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6. The art of managing weapons: The stinging behaviour of solitary wasps in the eyes of past, present and future research

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Abstract. Studies on sting morphology and venom chemistry of predatory wasps showed impressive progress in the last two decades; by contrast, researches dealing with the behaviour associated with prey stinging are comparatively less numerous and much of what we know come from 19th and 20th centuries up to middle 80s'. Neverthless, in the last 10 years a new interest in this topic appeared, and a number of stimulating studies involving an integrative approach using histological, biochemical, ethological and neurological methods was carried out, although mainly concerning two species (*Ampulex compressa* and *Liris niger*). Here one makes a temporally organized review of what we know about stinging behaviour and prey manipulation of solitary predatory wasps. On the whole, more or less detailed information on stinging patterns is available for species belonging to all the major groups of solitary predatory wasps: Ampulicidae, Sphecidae, Crabronidae, Eumeninae and Pompilidae. Neverthless, some important lineages of wasps preying upon taxa which could elucidate the role of prey

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neuroatonomy on the stinging behaviour were completely ignored. A detailed critical review of early observations (19th century) and comparisons with modern researches revealed that many of the hypotheses suggested by old classic studies were mostly confirmed using modern techniques of analyses. In particular, the “locomotory ganglia hypothesis”, which stresses the importance of distribution of prey ganglia on the spatial pattern of wasp stings, seems to probably unify the mechanism of stinging behaviour of predatory wasps. Nevertheless, exceptions to this rule emerged, and only new studies devoted to observe species neglected to date could confirm or reject it.

Introduction

Belonging to the Aculeata, solitary wasps which prey upon other arthopods to feed their offspring (Apoidea and Vespoidea) possess a sting, i.e. a strongly modified ovipositor with no egg-laying function [1]. The ability to paralyse the prey by means of the sting is certainly one of the features of Aculeata that made possible the evolutionary success of this group of Hymenoptera.

The sting apparatus of solitary wasps may be expected to have been under selection in relation with hunting behaviour, and modifications of the sting is expected to vary depending on the prey type and prey carrying [e.g. 2, 3, 4].

However, differently from research on the sting morphology and venom chemistry of the Aculeata, which showed impressive progress in the last two decades [e.g. 5, 6, 7], little is being currently done about the stinging behaviour of predatory wasps, in particular in relation to the position and frequency of stings on the prey body during the capture, although detailed data are now available for few species [8, 9, 10, 11, 12, 13, 14, 15, 16, 17].

Much of what we know about stinging behaviour of wasps from a comparative point of view date in practice before 1990, and no new reviews on this topic were carried out since 1986 [2]. However, the aim of the present study is by no mean that to give an exhaustive review of the ethology of the different ways how a solitary wasp may sting her prey. Such a work, at least up to the year 1986, has already been done by Steiner who, in the Tables I-V of his excellent paper, condenses all published information up that date regarding the stinging behaviour of “solitary wasps” (including some Terebrantia) [2]. Instead, the present work is focused to a few restricted points: 1) a rather detailed account of the oldest researches, confined within the 19th century; 2) a short exposition of different lines of researches developed since the last decades of the 20th century, and 3) a discussion regarding the “locomotor ganglia hypothesis”. According to it, the wasp directs a sting for each nerve centre involved in a locomotion, attack or defence of the victim. This would imply that for most predatory wasps stings are directed to the throat (suboesophageal ganglion) and three thoracic segments of their prey.

Deep in the past: Studies on wasp stinging behaviour in the 19th century

I will outline in this section four leading figures which operated within the last decades of the 19th century: Jean Henry Fabre, Charles Ferton, in Europe, and the Peckams, in the United States. Even if many other researchers working at the time could be considered, I believe that the four indicated above are those that better characterize this pioneering era of modern wasp ethology. Their researches will be examined in some detail, for the sake to show the variety of situations concerning the stinging patterns, the prey manipulation and the prey paralysis. Moreover, they may illustrate the first controversies regarding the fixity or variability of stinging behaviour, the relationship between stinging patterns and the location of prey nerve centers, the way how paralysis is induced, and other problems which will become matter of further analysis for the following century. For a more comprehensive understanding, in some cases the results of recent findings will be exposed, when they may either confirm or contrast those reported by the early literature.

Jean Henri Fabre (1823-1915)

According to Fabre, the wasp stinging behaviour is strictly correlated to the nervous anatomy of the prey. In the case of *Cerceris*, for example, since the stinging position is located between the first and the second pair of legs, the thoracic ganglia should be concentrated there. This is the reason why, always according the Fabre, the type of prey hunted by this wasp is restricted to the coleoptera of the families Buprestidae and Curculionidae, which present thoracic ganglia fused or located very close together (Curculionidae) or partially fused (Buprestidae). Other coleoptera with fused thoracic ganglia like Scarabeidae, Histeridae or Scolytidae should be excluded due to the difference in habitat with respect to that of *Cerceris* or to their small size. Inside the two families, prey of different size may be captured according to the size of the wasp [18-I: pp. 77, 84-87]. In fact, Fabre did not know of any other family preyed by *Cerceris* wasps (except the case of a species which captures Halictidae: see [18-IV: p. 240].

In the same period when Fabre wrote the first volumes of his *Souvenirs Entomologiques*, Marchal [19] (an extract may be found in [20: pp. 200-210]) wrote a memory devoted to the behaviour of *Cerceris rybyensis* (Linnaeus); this wasp hunts different bee species in the family Halictidae which, as all other Hymenoptera, have a relatively non concentrated ventral cord [21-II: p. 1239]. He was able to study in detail the stinging method placing a wasp with a bee under a bell glass. It is worthwhile to report here some of his considerations, either for what strictly concerns the way of stinging or for the more general implications they have with regard to Fabre's opinions

> At the moment of capture *Cerceris* seizes the bee brusquely, clasping the anterior part of the body with her mandibles. Her recurved abdomen darts the sting into the neck at the articulation of the head and thorax, the stroke being given vigorously as though it were of capital importance. For a few moments the two combatants roll on the ground; one or two quick strokes are given under the thorax, principally between the prothorax and the mesothorax, and the bee becomes motionless; then *Cerceris* holds her victim face to face with her, looks at it a few seconds, and turns it around so as to bring the neck opposite her mandibles. The bee being thus adjusted the wasp proceeds to dig at the nape of the neck, squeezing it for from two to four minutes...Marchal does not agree with Fabre in his belief that wasps are endowed not only with tools but with the method of using them, the gift being original, perfect from the beginning, not modified by past or future. The action of *Cerceris* does not imply any mysterious science. She runs the end of her abdomen along the under surface of the thorax of the bee and stings at the division of the segments – that is at the points where the sting can enter. The order in which the strokes are given is very variable, and if the neck is protected by gum-arabic, so that it is impervious, the stings between the pro- and mesothorax give just the same result. All that is necessary is that the sting shall reach the line of nervous matter that runs along the ventral face of the thorax. As a matter of fact it does not touch the ganglia, but enters just half way between them. The distance is small and the poison quickly reaches the nervous centers.
> The order in which the stings are given is variable. We may consider than there are four classes:
> In class A. the sting is given:
> 1st, at the neck.
> 2nd, at the articulation of the prothorax and the mesothorax.
> 3rd, at the neck.
> In class B. it is given:
> 1st, at the neck.

2nd, at the articulation of the prothorax and the mesothorax.
In class C. it is given:
1st, at the neck.
2nd, at the articulation of the prothorax and the mesothorax.
3rd, behind, toward the origin of the abdomen.
In class D. it is very probably given in a considerable number of cases, either only in the articulation of the prothorax and the mesothorax, or only in the neck.

