discriminate between prey and nonprey species. Using ablation experiments we have identified the sense organs responsible for prey recognition. Close-up video recordings helped to determine whether or not a contact with the potential prey is necessary. First attempts were made to characterize the chemical stimuli which are important for prey recognition in this digger wasp species.

MATERIALS AND METHODS

Animals

In all experiments mated *L. niger* females were used. They were raised in our laboratory throughout the year, without diapause, resulting in 10 generations/year compared to 1 generation/year in the field (Steiner, 1968). Mated *L. niger* females originally were captured in southern France near Orange. New females from the field were added to the culture each spring. The animals were kept in groups of several males and females in glass terraria (120 × 70 × 50 cm) with a sand layer, under an artificial 12/12-h L/D cycle. Feeding of the adult animals was ad libitum with honey and water. As a food supply for their offspring, we offered juvenile and adult crickets of the species *Acheta domesticus* (for details see Gnatzy and Heußlein, 1986). All experiments followed established principles of laboratory animal care and complied with the current German law of protection of animals.

Behavioral Studies

General Experimental Conditions. For the experiments the test animals were placed in glass terraria (25 × 50 × 30 cm) with a 10-cm sand layer on the bottom. In each terrarium, there was a removable sand-filled inset with preformed burrows to make control of egg laying easy (Fig. 1). Behavioral observations were made in the early afternoon, the animals' most active time period. Each experiment was conducted with a single, mated *L. niger* female. The motivation of *L. niger* females for prey capture, hereafter called the "hunting mood," was tested before and after the experiments by offering them an *A. domesticus*, which was removed when attacked. We did not use data from wasps that were not in the "hunting mood" after the experiments (< 1% of the experiments).

"Prey Specificity." These experiments included the following Orthoptera species, of which specimens of comparable size and weight (juveniles or adults) were offered to *L. niger* females: *Acheta domesticus*, *Acheta canariensis*, *Scap-*

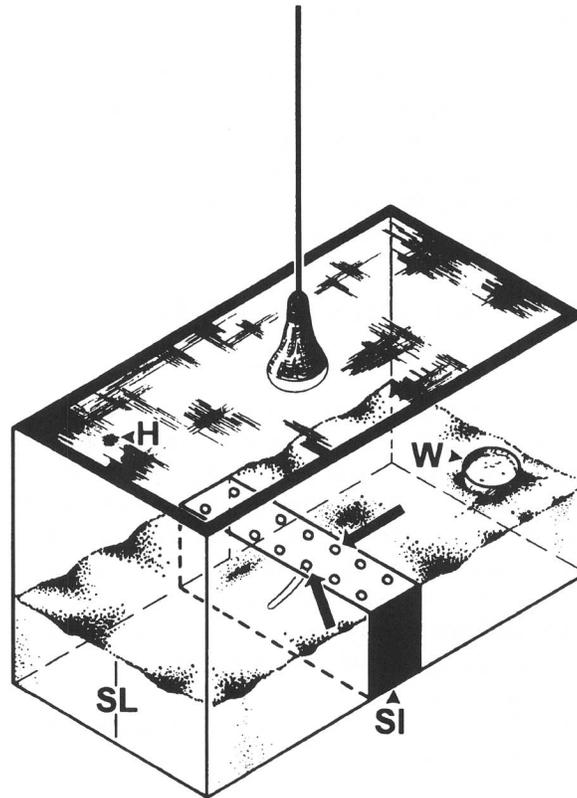


Fig. 1. Terrarium for behavioral observations. A glass terrarium filled with a 10-cm-thick sand layer (SL) was used for the experiments. A removable, sand-filled inset (SI) with preformed burrows (arrows) allowed easy control of egg laying. Water (W) and honey (H) were offered ad libitum.

sipedus sp., *Teleogryllus commodus*, *Gryllus campestris*, *Gryllus bimaculatus*, *Nemobius sylvestris*, *Locusta migratoria*, and *Tettigonia* sp. Five individuals of a potential prey species were offered to an *L. niger* female in each experiment. The behavior was observed during 1 h after the prey was introduced to the wasp. After 1 h all nonparalyzed prey items were removed from the terrarium, and after 1 or 2 days, we examined whether eggs had been laid on the paralyzed crickets. Thus we did not disturb the wasps directly after the experiments. Egg laying on at least one of the prey specimens was chosen as the criterion of acceptance of a prey species. Normally *L. niger* females paralyze only individuals of species on which they also lay eggs. In the first experiment, *L. niger*

females of unknown age, which had been in contact with *A. domesticus* as a prey species, were confronted with different potential prey species ($n = 4\text{--}30$ *L. niger* females per species). In the second experiment, as a control for host conditioning, freshly emerged *L. niger* females ($n = 11$), which had been raised on *A. domesticus*, were kept individually without prey contact for 25–40 days and were then confronted with *G. bimaculatus*.