Cerceris is far from using the exquisite method of malaxation followed by *Sphex*, as described by Fabre. She is on the contrary, quite brutal. She pricks and squeezes the neck of the victim and then lick off the juice that exudes. All the bees that have undergone this operation have the neck cut, some on the median line, some on both sides. The supposition that *Cerceris* proceeds differently with the bees which are destinated to feed the larvae, perhaps not malaxing them at all, or only delicately, proves to be incorrect, since half the bees that were taken from nests showed marks of being cut. The other half, upon which no sign of a wound was visible, had probably received lesions which resulted in death after a brief delay. Of five bees taken from nests where the eggs had not yet hatched...only one responded to stimulation of the electric current by flexing the anterior legs at the moment that it was applied, the others giving no reaction. Moreover of three taken from a nest not yet fully provisioned one gave no response to the electrical current (from the Marchal's memoir extract given by [20: pp. 201-203]).

However, half an hour after being stung, the bee shows a slight recovery whose extent depends upon the fact of having or not received malaxation (from the Marchal's memoir extract given by [20: p. 205]). In conclusion, according to Marchal

1. The effect of the sting is to produce inhibition; this permits *Cerceris* easily to carry the bee into her hole.
2. Inhibition ceases, but the nervous system has been so injured that the insect does not recover. Still motion may persist for a long time if there has been no malaxation.
3. Malaxation by the effusion of blood which it produces, and also by the lesion of the nervous chain, strikes a fatal blow at voluntary movement, and leads to the suppression of animal life in about twenty-four hours (from the Marchal's memoir extract given by [20: p. 207]).

Malaxation has an effect even on the preservation of the prey after they died (or at least cany movement was ceased): the malaxed bees dried up at once, while the others remained fresh for a longer period, maybe still maintaining a kind of vegetative life (from the Marchal's memoir extract given by [20: pp. 207]).

To validate his hypotheses Fabre performed a few experiments by inoculating a droplet of ammonia at prothorax junction behind the first pair of legs. The result was very variable according to the kind of insect: with Scarabeidae, Buprestidae and Curculionidae, which possess thoracic ganglia close to each other, a complete paralysis similar to that induced by the *Cerceris* was obtained and they remained in life for one or two months. In the case of insects with distant thoracic ganglia like Carabidae or Cerambycidae, instead, the effect was different and after some quick and uncoordinated convulsions recovered in a few hours or the day after [18-I: pp. 88-89, 91-92].

Ammophila shows a completely different pattern of stinging behaviour. Placed under a glass bell together with a caterpillar, an *Ammophila hirsuta* Scopoli stings it at the thorax level, from the third to the first segment, where it seems to be stung more insistently. After this first phase, the caterpillar is released and the wasp performs a very special behaviour which Fabre calls a "dance of triumph", then seizes the caterpillar from the dorsal side and stings it again ventrally at every segment from the anterior to the posterior end, with exception of those already operated, progressively shifting behind the position where the prey is seized. The caterpillar is released, then seized again by *Ammophila* with her mandibles at the level of the beginning of first thoracic segment (close to the nervous centers of the brain) and chewed for many minutes: the wasp mandibles movements are sharp, but distant one from the other, as if the wasp was checking their effect. When this last operation is terminated, the mandibles of caterpillar, which after the stings were still in movement, become quiet. The provided scheme admits a few variations, as the reduction of the stings of the first phase to two or even one (at the first segment). In such case the missed segments are stung at the beginning of the second phase, and sometimes the first segments are stung twice, in both the first ands the second phase. It is frequent that the last two or three segments are missed and rarely the direction of the stings is reversed from the posterior to the anterior end. The chewing of the first segment (malaxation) may be omitted in case the caterpillar mandibles, which could interfere with the transport, remain motionless after the stings [18-IV: pp. 248-251].

Exactly the same stinging behaviour has been observed in *A. apicalis* Brullé, preying on a geometrid caterpillar - two stinging phases spaced by the "triumph" dance, malaxation, same number and order of stings - notwithstanding the rather different external morphology of the caterpillars (like legs distribution). What makes Fabre to claim: “the anatomy of the game, rather than its form, controls the huntress' tactics” [18-IV: pp. 254-256].

A situation intermediate between that of *Cerceris* and that of *Ammophila* was depicted by Fabre in the chapter entitled “Three strokes of a dagger” [18-I: pp. 107-115]. It concerns *Sphex flavipennis* Fabricius which hunts young locusts. However there are some problems on the correct identification of this species: on the base of some drawings of Fabre, Berland [22] who examined Fabre’s material stated that it was *Sphex maxillosus* Fabricius (= *Sphex rufocinctus* Brullé), but Ferton excluded this possibility due to the kind of prey [23].

> She places herself body to body with her adversary, but in a reverse position, seizes one of the bands at the end of the cricket’s abdomen and masters with her forefeet the convulsive efforts of its great hindthigs. At the same moment her intermediate feet squeeze the pantaing sides of the vanquished cricket, and her hind ones press like two levers on its face, causing the articulation of the neck to gape open. The Sphex then curves her abdomen vertically, so as to offer a convex surface impossible for the mandibles of the cricket to seize, and one beholds, not without emotion, the poisoned lancet plunge once into the victim’s neck, next into the jointing of the two front segments of the thorax... [18-I: pp. 108-109].

This first stinging is followed by a second one between the first and the second thoracic segment and a third one at the junction with the abdomen. In fact, in the nervous system of the cricket the three thoracic ganglia are separated: this should be the reason of the three stings [18-I: pp. 109-112].

Only slightly different is the stinging behaviour of *Palmodes occitanicus* (Lepeletier & Serville), called by Fabre “*Sphex languedocien*”, a related species hunting *Ephippiger* females with ripe eggs. The paralysis seems to be less pronounced that in the preceding case and this should be related, according to Fabre, to the fact that *P. occitanicus*, differently from *S. flavipennis*, hunts one single prey. In fact in both cases the egg is laid in a position protected from the movements of the prey (close to the base of one of the posterior legs or more laterally between the first and second pair of legs, respectively) to which is attached, but could be stressed by the movements of an other non completely paralysed prey previously brought into the cell. A first sting is made in the thorax, the second behind the neck, but apparently the sting is pushed toward the thoracic ganglia. If the *Ephippiger* hinds the transport by means of its tarsi or mandibles, the wasp performs malaxation, chewing its brain centers with her mandibles inserted through the neck membrane. In such way a complete paralysis is induced which lasts a few hours, then one goes back to a less paralysed state [18-I: pp. 159, 172, 174-176, 178, 179, 183-184].

Among eumenine wasps, *Odynerus nidulatur* Saussure (= *Symmorphus murarius* (Linnaeus)) was found to turn the prey, a larva of leaf beetle (Chrysomelidae) close to pupation, ventral side up and to give one stings at the level of each of its thoracic ganglia. After stinging, *Odynerus* performs malaxation on the neck of the prey. In some cases the larva of the leaf beetle is stung in the terminal segments beginning from the most distal ones (i.e. in a reversed order with respect to the stings in the thoracic ganglia), and then the last three of them are chewed. In such way the liquid contained in the alimentary channel is pushed behind and is licked from the wasp. Then the prey is abandoned. However, this second behaviour is less regular than the standard one: sometimes the stings reach the thoracic ganglia, while in some other cases stings are absent and the wasp feeds on a non paralyzed prey [18-IV: pp. 196, 204-207]. Even Ferton observed a different *Odynerus* species malaxating her prey, but without discarding it: from time to time *Odynerus nobilis* Saussure stopped her provisioning work and took out from the cell his whole content; then the prey were reviewed, malaxated to extract the juice which was licked from the wasp, and re-introduced in the nest. Malaxation was observed even in *Odynerus alpestris* Saussure, from the anterior to the posterior extremities of the preyed caterpillar [24].

Philanthus triangulum Fabricius equally feeds on the content of the crop of her prey, the honey bee worker. The prey is stung ventrally, between the head and the neck (prothorax). The sting reaches the cerebral ganglion inducing a real death, not only a locomotory paralysis. Next follows a special kind of manipulation, consisting in chewing and squeezing the bee neck and abdomen in a way which allows nectar contained in the crop to escape from mouth to be licked from the wasp. The rational of the behaviour to kill the prey, instead of paralyzing it, consists in the fact that in dead prey any muscular tonicity is abolished and the flowing back of the nectar made easier. This is the reason to sting below the neck instead than in the thorax. But there is an additional reason to empty the bee from its nectar, due to the rigorous carnivorous diet of wasp larvae which, after a first acceptance, soon disliked experimentally killed bees non previously emptied [18-IV: pp. 214-216, 221, 225, 231]. In accordance with this consideration, Fabre expected that any wasp hunting bees would behave in the same way [18-IV: p. 240]. One has seen that for *C. rybyensis* this prevision is only in part confirmed. Instead, for the other species of beewolves (*Philanthus*) preying upon bees, according to Evans & O’Neill [25: p. 25] this behaviour has never been observed in any of the North American species. However, Kurczewski and Miller [26] found that females of *P. sanbornii* Cresson, before introducing the hunted bees in the cell, probably cleaned them from the pollen they bored, sealing it at the end of a side passage.

Regarding the other statements made by Fabre concerning *P. triangulum*, many of them did not receive confirmation. According to Rathmayer [27], the bee results to be stung behind the front coxae instead than under the neck and it is not killed. Moreover, provisiong is massive and non progressive and oviposition is made on the last instead that on the first prey, as supposed by Fabre [18-IV: pp. 228-230; 28].

The stinging method used by *Scolia* (Hymenoptera: Scoliidae) is of special interest since the prey is a larva of *Cetonia* or other scarabeid beetles for which one would expect a serial pattern of stings, as in the case of *Odynerus* or *Ammophila*. Instead, these larvae present a particular nerve cord which, as in the corresponding adult insects, shows a fused ganglionar organization since thoracic and ventral ganglia form an unique mass. In accord with this finding, Fabre foresaw that the stinging behaviour should consist in only one sting in the ganglionar mass. On the other hand additional stinging would be hard to realize in the difficult conditions of hunting under the ground [18-III: pp. 48-53].

To check what effectively happens during the underground hunt of *Scolia* is hardly feasible, but Fabre succeeded to study under a glass bell the case of *S. bifasciata* van der Lind. (= *S. hirta* (Scrk.)?) and *Scolia* (*Colpa*) *interrupta* Latreille, preying respectively on *Cetonia* ed *Anoxia*. The results of the observation agreed with the expected ones: the scarabeid larvae were effectively stung only once ventrally, as predicted on the base of the anatomical findings: between the first and the second thoracic segment for the first species; under the neck for the second one [18-IV: pp. 258, 260, 266]. The result is that of a very deep paralysis, “Never, since my remotest investigations, have I witnessed so profound a paralysis” (18-III: p. 47). In fact, the unique stroke in the ganglionar mass paralyzes at the same time the legs and the whole animal.

Even in the case of the mantis-killing *Tachytes* (probably *Tachysphex jullianii* Kohl, see [29: note 1 of the eds., p. 128], the anatomical structure of the prey nerve cord determines the stinging pattern of the wasp. In fact, in the mantis species the second and third locomotory ganglia are widely spaced from the first one, matching the long prothorax which divides the front legs from the four other ones. The first ganglion is the largest of the three, and is also that where the first stroke will be delivered in order to abolish any offensive movement of the front raptorial legs. Subsequently, the wasp retreats along the mantis’ back and stings two closed points, corresponding to the ganglia which innerve the second and third pairs of legs. What is more striking is the unsymmetrical pattern of the stings distribution which, unlike what observed in other cases, as in *Ammophila*, are not uniformly distributed along the mantis length, in conformity with the anatomy of the prey. A different prey, as a small grasshopper, even if accepted as a diet from *Tachytes*'larvae, will not be stung by the mother since, according to Fabre, she does not know the way to operate upon a different nervous structure [18-III: pp. 242, 254-261].

Lastly, for what concerns spider wasps, Fabre observed under a wire-gauze cover the stinging behaviour of *Cryptocheilus sexpunctatus* Fabricius (called by Fabre *Calicurgus scurra*) in regard of *Epeira fasciata* Walck. Contrary to what expected according to the nervous organization of the spiders cord, consisting in only one ventral ganglion, the first blow is not aimed at the spider's cephalotorax. In fact the wasp, after having overturned the spider on its back and mounted on its top, mastering its legs with her legs and hanging the cephalotorax with the mandibles, drives her sting into spider's mouth "with minute precaution and marked persistency". Immediately, the paralyzed poison-fangs close, leaving the spider harmless. A second sting made at the level of the cephalotorax-abdomen attachment paralyzes all eight legs at once. Even if the ganglion is located a little above this point, it should be reached due to inclination of the sting. The overall effect of the two stings is to paralyze both fangs and legs. The first ones will stay so for ever, while the legs movements, although in a quite uncoordinated manner, may recover after some time. The palpes, instead, will remain irritable and mobile [18-III: pp. 270, 276-281].

What is very surprising is the inability of the spider to defend itself efficiently by the wasp

> The Lycosa is soon seen. The Calicurgus approaches her without the least sign of fear, walks round her and appears to have the intention of seizing one of her legs. But at that moment the Tarantula rises almost vertically on her four hinder legs, with her four front legs lifted and outspread, ready for the counterstroke. The poison-fangs gape widely; a drop of venom moistens their tips. The very sight of them makes my flesh creep. In this terrible attitude, presenting her powerful thorax and the black velvet of her belly to the enemy, the Spider overawes the Pompilus, who suddenly turns tail and moves away. The Lycosa then closes her bundle of poisoned daggers and resumes her natural pose, standing on her eight legs; but, at the slightest attempt at aggression on the Wasp's part, she resumes her threatening position.
> She does more: suddenly she leaps and flings herself upon the

> Calicurgus; swiftly she clasps her and nibbles at her with her fangs. Without wielding her sting in self-defence, the other disengages herself and merges unscathed from the angry encounter. Several times in succession I witness the attack; and nothing serious ever befalls the Wasp, who swiftly withdraws from the fray and appears to have received no hurt. She resumes her marching and countermarching no less boldly and swiftly than before.
>
> Is this Wasp invulnerable, that she thus escapes from the terrible fangs? Evidently not. A real bite would be fatal to her. Big, sturdily built Acridians succumb; how is it that she, with her delicate organism, does not! The Spider's daggers, therefore, make no more than an idle feint; their points do not enter the flesh of the tight-clasped Wasp. If the strokes were real, I should see bleeding wounds, I should see the fangs close for a moment on the part seized; and with all my attention I cannot detect anything of the kind.
>
> Then are the fangs powerless to pierce the Wasp's integuments? Not so. I have seen them penetrate, with a crackling of broken armour, the corselet of the Acridians, which offers a far greater resistance. Once again, whence comes this strange immunity of the Calicurgus held between the legs and assailed by the daggers of the Tarantula? I do not know. Though in mortal peril from the enemy confronting her, the Lycosa threatens her with her fangs and cannot decide to bite, owing to a repugnance which I do not undertake to explain [18-IV: pp. 271-272].

The chapter entitled “Objections and rejoinders” [18-IV: pp. 287-302] was devoted to discuss the “discovery of the surgical methods” in wasp stinging behaviour and to reply to the many criticisms met from the entomological community. It is worthwhile to expose in some detail this discussion, which at great extent maintains its validity even nowadays.