Rearing Experiments. To find out if there is preimaginal conditioning for certain prey species, *L. niger* larvae were artificially reared on *G. bimaculatus*, partly through transferring young wasp larvae from *A. domesticus* to decapitated *G. bimaculatus* ($n = 88$) and partly by using eggs laid on *G. bimaculatus* directly by *L. niger* females without antennal flagella ($n = 79$). For comparison, the development of *L. niger* raised on *A. domesticus* was observed ($n = 267$). Since relatively few individuals of other cricket species were available, only anecdotal reports can be given on the survival of wasp larvae on these species. In all cases development of wasp eggs and larvae was observed on prey dug out of the sand. Larvae and cocoons were kept individually in sand-filled glass vials. The adult *L. niger* females raised on *G. bimaculatus* were confronted with *G. bimaculatus* for only 5–30 days ($n = 5$). After that they were tested in a choice experiment with both *G. bimaculatus* and *A. domesticus*.

Ablation Experiments. For localization of the sensilla responsible for prey recognition, we performed a series of ablation experiments. Before operation, female *L. niger* were anesthetized by chilling to $+4^{\circ}\text{C}$ on a Peltierelement (Peltron). Then either the eyes were covered with Leit-C ($n = 6$) or the labial and maxillary palps ($n = 5$) or the antennal flagella were severed ($n = 22$) or covered with wax ($n = 15$). In a number of females, parts of the antennae were covered with wax selectively: either the under ($n = 5$) or the upper ($n = 5$) side of the antennal flagellum. In six females the seven distal flagellar segments were covered with wax, in five females only six distal flagellar segments were covered, and in five females the whole flagella except distal most segment were covered with wax.

In the first set of experiments, five *A. domesticus* and five *G. bimaculatus* were offered either to unmanipulated females (control group) or to manipulated *L. niger* females. Their behavior was observed for 1 h and the following parameters were recorded: (i) attacks, i.e., active approach and antennal contact, but no paralysis; (ii) incomplete paralysis (at least one sting); (iii) complete paralysis (four stings); and (iv) egg laying.

In the experiments after complete ablation of the antennal flagella, the number of events within 1 h was analyzed for each behavioral parameter and a Mann–Whitney test was performed for each single parameter to compare (i) the behavior of unmanipulated versus ablated *L. niger* females toward the two cricket species and (ii) the behavior of unmanipulated versus ablated individuals toward each of the two cricket species. Mean values of the different experiments per-

formed with each single female were used for the statistical test. For the following experiments, "no attack" of *G. bimaculatus* was regarded as intact-like behavior, whereas paralysis of *G. bimaculatus* was regarded as antennectomized-like behavior. Some of the latter experiments (three females with covered eyes and covered palps, respectively) were performed with single females kept in jars of ca. 12-cm diameter, with a sand layer, differing from the general experimental protocol at a later time, when the other experimental setup was not available. In these cases, we first offered one *G. bimaculatus* for 10 min and then added one *A. domesticus* for another 10 min as prey. For all ablation experiments, each treated *L. niger* female was tested in at least three experiments on 3 days. The results for each single female did not differ in consecutive experiments.

Range of the Stimulus. To examine if *L. niger* females have to touch the potential prey with an antennal flagellum to accept or reject a prey, we first made video recordings of the hunting procedure ($n = 5$) with a CCD video camera (Panasonic Type F 15, recorder Panasonic NV-FS 100). Single video frames were printed out (color video printer, Panasonic NV-MP1). To locate frames we used a video recorder (Panasonic NV-FS 88 EG) equipped with a jog shuttle. In the second step we wrapped *A. domesticus* in gauze pieces of different mesh size and offered the wrapped crickets to *L. niger* females in the "hunting mood." The mesh size was either smaller (<0.2 mm) than the diameter of the antennal flagellum ($n = 8$), about the same (0.2 mm) ($n = 9$), or bigger (>0.2 mm) than the diameter of the antennal flagellum ($n = 15$).

Characterization of the Stimulus. To test whether or not there is an attractive or a repellent substance on the cuticle of *A. domesticus* and *G. bimaculatus*, respectively, which allows the wasps to discriminate between prey species, we did two series of experiments. In the first experiments, we tried to transfer behaviorally active substances from the cricket cuticle to pieces of filter paper. In each case one *A. domesticus* or *G. bimaculatus* was rubbed with pieces of filter paper (1 × 2 cm) for 1 min. In each experiment, one female *L. niger* was kept in a small Plexiglas box (20 × 5 × 10 cm) with a sand layer. Two filter papers, each on an insect pin, were offered to the wasp in different combinations: filter papers rubbed on *A. domesticus* were tested against an untreated control paper (11 females in 20 experiments), *G. bimaculatus* was tested against a control (13 females in 17 experiments), and both cricket species were tested against each other (10 females in 12 experiments). The antennal contact time of the wasp to each of the filter papers during a 3-min observation time was registered with the help of a video system. A Wilcoxon signed-rank test was performed on the data using mean values for each animal tested.