Firstly, Fabre examines the thesis of those who state that “the sting...is directed at one point rather than another because that is the only vulnerable point”, i.e. “accessible..., penetrable”. Such point may be supported in the case of the weevils and the buprestids preyed by *Cerceris* and stung behind the prothorax (even if there is a different spot, located under the throat, equally accessible but disregarded by the wasp). But “What are we to say of the Grey Worm and other caterpillars beloved of the Ammophilae? Here are victims accessible...everywhere with the same facility, excepting the top of the head. And of this infinity of points, which are equally penetrable, the Wasp selects ten, always the same, differing in no way from the rest, unless it be by the close proximity of the nerve-centres. What are we to say of the

Cetonia- and Anoxiae- larvae, which are always attacked in the first thoracic segment, after long and painful struggles, when the assailant can sting the grub freely at whatever point she chooses, since it is quite nake and offers no greater resistance to the lancet at one point that at another?" [18-IV: pp. 288-289].

Similar things may be said for the *Sphex*, whose prey are stubbed three times below the well defended thorax, neglecting the soft and bulky abdomen, and for the *Philanthus* "who takes no account either of the fissures beneath the abdominal plates or of the wide hiatus behind the corselet but plunges her weapon, at the base of the throat, through a gap of a fraction of a millimetre". Analogous considerations may be made for the mantis-killer *Tachytes* and for the spider wasps, since if "there is one point about the Tarantula and the Epeira that is dangerous and difficult to attack it is certainly the mouth which bites with its two poisoned harpoons" [18-III: pp. 289-290].

Then Fabre argues against the supporters of the fact that the wasps' stings may be directed thereabout at the nerve centres, not necessarily exactly where they are. To reject this possibility, it is necessary to consider the details of the stinging technique. In the case of *Scolia interrupta*, for example, in the preliminary conflict that precedes the stroke, the adversaries appear as "two rings interlocked not in the same plan but at right angles....Owing to her transversal position, the assailant is now free to aim her weapon in a slightly slanting direction, whether towards the head or towards the thorax, at the same point of entry in the larva's throat". However, the choice between the two different directions will not be uninfluential, since in the first case the sting will be driven into the cerebral ganglia what will cause the death of the prey: the way how the *Philanthus* kills her bee, by a slanting sting from below under the chin. With a slant towards the thorax, instead, the thoracic ganglion will be reached and the paralysis will follow: "A millimetre higher kills; a millimetre lower paralyses. On this tiny deviation the salvation of the Scolia race depends. You need not fear that the operator will make any mistake in this micrometrical performance: her sting always slants towards the thorax, although the opposite inclination is just as practicable and easy. What would be the outcome of a there or thereabouts under these conditions? Very often a corpse, a form of food fatal to the grub" [18-III: pp. 217, 290-292].

Additional support to his statements was found considering the case in which a *S. bifasciata* committed a slight mistake, a very rare thing: her sting entered a little laterally, about one millimetre, from the right spot between the first two thoracic segments, however always correctly on the median line. This small error of position is large enough to determine the paralysis only of the left side legs, while the others maintain their movement. Soon the paralysis gains even the right legs which become motionless. But, differently from those of the other side, they remain able to contract to any stimulation.

In this situation the wasp egg will be unsafe, easily crushed or detached from its position. However, "it is extremely unusual for the operator, no matter what her prey or her method, to make a slight mistake and sting merely somewhere near the requisite point. I see them all groping with the tip of the abdomen, sometimes seeking persistently, before unsheathing. They thrust only when the point beneath the sting is precisely that at which the wound will produce its full effect. The Two-banded Scolia in particular will struggle with the Cetonia-grub for half an hour at a time to enable herself to drive in the stiletto at the right spot.... The sting, by straying less than a millimetre, would leave the Scolia without progeny" [18-IV: pp. 292-294].

One has already said as the first stroke, which is given in the mouth, given by the pompilid wasp *C. sexpunctatus* on *E. fasciata*, causes the paralysis of the fangs but non of the palpi, very near to them. A similar result was observed in the case of the lycosid spider preyed by the spider wasp *Cryptocheilus annulatus* Fabricius. Fabre considered as more probable that fangs and palpi were innervated by the same ganglion, rather than from two different ones. In such case the different effect of the sting implies that the two thin ("as fine as a hair") filaments innervating the fangs, and only them, must be injured directly. It is of special importance do not affect the cephalic centres, what will cause death instead of paralysis: "The Calicurgus [= pompilid wasp] has to reach them one after the other, to moisten them with her poison, possibly to transfix them, in any case to operate upon them in a very restricted manner; so that the diffusion of the virus may not involve the adjoining parts. The extreme delicacy of this surgery explains why the weapon remains in the mouth so long; the point of the sting is seeking and eventually finds the tiny fraction of a millimetre where the poison is to act. This is what we learn from the movements of the palpi close to the motionless fangs; they tell us that the Calicurgi are vivisectors of alarming accuracy." On the contrary, if palpi and fangs were linked to two different ganglia, as it actually happens (palpi have their nervous centre in the subesophageal mass, while cheliceral ganglia are fused with the brain [21: pp. 884, 887]), the difficulty will be only a little less [18-IV: pp. 269, 271, 280, 294-295].

Fabre enumerates three different possibilities used by wasps to induce paralysis in her prey, which he is not able to discriminate: the ganglion directly injured; the venom drop deposed over the ganglion or in its immediate neighbourhood. Even if it is true that the effect of the venom may diffuse, as it has been mentioned above, the process takes some time, while the wasp egg requires absolute safety from the very first [18-IV: pp. 295-296].

A further example regarding the wasps stinging method concerns *A. hirsuta*: “the three thrusts in the caterpillar's thorax and especially the last, between the first and second pair of legs, are more prolonged than the stabs distributed among the abdominal ganglia. Everything justifies us in believing that, for these decisive inoculations, the sting seeks out the corresponding ganglion and acts only when it finds it under its point. On the abdomen this peculiar insistence ceases; the sting passes swiftly from one segment to another. For these segments, which are less dangerous, the *Ammophila* perhaps relies on the diffusion of her venom; in any case, the injections, though hastily administered, do not diverge from a close vicinity of the ganglia, for their field of action is very limited, as is proved by the number of inoculations necessary to induce complete torpor...”. The last point is proved by a caterpillar which has been removed by the *Ammophila* after it has received its first sting in the third thoracic segment. Only the legs of this segment remain paralysed, while the other maintain their usual mobility for two weeks, during which the caterpillar performs a normal life, walking, burrowing and retaining perfect liberty of action until it dies accidentally, not of its wound: during this time the effect of the venom is remained confined to the stung segment [18-IV: pp. 296-297].

Charles Ferton (1856-1921)

Large part of the entomological community did not agree with Fabre’s opinions. The innate knowledge by part of the hunting wasp of the precise nervous structure of her prey seemed too difficult to be accepted. Among the many opponents was Charles Ferton who, however, maintained always in great consideration the opinions of “the Great Fabre” and always tried to conciliate, as far as possible, his own observations with those of the other, of whom he even wrote a short bibliography [29: Appendix]. The same may not be said for the angry intolerance of other entomologists as Rabaud and Picard who, in their preface to a collection of Ferton’s works, spoke of the “legends” spread by Fabre [29: Preface, p. VII].

Sphex. However, Ferton did not agree with the opinions of Fabre regarding the fact that the wasp stings should reach the thoracic ganglia of the prey, at least in some cases as that of the sphecid wasp *Sphex subfuscatus* Dahlbom (now *Prionyx subfuscatus* (Dahlbom)), which preys the orthopterous *Calliptamus italicus* (Linnaeus). Differently from Fabre’s description of *Sphex flavipennis*, Ferton did not report the “three strokes of a dagger” but only two: the first through the membrane which surrounds the anterior legs; the second at the origin of the middle or, more frequently, posterior legs. No attempt was observed, by part of the wasp, to wide the articulation between the head and the first thoracic segment and to maintain the prey motionless (see above). But two other more important differences are to be taken into account. Ferton thought that the possible positions of the stings were necessarily restricted to a few points, where they had effectively been observed, since the sternum of the prey is protected from a chitinous plate which prevents any other possibility. However, Ferton did not attribute these differences to a mistake in Fabre's observations, but only to an interspecific diversity between the two *Sphex* species. Moreover, it appeared improbable that the second sting could reach the thoracic posterior ganglion and it seemed more plausible that the paralysis of the rear legs was caused by the diffusion of the venom rather than by a direct lesion due to the sting of the nervous centre. After paralysis, the wasp licked the liquid flowing out from the prey's mouth [30].

For what *P. occitanicus* is concerned, Ferton observed the wasp carrying to her nest a male of *Tettigonia viridissima* Linnaeus, contrary to Fabre who observed only females of *Ephippiger*, and was unable to make the wasp accept, through an experiment of substitution, a prey of the other sex. The disagreement is explained by Ferton assuming that the females prey are destinated to females new born wasps; male prey, instead, which have a smaller size, to males. Substitution may not be accepted by a wasp that has "decided" to lay an egg which will give rise to a female and for which a larger cell had been build [18-I: pp. 172-173; 31, 29: p. 120]. Ferton noted that the prey of *P. occitanicus*, as well as those of other Orthopteroids hunter wasps like *S. maxillosus* or *Notogonia pompiliformis* Panzer (the prey of the last species appeared to be very imperfectly paralyzed), often show mutilation of some legs. This possibly could be due to a process of autotomy, maybe consecutive to bites of the wasp [23, 32, 31, 29: note 1 of the eds., p. 121].

Spider wasps. In the case of spider wasps, the data of Ferton do not always match those of Fabre. He too observed "the spider to show, in regard to the assailant, an instinctive fear, at the most tossing about to take to flight" [33, 34]. In case of spiders leaving inside a shelter, they are in general dislodged before being attacked. This had already been observed by Fabre in the case of *C. annulatus* chasing a tarantula

> ...the Calicurgus, without the least fear, descends into the Tarantula's den and dislodges her. I imagine that things happen in the same fashion outside my cages. When expelled from her dwelling, the Spider is more timid and more vulnerable to attack.
> Moreover, while hampered by a narrow shaft, the operator would not wield her lancet with the precision called for by her designs. The bold irruption shows us once again, more plainly than the tussles on my

> table, the Lycosa's reluctance to sink her fangs into her enemy's body. When the two are face to face at the bottom of the lair, then or never is the moment to have it out with the foe... Quick, my poor Lycosa, quick, a bite; and it's all up with your persecutor! But you refrain, I know not why, and your reluctance is the saving of the rash invader [18-IV: pp. 275-276].

Something similar had been observed a few years before, in the case of a spider of the genus *Segestria* chased by a *Pompilus apicalis* Van der Linden, which dragged the prey out from its silk tube before stinging it [18-II: pp. 230]. However, Ferton observed that in some cases the spiders were attacked inside their own shelter or in another where they were hidden [33]. A very interesting case is that showed by *Pompilus vagans* Costa (=*Anospilus orbitalis* (Costa)) which hunts the trap-door spider *Nemesia* in two different ways. Early in the season (in Corse), when the prey uses a single channel nest, the wasp enters inside, gives a light sting to the spider which induces a very short paralysis and lays its egg upon it. Later on in the season, when the *Nemesia* has added a second door to the nest which opens in a secondary channel, the wasp tactics is completely different: she enters the nest to dislodge the spider, which escapes from one or the other door but is soon reached and stung [34].

However, if in general the spider is powerless with regard to the pompilid which is its usual enemy, this may not be true with different spider wasps, as it was observed by Ferton in an experimental situation where a *Gnaphosa alacris* E. Simon was collocated close to a *P. vagans*: the wasp was attacked and killed. Instead, its usual prey *Nemesia*, even if much larger than the wasp, seems unable to fight with her [35].

For what concerns the stinging pattern, Ferton observed a wide range of situations: from random and ineffective stinging anywhere on the body to stinging in precise positions, as illustrated by Fabre for *C. sexpunctatus* (and suspected for *C. annulatus*), in *Priocnemis affinis* [33]. In addition, *Pompilus effodiens* Ferton, layd her egg on a Lycosid spider inside its own shelter, possibly without giving any sting to it [33, 34]. However, it is possible that when imprecise and random stings were observed, this was due to the impossibility to reach the right position. In fact, *Priocnemis leucocelius* Costa, at the beginning stung her *Nemesia* randomly and without any effect everywhere but eventually succeded to paralyze it giving her stroke in a point close to the mouth. A similar observation concerns a *Pompilus republicanus* Kohl which had firstly stung her prey, fallen on its back, on the dorsal side, without effect; subsequently in the right spot close to the mouth. This should be the “weak point” of any spider, as already illustrated by Fabre for

C. sexpunctatus and *C. annulatus*) (see above), and which most pompilids should try to reach. The effective result greatly depends on the spiders behaviour: some of them, when overtaken by the wasp, try to escape in any possible way, making it difficult to sting them with the due precision; others, instead, remain motionless, making the job easier. Ferton agrees with Fabre even for the presence of secondary strokes given more often under the thorax, after the first main stroke near the mouth [34, 36].

An interesting point regards the possibility, for pompilid wasps, to use the tip of the abdomen as a receptor organ. In some cases, as in *Pompilus wesmaeli* Thomson, Ferton observed the tip of the abdomen to explore without stopping the body of the preyed spider, possibly without unsheathing the sting, until it eventually reached the right position for the stroke, close to the mouth. And *A. orbitalis*, when has discovered the nest of her prey *Nemesia*, frequently inserts her abdomen in the nest opening, probably either to induce the spider to dislodge or to better localize it [36].

However, in some cases the stinging pattern is different, as it was seen in the case of an *A. orbitalis* which was trying to open a *Nemesia* single-channel nest. Offering to the wasp an already paralyzed *Nemesia*, she accepted the substitution giving to the spider, which was maintained with its axis perpendicular to that of the wasp, a first sting in the middle of the thorax. A second, and sometimes a third, stroke was given in the anterior and posterior part of the thorax, on a side. After the stings, the wasp performed malaxation of legs and thorax, close to the attachment with the abdomen [35].

A previous observer (Goureau, 1839, quoted by [33]) had already observed that some pompilid wasps cut the legs of their prey. Cutting or biting the legs of the spider, probably to make easier the transport either amputating or dislocating a few limbs, was confirmed by Ferton [33, 34, 23]. A special kind of mutilation was observed in *Pompilus sexmaculatus* Spinola (probably *Aporinellus s. sexmaculatus* (Spinola)), which depilates a small surface of the spider tegument where to attach the egg [37].

According to Ferton, the effect of the stings was very variable, and the prey may remain paralyzed or recover either slower or shortly after the closing of the nest. In some cases, after a few hours the spider was completely re-established, able to feed and to weave its web. This means that the wasp larvae may devour whether paralyzed or active spiders, which are prevented to escape only because confined inside a narrow cell. Among the spiders collected from 29 pompilid species, eight spider species were found re-established [34, 36]. The different effects may be due whether to the kind of sting or maybe the different quantity of inoculated venom: *Nemesia badia* Ausserer stung by *A. orbitalis* remains paralyzed for a short period; by *Priocnemis leucocoelius* Costa, instead, it recovers very unfrequently. But it may depend even on the species of spider: the *Lycosa*, for example, seem to recover more easily than the other species [36].

Bembix. Among the many works devoted to sphecid wasps, one is exclusively concerned wih the flies hunters of the genus *Bembix* [38]. Fabre had already revealed a very interesting fact, quite unusual for the solitary wasps. It consisted in what will be later called "progressive provisioning", to be distinguished from the fairly more common "mass provisioning" [18-I: pp. 264-267], a habit which the genus shares with social wasps.