In the second series of experiments, we tried to transfer behaviorally active substances from one cricket species to the other. Single *G. bimaculatus* were kept together with 15 adult individuals of *A. domesticus* in a small jar for 30

min, so that close body contact was guaranteed, then offered as prey to examine if a potentially attractive substance can be transferred from *A. domesticus* to *G. bimaculatus*. Also, single *A. domesticus* were kept together with 15 *G. bimaculatus* for 30 min in the same way to see if a potential deterrent substance can be transferred from *G. bimaculatus* to *A. domesticus*. In both cases (*A. domesticus*, $n = 15$; *G. bimaculatus*, $n = 18$) the crickets treated in this way were offered to a single *L. niger* female for 10 min. If they were not attacked, one untreated *A. domesticus* was offered thereafter for control of motivation. Only experiments in which either cricket was paralyzed were taken into account.

Light and Electron Microscopy. The distribution pattern of the antennal flagellar sensilla of *L. niger* females was studied in the following way. Antennal flagella ($n = 4$) were boiled in 10% KOH solution; then single flagellar segments were cut into pieces and embedded in W15 (Zeiss). The antennal sensilla were examined and counted with a Zeiss light microscope.

Preparation for scanning electron microscopy was conventional (see Gnatzy *et al.*, 1987). The critical point-dried specimens were examined with an Hitachi SEM (Model S-4500) at an accelerating voltage of 2 kV (cold-field emission electron source) without any precoating.

RESULTS

Prey Specificity

The acceptance of the offered cricket species by *L. niger* females raised on *A. domesticus*, defined by paralyzing and laying an egg on at least one of the individuals of a respective species, can be divided into three categories (Fig. 2). The first category comprises the cricket species *Acheta domesticus*, *A. canariensis*, and *Nemobius sylvestris*, whose individuals were accepted as prey by all *L. niger* females tested. The second category refers to species whose individuals were accepted by only less than half of the females tested (30–45%); this category includes the cricket species *Scapsipedus* sp., *Gryllus campestris*, and *Teleogryllus commodus*. Individual *L. niger* females varied, however, concerning the acceptance of different species within the second category in that they accepted different numbers and combinations of prey species. For example, one female accepted *Scapsipedus* sp. and *T. commodus* as prey, whereas two other females accepted *Scapsipedus* sp. but rejected *T. commodus*. The species in the third category are those species whose individuals were rejected by all *L. niger* females tested; among these are the Mediterranean cricket *G. bimaculatus*, a migratory locust (*Locusta migratoria*), and a bush cricket (*Tettigonia* sp.). All “category 3” species were rejected, even when *L. niger* females were kept prior to the experiments individually without any prey for 25–40 days.

The larval development of *L. niger* raised on *G. bimaculatus* was normal

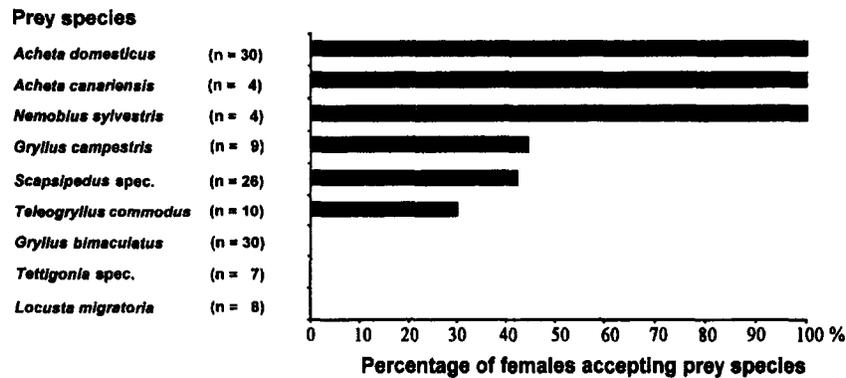


Fig. 2. Prey specificity in *Liris niger*. The percentage of *L. niger* females accepting one potential prey species varies between the different Orthoptera species. From all cricket species tested in the experiments, only *G. bimaculatus* was rejected by all females; number of females tested given in parentheses for each prey species.

in terms of development time and spinning of the cocoon, but fewer adult wasps hatched from the cocoons, compared to wasps raised on *A. domesticus*. Thirty-five percent of the eggs laid on *A. domesticus* ($n = 267$) developed into adult wasps under the given laboratory conditions (46 females, 47 males), but only 9% of the eggs laid on *G. bimaculatus* ($n = 79$) (3 females and 1 male hatched). If the larvae were transferred from *A. domesticus* to *G. bimaculatus* ($n = 88$), only 4.5% (2 females, 5 males) of the adult wasps hatched, probably due to the handling of the young larvae. Larvae developed on *A. canariensis*, *Nemobius sylvestris*, *G. campestris*, *Scapsipedus* sp., and *Teleogryllus commodus* up to the pupal stage, but only individuals raised on *G. campestris* and *Scapsipedus* sp. resulted in adult wasps hatching.

L. niger females raised on *G. bimaculatus* ($n = 5$) showed the same hunting behavior as those raised on *A. domesticus* in that they never accepted *G. bimaculatus* as prey, even if no other prey species was offered for as long as 30 days, but immediately attacked *A. domesticus* if available.