A part from this main result, which will be confirmed by all subsequent researches, Fabre stated that the diptera preyed by *Bembix* were killed by the wasp. Moreover, in a few cases the victim showed the head turned of 180 degrees around the neck, the abdomen open by a stroke of the mandibles, the wings rubbed, indicating an uncoordinated and very rapid struggle with a much larger and stronger enemy [18-I: pp. 278, 280]. Even if Fabre does not tell us the species of *Bembix* which was effectively concerned, this last finding results quite anomalous with respect to those of other observers. If Wesenberg-Lund (quoted by [38]) confirms, for *Bembix rostrata* (Gmelin), the fact that the food supply consists of killed and crushed flies, most other researchers maintained a different opinion, starting from Lepeletier who studied the same species (1841, quoted by [38]). Also Ferton found that, either in the case of *B. rostrata* or *Bembix oculata* Panzer, the largest number of hunted prey remained alive. Moreover, he never observed the brutalities on their bodies reported by Fabre or any indication that they were seized hastily and in a random fashion [38].

Later researches will not confirm Fabre's and Wesenberg-Lund's findings, since most flies were found paralyzed, only occasionally killed or crushed [39: pp. 112-113; 40: p. 13; 41: p. 356]. However, the paralysis increases gradually and in a few days kills the prey: "There can be no doubt that the flies do not remain in a fresh condition as long as do the prey of many other digger wasps" [40: p. 13]. What was already established by Fabre, who observed that flies withdrawn from the *Bembix*, differently from those taken from other wasps, soon became mouldy and rotted. However, this problem is overcome thanks to the progressive method of provisioning [18-I, pp. 275-277]. On the contrary, sometimes one has observed some flies to escape while the wasp was opening her nest or immediately after having been introduced [39: p. 111; 40: p. 73; 42]. In some cases (*Bembix* near *capensis* Lepeletier) this result is far from being rare, involving about the 30% of the hunted prey [42] and may not attributed to the breakdown of the venom stock, as supposed by Nielsen [39: p. 111], since it does not result correlated to the frequency of provisioning trips. The state of the prey may therefore result very variable: they may be killed, either immediately (as it happens for

Bembix texana Cresson or *Bembix nubilipennis* Cresson [41: p. 356; 43: p. 23, 32]) or, more frequently, after a short period. But it may even happen that the induced paralysis is very light (as in *B. belfragei* Cresson and *B. hinei* J. Parker [41: p. 356]) or completely absent. However, one may not exclude a possible modulation according to the environmental conditions or the different use of the prey, as stated by Tsuneki (1956, 1958, quoted by [41: p. 356]) who found, in *B. niponica* F. Smith, that the first prey (that used as pedestal for the egg) was killed, the subsequent others paralyzed. A different explanation (for *B.* near *capensis*) could be that of a “volutary” lack of stinging, in order to let escape the fly and so to mislead a satellite parasite present in the site [42].

According to Ferton, probably the wasp stings her prey in flight [38]; some species (as *B. handlirschi*) have developed particularly short wings, what make easier for them to follow the evolutions of flies flights [37]. However, it is very difficult to study the stinging method in a natural condition. What can be done is to induce a re-sting of the prey that has been withdrawn and then given back to the wasp. In such case *B. rostrata* may take off again to re-sting the fly in the air or may do the job on the ground or on a branch close to the nest. In the last two cases Ferton observed the wasp to seize the prey anteriorly, in a way to maintain it below and perpendicular to her own body. Then she bended her abdomen below the fly and stung it slowly and repeatedly just a little behind the mouth [38]. More recent findings regarding whether the position of the sting - close to the mouth or in the thorax [39: pp. 102-104; 40: pp. 12, 73] - or the fact that the in natural condition they capture flies either in flight or at rest [40: p. 12] - confirm at a large extent the observations of Ferton.

Fabre maintained to have observed the *Bembix* to chew the head and the thorax of the prey, in order to give the “finishing stroke” needed to kill them [18-I: pp. 280-281]. Ferton disagreed even on this point, since he never observed anything like this. He reports sometimes malaxation, in order to feed on the prey internal juices. But in these cases the fly was brought into the nest only if still intact and living, otherwise it was abandoned [38].

Moreover, according to Ferton the quickness of the attack may not justify any imprecision in the stroke and the subsequent paralysis, as stated by Fabre [18-I: p. 280] and other authors, since in some cases, as in that of *A. orbitalis*, an extremely quick stroke induces a complete paralysis of the prey [38]. The fact that the *Bembix* prey remain fresh for a shorter period should be not due to an imperfect way of stinging, but to some reasons inherent to the flies themselves, maybe because they become dry more rapidly. This could explain why the large flies live more than the small ones [38].

Orthopteroids hunters. Ferton was very involved in studies regarding Orthopteroids hunters. For what regards *Stizus faciatus* (Fabricius), it is only said that the prey is stung below the thorax [44]; for *Tachysphex mediterraneus* Kohl, instead, a more precise description of the stinging pattern is given: a first sting between the two last pairs of legs; a second between the legs of the first pair; a third close to the throat [35]. Remark the difference from the description reported above from Fabre for the mantis-killing *Tachytes*.

In *Dolichurus haemorrhous* A. Costa, a blattids hunter, the observed effect of the sting was very light, only able to reduce a part of the force of the prey which maintained its capability to run and to jump. Its vitality remained intact the first days after eclosion and the wasp larva ate a living and moving prey that, however, was unable to get rid of it [45].

Among the orthoptera-hunter wasps the most interesting observations concern the sphecid *Sphex*, already been long studied by Fabre. The method of stinging employed by the *Sphex (=Prionyx) subfuscatus* Dahlbom, restricted to only two strokes in the thorax of a female of *Calliptamus italicus* (Linnaeus), results more simple than that described by Fabre for *S. flavipennis*. One does not observe any stroke in the neck preceded by a widening of its articulation; neither any preliminary immobilization of the prey hindlegs. In the present case, the wasp rushed into the prey giving a first sting across the membrane which surrounds the anterior legs; then her abdomen moved to give a second and last sting at the origin of the second pairs of legs or, more commonly, at that of one of the legs of the third pair [30]. What is more important is the observation that “In spite of the great length of her sting, the *Sphex* probably does not reach with the second stroke the nervous centre of the two hind legs: it is by mean of the venom which it infuses, rather than by the lesion itself, that the legs paralysis is realized” [30].

Moreover, the position of the sting is determined by the fact that “the only vulnerable points are the soft membranes which surround the two anterior legs, and those of the articulations of the 4 posterior ones” [30]. The choice of the right target where to sting should not be attributed to an inborn knowledge of the internal anatomy of the prey, as supposed by Fabre [18-I: pp. 111-112], but to mere necessity. However, Ferton was far to doubt of Fabre’s findings, and ascribed the observed differences to interspecific diversity [30]. After having paralyzed her prey, *S. subfuscatus* female licks the liquids vomited from it. This operation seems to be useful either to the better conservation of the prey or to the feeding of the wasp [30].

Oxybelus. Offering an already paralyzed prey to an *Oxybelus melancholicus* Chevrier (= *O. haemorrhoidalis* Olivier) which is leaving her nest after having provisioned it, she hangs the new prey with her mandibles, curves the abdomen and stings the fly below the thorax close to the throat. Then she straightens her body and without drawing out the sting carries in flight the fly impaled on it, keeping it with the hind legs. Probably the single sting in the thorax is the only one received by the prey. More than half of the flies collected from the wasp nest gave signs of life when stimulated. [23].