Prey Recognition

Unmanipulated females in the "hunting mood" were observed to run toward conspecific males or females, other small insects or moving objects from a distance of up to about 15 cm in the big rearing cages, but they never actually attacked any of these, whereas prey items (*A. domesticus*) were readily attacked. In choice experiments using unmanipulated *L. niger* females ($n = 34$), a significant difference was found between the behavior toward *A. domesticus* and *G. bimaculatus* in all four parameters analyzed (attacks, no paralysis following;

incomplete paralysis; complete paralysis; and egg laying) (U test, $P = 0.05$ for each parameter) (Fig. 3a). *G. bimaculatus* was briefly attacked in one experiment but never paralyzed.

L. niger females with covered eyes did not hunt a prey when observed for up to 42 days in the experimental terraria ($n = 4$), even though they were showing normal feeding and digging behavior. Two *L. niger* females with covered eyes kept in very small glass jars (12-cm diameter) together with the prey (one *A. domesticus* and one *G. bimaculatus*) paralyzed *A. domesticus* after 8 and 10 min, respectively, after a large number of random antennal contacts with both cricket species. *L. niger* females which had the labial and maxillary palps covered with wax ($n = 5$) never attacked *G. bimaculatus* during the observation time (1 h/10 min), but all females paralyzed *A. domesticus* within 5 to 10 min.

L. niger females with bilaterally severed antennal flagella ($n = 37$) changed their behavior clearly compared to unmanipulated animals (Fig. 3b) and accepted *G. bimaculatus* as prey. The behavior toward *G. bimaculatus* was, however, significantly changed in all four parameters recorded from unmanipulated to operated females (U test, $P = 0.05$ for each parameter) (Figs. 3a and b). Furthermore, one *L. niger* female with the flagella removed paralyzed a bush-cricket and some females even tried to paralyze locusts, but did not succeed, because the intersegmental membranes in locusts are too narrow to be penetrated by the sting of the wasps.

Female *L. niger* without antennal flagella performed significantly more attacks without success and more incomplete paralyzes than unmanipulated females (U test, $P = 0.05$ for both parameters), whereas the number of complete paralyzes and egg laying did not change significantly after amputation (Fig. 3). The whole process of hunting was disturbed after ablation of the antennal flagella. For example, a paralyzed prey was often left behind, while another prey was attacked. The otherwise so clearly defined "chain of behaviors" (see the Introduction) was disturbed in females without antennal flagella, resulting in uncoordinated behavior. Unilateral ablation of the flagellum had no effect on the prey recognition of *L. niger* females. Animals treated in this way displayed behavior similar to that of unmanipulated females.

In order to locate the sensilla involved in prey recognition, different parts of both antennal flagella were ablated in the following experiments (Fig. 4). *L. niger* females in which the seven distal flagellar segments were ablated behaved like animals without flagella, while those with only six distal flagellar segments ablated were able to discriminate between the two cricket species like unmanipulated animals. Four of five *L. niger* females in which the whole flagella except the distal most flagellar segments were covered with wax behaved like unmanipulated animals. One *L. niger* female in this group paralyzed *G. bimaculatus* after having paralyzed an *A. domesticus* in most experiments. *L. niger* females also rejected *G. bimaculatus* if only the underside of the flagella was