Eumenine wasps. Fabre had discovered that both *Odynerus* and *Eumenes* possess a “hanging egg” [18-II: p. 94]. He claimed to have obtained this result, confirmed by his own observation, by mean of a deductive way of reasoning. The paralysis was very incomplete, whether for the caterpillar or the larve beetles preyed by *Odynerus* or *Eumenes*: from a nest of *E. amedei* Lepeletier were extracted caterpillars half transformed in chrysalides. The wasp egg would have very easily been destroyed in the middle of the actively moving caterpillars which completely fill the cell [18-II: pp. 73, 76, 77, 82, 92]. Unless

> As I foresaw by my process of reasoning, the egg is slung from the ceiling of the cell. A very short thread fastens it to the top wall and lets it hang free in space. The first time that I saw this egg, quivering at the end of its thread at the least jerk and confirming by its oscillations the correctness of my theoretical views [18-II: pp. 93-94].

Re-examining the problem, Ferton confirmed the high vitality and mobility of the prey of the vespid wasps *Odynerus* [24, 23], *Pterocheilus* [31], *Eumenes* [23, 31]. But he could not agree with the Fabre’s opinion concerning the reason of the “hanging egg”. Firstly, because in six observations regarding one of the same species considered by Fabre, *E. pomiformis* Fabricius, only one case showed an egg maintained vertical; but it had been pushed against the wall of the cell by the mass of the stored prey. In the other five cases, the egg lay horizontal, resting above the prey layer and in contact with the cell ceiling. In all cases the egg was attached to its thread. Ferton reported that the different situations did not influence the egg eclosion and the initial feeding of the larva, not differently from what resulted in the case of an egg hanging freely in a cell from which part of the caterpillars were removed. The same result was obtained when the egg, always resting on the prey layer, was detached by its thread [30].

Similar findings were obtained for *Odynerus* and *Pterocheilus*: in both cases Ferton, differently from Fabre, was able to carry the content of the cell - egg and prey – thrown without any caution in a box where the wasp larva regularly developed [18-II, pp. 94-95; 23, 31].

Moreover, the egg of *Eumenes* is not as frail as supposed by Fabre. At the contrary, one may manage it without many cautions, to let it fall or to carry it detached by its thread without, in general, any damage [18-II, pp. 77; 30]. The fragility was an important reason invoked to explain the presence of a "hanging egg", which otherwise would have been crushed or destroyed by the prey. The reason of the "hanging egg" should be found elsewhere: to escape humidity or, maybe, parasites of the wasp egg [23].

However, the incomplete paralysis could have an adaptative value and not merely be due to an imperfection of the stinging method. In fact, Ferton observed that in *E. pomiformis* the mobility of the caterpillars may be of help for introducing them in an already crowded and full cell [30].

George Williams (1845-1914) and Elizabeth Maria (1854-1940) Peckam

One will review here a few findings concerning the stinging behaviour of North American solitary wasps which were revealed by the Peckam's.

Spider-wasps (Pompilidae) – *Pompilus*. The Peckam's were unable to determine the method of stinging. The experiments of substitution of the prey failed, while in natural conditions the movements were too rapid to be observed. All they saw was a violent struggle with "both the combatants rolling over and over upon the ground". After an instant it was over, the spider lying motionless on the ground. Before beginning the transport, the wasp checked her prey: "With the utmost circumspection she settled down upon the spider and made a prolonged and careful examination of the mouth parts" [20: pp. 131-132, 149-150]. However, the conditions of the prey stung by *Pompilus quinquenotatus* Say is very variable: they may be killed or very deeply paralyzed or alive and survive from a few days to more than forty [20: pp. 129, 136-137, 143]. This contradicts Fabre's statements

> Fabre bases his very strongest arguments for the exactness of the method by which wasps sting their victims, upon the action of one of the Pompilidae, but certainly *P. quinquenotatus* can make no claim to nice workimanship, for if she occasionally stings in such a way that life is preserved for some time it seems to be a matter of chance rather than of skill. In one respect, however,...she is very successful...Even in the case of the spider that lived forty days the power of motion did not return, to any extent, during the first ten or twelve days, and before this time in the natural course of events, there would have been nothing left to move [20: p. 138].

Spiders collected by the Peckams's from *Pompilus biguttatus* Fabricius confirm the wide range of possible situations.

> Of three spiders stung by *bigutattus*, one was dead when taken from the wasp. The second, taken on July sixth, seemed to be quite dead until the eighth, when it gave a slight response to stimulation. From this time it improved, at first slowly and then rapidly, until on July fifteenth it drank water and moved all its legs without stimulation. On the eighteenth it began to walk, and by sixth of September it had entirely regained its health and was released. The third spiders, which was taken with the egg upon it, lived until it was destroyed by the larva [20: p. 140].

For other species one observed similar situations: spiders either dead or living for a few days (*Pompilus calipterus* Say); dead or alive from one to twenty days, but even a dead spider in sufficient good conditions to be used as food for eleven days (*Pompilus marginatus* Say); one dead spider (*Pompilus interruptus* Say). [20: pp. 144, 145-147, 149, 152, 153].

A big Lycosid spider captured by a *Pompilus scelestus* Cresson appeared immobile and dead after being stung, but after a few hours some quivering of tarsi, when stimulated, began to appear and one day later the prey was almost recovered. A few days later it fed on flies, was able to run and showed co-ordinated control of its movements. However it remained more sluggish than usual and permanently blind. A second case gave similar results: at first the spider taken from the nest was motionless and did not give any response to stimulation, but within twenty four hours the effect of the venom was at great extent passed off and in the third day the spider recovered its health and was released. According to these findings, the Peckam's stated that the cephalotorax ganglionar mass should have been affected by the poison whose diffusion in the whole ganglion explained the complete paralysis. The main damage should not be structural, at exception of the region directly pierced by the sting that, in the first case, should have been responsible for permanent lesions at the optical centres. In fact, pushing a fine needle (six or seven times as thick as the sting of the wasp) through the ventral ganglion of a spider, one obtains a result which may not be comparable with that of the sting: a partial paralysis for a period of six days, ensued by the death [20: pp. 153, 160-163].

In some cases the Peckam's observed the wasp biting the spider legs, which often appear to be cut off. For *Pompilus fuscipennis* St. Fargeau, this is a common habit, even if the behaviour is very discontinuous: four out of ten spiders had all their legs, the others being deprived of one or two of them. Biting at the spider legs is made at intervals, starting from the moment when the prey is captured, but becomes particularly frequent at the moment to introduce it into the nest. This operation is possibly related to the narrowness of the pompilid nest and cell which makes necessary for the spider "to be shifted and turned and tugged”. It may not be necessary to remove the legs, but only to render the limbs more flexible, making it possible to drag the spider down. In fact, the legs of intact spiders were sometimes squeezed, showing the marks of the wasp mandibles [20: pp. 140-141, 143-144, 164]. Even *Pompilus scelestus* Cresson was observed biting and squeezing the spider legs [20: pp. 154, 155, 158]. During one of the frequent pauses made by the wasp while she was dragging her spider

> She seemed to be biting the legs, near the body, beginning with an anterior leg on one side and working backward and then repeating the operation on the other side. She went through this squeezing process again and again, and to us it looked as though she might be trying to force back the juices from the legs into the body preparatory to cutting them off, but after a time she would seize her prey and start on again [20: p. 154].

The high mobility frequently showed by the stored spiders after their recovery, should be one of the reasons of the narrowness of the cell: “The prey is buried alive in the fullest sense of the term but is wedged in so tightly that not the slightest movement is possible, and thus the egg is protected” (Peckam & Peckam, 1898, p. 163) [20: p. 163].