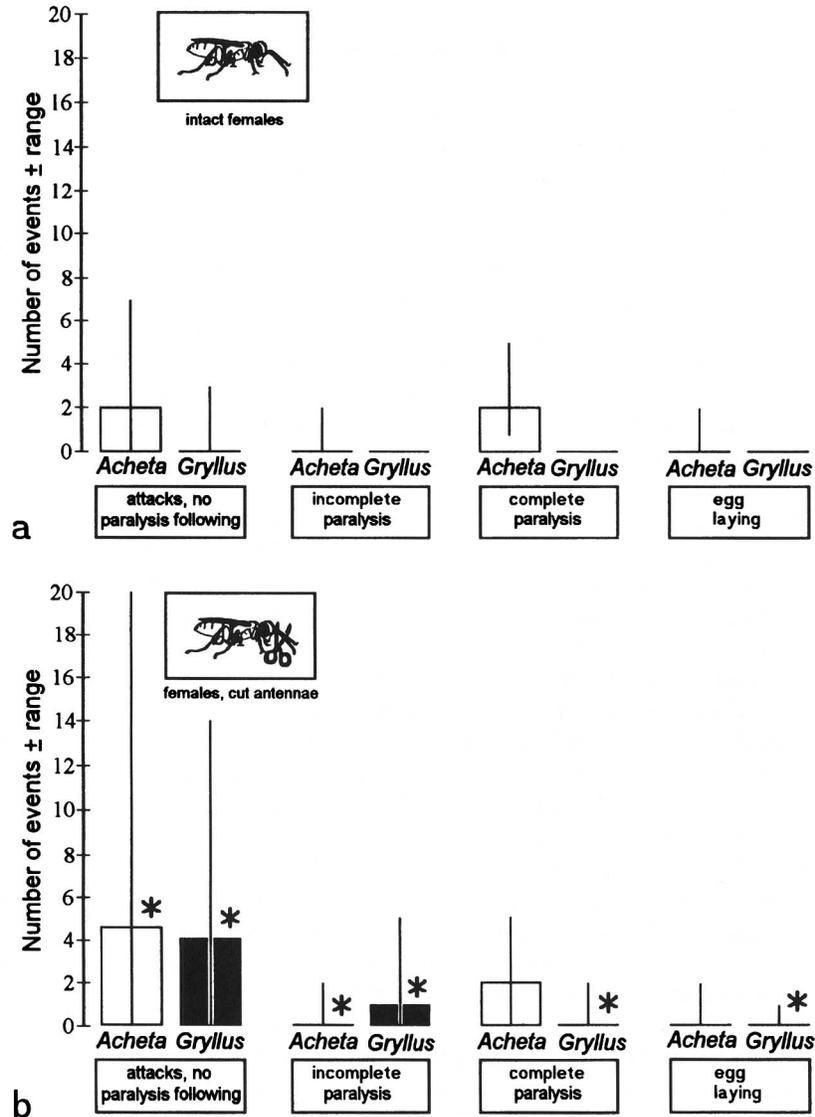


Fig. 3. Behavior of *L. niger* females versus two cricket species *A. domesticus* (*Acheta*) and *G. bimaculatus* (*Gryllus*), during 1 h of observation. (a) Behavior of unmanipulated *L. niger* females. (b) Behavior of *L. niger* females from which antennal flagella had been removed. Significantly different from a (asterisk). The median number of events (bars) and the range (lines) are shown for four parameters. In contrast to unmanipulated animals, *L. niger* females without antennal flagella were not able to discriminate between the two cricket species.

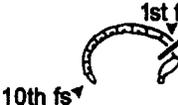
Operation		Ability to recognize prey
Flagellum completely ablated		-
Flagellum completely covered with wax		-
6 flagellar segments ablated or covered with wax		+
7 flagellar segments ablated or covered with wax		-
Tip segment of flagellum (arrow) intact		+
Ventral side of flagellum covered with wax		+
Dorsal side of flagellum covered with wax		-

Fig. 4. Behavior of *L. niger* females in ablation experiments. The ability to discriminate between *A. domesticus* and *G. bimaculatus* after ablation of different parts of both antennal flagella is shown. The experiments show that one of the six distal flagellar segments (flagellar segments 5-10) or the upper side of the antennal flagella has to be unmanipulated, to enable the wasps to discriminate between *A. domesticus* and *G. bimaculatus*. Discrimination possible (+); discrimination not possible (-); first flagellar segment (1st fs); tenth flagellar segment (10th fs).

covered with wax ($n = 5$), whereas animals with only the upper side covered ($n = 5$) paralyzed *G. bimaculatus* as well as *A. domesticus*.

The results of the ablation experiments correspond with the distribution of sensilla basiconica on the antennal flagella of *L. niger* females (Fig. 5). These sensilla are found only on the upper side of the flagellum. Their number decreases abruptly on the proximal flagellar segments. A high number of sensilla basiconica is found on the distal flagellar segments, very few are located on the third flagellar segment, and no sensilla basiconica are found on the two proximal (first and second) flagellar segments. Each sensillum basiconicum has at its tip a smooth, perforated oval plate (Fig. 5a).

Video recordings of the hunting behavior of *L. niger* females showed that, while hunting and tapping the potential prey, the antennae are held more or less

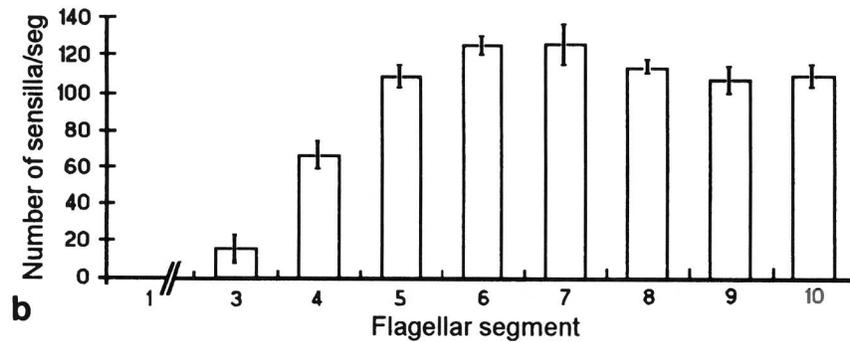
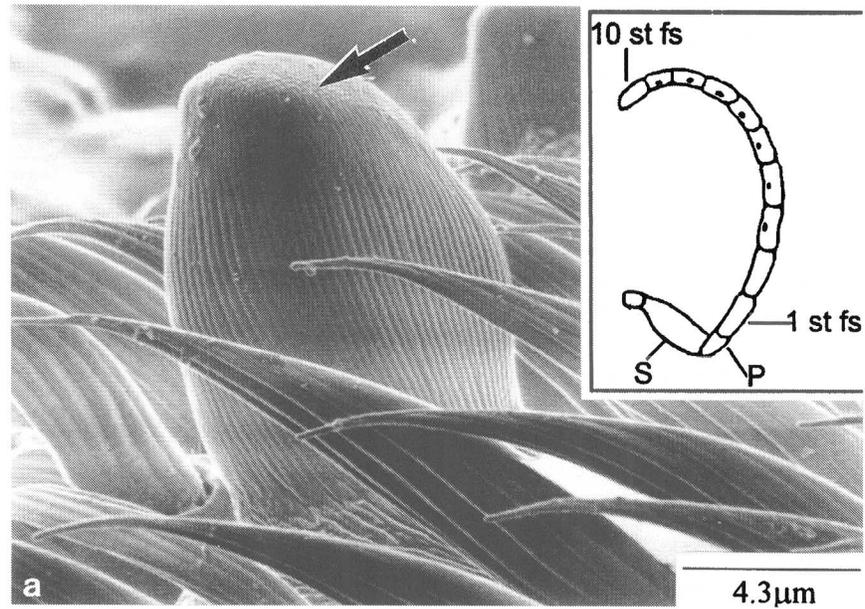


Fig. 5. Female of *L. niger*: topography and structure of antennal sensilla basicica. (a) High magnification of a sensillum basicicum on the dorsal side of a flagellar segment; SEM micrograph. Note that the cone-shaped hair shaft is grooved and has a smooth, multiply perforated pore plate (arrow). Inset: Gross morphology of a *L. niger* female antenna. Scapus (S), pedicel (P), first (1st) to tenth (10th) flagellar segment (fs). (b) Number of sensilla basicica on the 10 flagellar segments of an antenna. The number of sensilla basicica increases rapidly from the third to the fourth flagellar segment and remains high on all distal flagellar segments.

horizontally away from the head, with the flagella slightly curved outward. It was shown that the females touch their prey for 20–40 ms only with the upper side of the flagellum before they reject it (turn away from) or start paralysis (Fig. 6), depending on the cricket species.

The behavior of *L. niger* females toward individual *A. domesticus* wrapped in gauze of different mesh sizes was dependent on if a contact between the antennal flagellum and the potential prey was possible. *L. niger* females were highly interested in these crickets only if they could touch them with the antennal flagella through the mesh. In the eight experiments with a mesh size smaller than the antennal diameter ($\phi \approx 0.2$ mm), no contact between the antenna and the cricket was possible and the *L. niger* females did not show any interest in

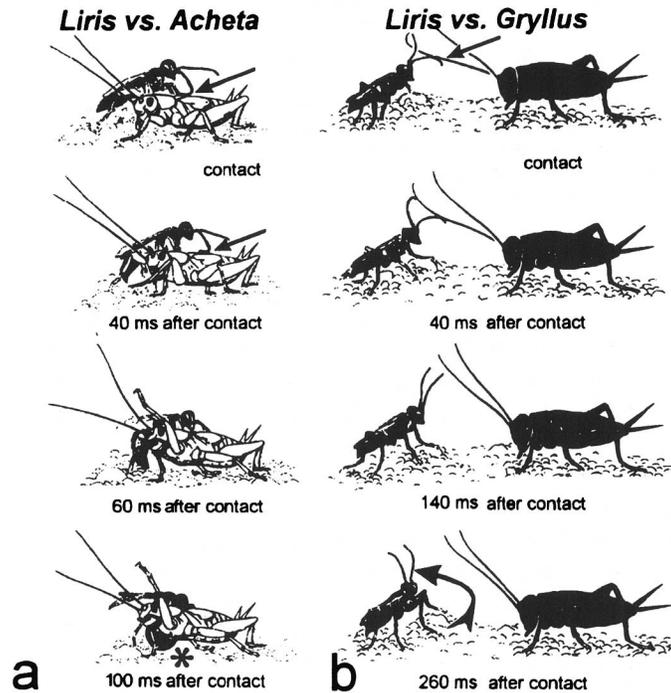


Fig. 6. Prey recognition of *L. niger* females after antennal contact with the two cricket species *A. domesticus* and *G. bimaculatus*. Drawings after video single frames. (a) Acceptance and start of paralysis (asterisk) of an *A. domesticus*. (b) Rejection and turn-away (arrow) from a *G. bimaculatus* after touching.

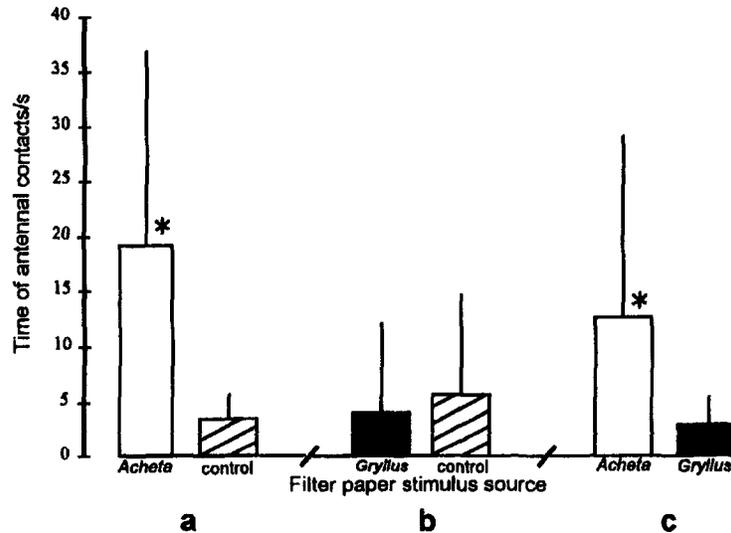


Fig. 7. Duration of antennal contact of *L. niger* with pieces of filter paper. Filter paper rubbed on the cuticle of one cricket species or left untreated (control). (a) *A. domesticus* versus control (b) *G. bimaculatus* versus control. (c) *A. domesticus* versus *G. bimaculatus*. The mean time (bars) and standard deviation (lines) are shown. (*) Significant difference between tested filter papers.

the cricket. In nine experiments with a mesh size about the same as the flagellum diameter, only one antennal contact with the cricket was observed, and in this case the *L. niger* female tried to attack the prey. In 15 experiments with a mesh size clearly bigger than the antennal flagellum diameter, 14 antennal contacts with the prey were observed, and in all 14 cases the animals tried to attack the wrapped cricket.

Behavioral observations in choice experiments showed that pieces of filter paper rubbed on *A. domesticus* were touched for significantly longer times by *L. niger* females than untreated control papers (Wilcoxon signed-rank test, $P = 0.004$) and than those rubbed on *G. bimaculatus* ($P = 0.005$) (Figs. 7a and c), whereas no difference was found between the attractiveness of *G. bimaculatus* and that of the control ($P = 0.754$) (Fig. 7b).

Living *G. bimaculatus* kept in a box crowded with *A. domesticus* were paralyzed by 72% of the unmanipulated *L. niger* females ($n = 18$). *A. domesticus* treated in the same way with *G. bimaculatus* were rejected by 47% of the unmanipulated *L. niger* females ($n = 15$), all of which attacked an untreated *A. domesticus* afterward.

DISCUSSION

The Inflexibility of Prey Specificity

“Prey specificity” varies slightly between individual *L. niger* females under laboratory conditions. The number of accepted prey species was different for individual females, but *G. bimaculatus* was rejected by all *L. niger* females. The laboratory experiments, using some species which occur in the same habitat as *L. niger*, but not testing all potential prey species, allow only limited assessment of the prey specificity in the field. However, the experiments show that *L. niger* is not a highly specific species, compared to some digger wasp species which hunt only one prey species (Evans, 1958; Gess, 1984). The rejection of *G. bimaculatus* by all *L. niger* females, which otherwise varied individually in their prey discrimination, provided a tool to study the determination of this rejection behavior and mechanisms involved in prey recognition.

The rejection of *G. bimaculatus* as prey by *L. niger* females is not subject to host conditioning. Conditioning or imprinting through feeding of the *L. niger* maggots on the normally rejected cricket species *G. bimaculatus* or through early contact of adult wasps with this species has clearly been disproved. Previous experience does not change the host preferences and even increasing motivation in *L. niger* females deprived of preferred host specimens does not reduce host discrimination. The rejection of *G. bimaculatus* might have its origin in that other prey species like *A. domesticus* are more suitable for raising offspring than *G. bimaculatus*, as shown in our experiments. Although Thorpe and Jones (1937) showed preimaginal conditioning of the ichneumonid wasp species *Nemeritis canescens* to two moth species by feeding on them as larvae, more recent work suggests that associative learning happens mostly in connection with cues of the host's environment, whereas directly host-derived cues tend to be more congenitally fixed (for review see Turlings *et al.*, 1993). As the chemical cues of the prey used by *L. niger* females seem to be directly “prey derived,” our results are in accordance with the literature.

Mechanisms of Prey Recognition

Orientation behavior toward the prey and prey recognition in *L. niger* females can be divided into three steps. In the first step wasps in the “hunting mood” run undirected on the ground (searching behavior). The second step is the visual orientation from a distance of up to 15 cm toward moving objects or animals, as observed in the laboratory (Gnatzy and Heußlein, 1986) and in the field (Gnatzy, personal observations). The third step is the chemical recognition of a prey species according to our experiments.

Visual orientation is supported by our ablation experiments, which show

that *L. niger* females with covered eyes do not hunt any more but can show normal feeding and digging behavior. Although visual input seems to be important, moving of the potential prey is not obligatory, because under laboratory conditions, wasps in the "hunting mood" also attack freshly shed exuviae and immobile, freshly killed crickets.

The results of video analyses and gauze experiments show that prey is attacked only after the antennal flagellum of the wasp has been in contact with the prey. These behavioral results imply that mechano- and/or chemoreceptors are responsible for prey identification.

In Hymenoptera, the combination of visual long-range orientation and chemical (mostly olfactory) near-field identification is a widespread phenomenon. The bee wolf, *Philanthus triangulum*, orients visually during prey capture at a distance of up to 1 m, and by olfaction in the near-field (Tinbergen, 1932, 1935). Another sphecid wasp, *Philanthus bicinctus*, uses chemical and mechanical cues to decide if a prey will be accepted (Armitage, 1965). Males of different sphecid and pompilid wasp species orient toward female in using visual and chemical information (Olberg, 1959).

The uncoordinated behavior of female *L. niger* without antennal flagella certainly originates in lost feedback through sensory information during hunting behavior. Unsteadiness following the amputation of the antennae has also been described for the digger wasp *Ammophila campestris* (Baerends, 1941). The bee wolf, *P. triangulum*, stops prey capture completely when the antennal flagella are cut off (Tinbergen, 1932).

Nothing was known about the types of sensilla involved in prey recognition in any sphecid species up to now. The ablation experiments in this study show a clear correlation with the topography of one type of cuticular sensillum on the flagella, the sensillum basiconicum. Sensilla basiconica are found in high numbers only on the upper side of the six distal segments of the antennal flagellum of *L. niger* females. Only these parts of the flagellum are involved in prey recognition, as shown in video analyses and in ablation experiments. The importance of sensilla basiconica for prey recognition in females is further supported by the fact that in males, which do not hunt, these sensilla occur in only low numbers on the antennal flagellum (Gnatzy and Anton, unpublished data). Most likely, sensilla basiconica are important receptors involved in prey recognition, although there are three other types of contact chemoreceptive hairs on the antennal flagella of males and females. The topography of these other sensilla types, however, is not correlated with the ablation experiments (Gnatzy and Anton, unpublished data).

Sensilla basiconica are common sensilla on the antennae of several insect orders, especially in Lepidoptera and Hymenoptera. In sphecid wasps (Martini, 1986) and also in *L. niger* (Knepel, 1995; Gnatzy, 1996), the fine structure of

the sensilla basiconica is identical. Each sensillum basiconicum is innervated by about 120 bipolar sensory cells, the outer dendritic segments of which show extensively branched dendrites ending below the perforated oval plate at the tip of the sensillum hair shaft. In that way the flagellar sensilla basiconica of sphecid wasps differ substantially from typical olfactory sensilla (whole hair shaft perforated) and from typical contact chemoreceptors (only one gustatory pore reached by dendrites) (for review see Altner and Prillinger, 1980). The need for contact between the flagellum of *L. niger* females and the prey supports our hypothesis that the flagellar sensilla of *L. niger* function as contact chemoreceptors. The same role of sensilla basiconica on the antennae of the honey bee, *Apis mellifera*, in another behavioral context (topochemical orientation) has been discussed by Martin (1965). The topography of sensilla basiconica on the antennae was also correlated with host finding behavior in *Cardiochiles nigriceps* (Hymenoptera, Braconidae) (Norton and Vinson, 1974) and in *Itopectis conquisitor* (Hymenoptera, Ichneumonidae) (Borden *et al.*, 1973). Electrophysiological experiments to determine the adequate stimulus for the sensory cells housed in sensilla basiconica in *Apis mellifera* (Hymenoptera, Apidae) and *Lasius fuliginosus* (Hymenoptera, Formicidae) did not succeed (Lacher, 1964; Dumpert, 1972).

Characterization of the Stimulus. An attractive substance of the cuticular surface of *A. domesticus* seems to be important for prey recognition. This substance was transferable to filter paper and to *G. bimaculatus*. A substance on the cuticle of *G. bimaculatus* which might serve as a repellent can only be a weak stimulus, as pieces of filter paper rubbed on *G. bimaculatus* are not less attractive than the control and *A. domesticus* can be made unattractive to only a few *L. niger* females by keeping them with *G. bimaculatus*. The latter result could also be explained by the attractive substance on the cuticle of *A. domesticus* being rubbed off during the close body contact with a large number of *G. bimaculatus*.

The role of cuticular lipids as intra- and interspecific messengers has been shown in several insect species (for review see Howard and Blomquist, 1982). In honey bees (*A. mellifera*) kin recognition is supposed to be based on close-range chemicals, whereas volatiles are important messengers in most other behavioral contexts (Getz and Page, 1991). In crickets contact chemical cues are known to be used in intraspecific communication. In several cricket species sex recognition is based on contact chemoreception if no acoustic communication is possible. Male *T. commodus* and *T. oceanicus* and also *G. bimaculatus* start courtship if they contact a conspecific female with the antennae (Loher and Rence, 1978; Hardy and Shaw, 1983; Adamo and Hoy, 1994). Male *A. domesticus* show the same behavior only when touching the antennae or cercal region (Hardy and Shaw, 1983). Filter-paper pieces stored in a box with females were

touched much longer than controls by males in *T. oceanicus* and *A. domesticus* (Hardy and Shaw, 1983). The substances used in intraspecific communication by crickets might also be used by predators to recognize their prey. Using behavioral and anatomical methods we were able to describe prey discrimination in *L. niger* females and we found receptors on the antennal flagella potentially involved in prey recognition. Our next goal is to characterize the stimulus and the sensory physiology of the antennal sensilla involved in prey recognition.

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