Spider legs amputation (see also above) was observed by many other researchers. Recently, Andrietti et al. [46] have confirmed the high variability of this habit in *Anoplius infuscatus* (Vander Linden), which may be alternatively present or absent either in individuals of the same species or in single individuals. In the same work is reported the high mobility of the stung spiders and the fact that in some cases one observes a certain difficulty in the introduction of the spider into the nest, what could justify the reason why legs may be cut. On the contrary in *Episyron* sp., where leg cutting is quite rare, no obstruction in spider introduction into the nest was ever observed [46].

Apart from *Pompilus*, the Peckam’s observed more briefly other spider-wasps genus obtaining similar results, either for what regards legs amputation (*Agenia*) or the condition of the prey: dead (*Agenia*, *Salius*) or alive and in general recovering from paralysis in a few days (*Aporus*) [20: pp. 54-57, 164-166]. In the case of *Salius conicus* Say, it was even possible to observe the method of stinging, consisting in seizing the spider by its head, to bend the abdomen around and below the prey and to sting in the middle of the ventral side of the cephalothorax. The spider collapsed almost immediately and the wasp repeated the operation a second time in the same manner. In a second observation, the spider was stung to one side of the middle of the ventral side of the thorax [20: pp. 53-54].

Astata. Is a genus of bug-hunters. The method of stinging of *Astata bicolor* Say consists in seizing the bug head in her mandibles, holding it dorsum up, and to curve her abdomen to sting it below the thorax, presumably in the thoracic ganglia. Malaxation is common, and in some cases it could substitute stinging since some bugs appear quite alive. But most of them appear to be dead or will die in a few days, and wasp larvae feed mainly on dead prey. Similarly quite dead were the bugs taken from *Astata unicolor* Say [20: pp. 92, 95-97].

Instead an European species, *Astata picea* A. Costa, according to Ferton stings her prey in the throat, and then performs malaxation to feed on the prey juices or maybe to complete the effect of the sting [23]. According to more recent findings, *Astata* prey are completely paralyzed [47: p. 212].

Cerceris. *Cerceris clypeata* Dahlborn hunts beetles (weevils) which stings a first time under the neck, and then behind the first pair of legs. The prey is picked up again and stung in the same manner four times in sequence, with intervals of five or six minutes between. For what concerns the condition of the stored beetles, most of them appear to be dead. The same may be said for the beetles hunted by *C. deserta* Say and *C. nigrescens* Smith, which mostly appear to be dead or quite dead [20: pp. 114, 116-117].

Sceliphron. To determine the stings pattern of the maud-dauber wasp *Sceliphron*, a hunting spider sphecid wasp, is quite hard. In his chapter entitled "Les Vivres du Pélopée" ("The provisions of *Pelopaeus* (= *Sceliphron*)" [18-IV: Ch. 2], Fabre clearly admitted the difficulties he met to discover the hunting method of *Sceliphron*. He could only observe

> …the wasp swoop down upon a spider, clasp it, and carry it away, almost without pausing in her flight. Other hunters alight on the ground, make their fastidious preparations sedately, and distribute the strokes of the sting with the calm deliberation which a delicate operation demands. This one darts down, seizes her victim and departs, something after the manner of *Bembex*. So rapid is the abduction that it may be presumed that the mandibles and sting are only used while the wasp is on the wing. This impetuous method, incompatible with learned surgery, explains to us, even better than the narrowness of her cells, the predilection of *Paelopeus* for small spiders. A larger and more powerful victim may put the wasp in danger. The faultiness of her art makes a weak victim necessary to her. We must suspect that a spider so hastily and carelessly taken, may be killed. As a matter of fact careful and repeated scrutiny of these cells, when the egg was not yet hatched, confirms these suspicions. There is never any trembling either of palpi or tarsi, and in about ten

> days they decompose. This then is what is stored in the cells of *Pelopaeus*, spiders dead or nearly dead [18-IV: pp. 27-28] (free translation from [20: pp. 190-191]).

A few years later Peckham & Peckham made intensive researches in this same topic. After many failures in the attempt to reproduce experimentally controlled situations [20: p. 181], they partially succeeded in observing direct attacks on the spider *Epeira strix* Hentz (= *Larinioides cornutus* (Clerck)) by part of the American species *Pelopaeus coeruleus* (= *Chalybion caeruleum* Linnaeus ?). In most cases, at the arrival of the wasp spiders were dislodged and dropped. Sometimes they were followed and were caught on the floor but more often the wasp left them escape continuing her searching on the wall, eventually succeeding in catching a victim. It appeared that the stinging was made in two steps: a first time, after being seized by the jaws and first legs of the wasp, the spider was stung under the abdomen or more commonly at the cephalotorax, underneath or at one side. The general impression was that this first sting was given at random anywhere, in order to induce a temporary quiet in the prey and to allow to perform the second step. It consisted in alighting upon a nearby object and to give a second sting, “either resting quietly or rolling the spider around and around” [20: pp. 182-183].

On a large sample, made mainly of *P. coeruleus*, but even of *Sceliphron cementarium* (Drury), which is a species now present in Europe too, the Peckham’s found that the 33 % of the spider were paralysed, the remainders being killed. The number of paralysed spiders was higher of that found by Fabre in France. Comparing their own data with those of different authors and related to different mud-dauber wasps, the Peckham’s found a variety of situations: some species killed almost all spiders, other more than half of them, others left them alive but in a helpless situation [20: pp. 176, 186-187].

Contrary to what happens for other ravishers like the spider wasps, the stinging method used by *Sceliphron* appears primitive and imprecise.

> Is the learned art of paralyzing practiced by *Calicurgus* upon the tarantula, which keeps it fresh for seven weeks, unknown here? Have we here to deal, not with a delicate operators who knows how to abolish movement without destroying life, but with a brutal worker who kills for the sake of rendering the victim immovable? Both the withered aspect and the rapid deterioration of the victims bear witness that this is true [18-IV: p. 28] (free translation from [20: pp. 191]).

The way of consuming the prey is made in accord to this situation, namely the shortness of the period of *Sceliphron* larval life and the use of a large number of relatively small spiders, which are devoured rapidly one after the other before decomposition can progress. On the contrary, as stated by Fabre, when a large prey is utilized, it is necessary to maintain them alive “and a special art must also be observed to eat it”, otherwise early decomposition will advance rapidly in the half eaten and disorganized body mass [18-IV: p. 28-29].

In any case

> Our study of the eating habits of these larvae has led us to the conclusion that they are not in the least fastidious as to whether the food is hard or soft, fresh or dry....On several occasions when playing nurse-mother to a number of growing larvae, which we kept in little glass saucers, where we had not provided a large enough food supply we made good the deficiency by adding a number of dead and dry spiders that we had had on hand for some three weeks [20: p. 189].

From a sample of 11 cells (*Paelopeus coeruleus* + *S. caementarium*), the Peckham’s found 75 spider killed and 84 alive, which mostly died within the first seven days from the moment when they were stored. Since dead spiders, even if drying up, remain in good condition for 10-14 days [20: pp. 186, 195-196], one sees that for the larvae there is not a big difference in the use of dead or living prey.

The Peckham’s performed a certain number of experiments in order to determine the effect of the wasp venom. By mean of a *S. caementarium*, which was induced to sting a few spiders at the spinnerets, a point far from the central nervous system, they obtained the following results

> The spider was paralyzed at once...but in five minutes it recovered somewhat and was able to stagger about. This continued for ten minutes when the wasp was allowed to sting it again in the same place with the same immediate results as before but this time there was no recovery and in thirty minutes the spider was dead....All of these spiders and many others were killed by the general diffusion of the poison through the system and not by any wounding of a ganglion. When we allowed a considerable amount of venom to be injected death followed almost immediately but if a smaller amount was used the spider lived a day or more [20: pp. 194-195].

Trypoxylon. In *Trypoxylon albopilosum* W. Fox (now *T. striatum* Provancher) the stung spiders resulted either be killed at the moment of the capture or, if only paralyzed, they died in the nest from day to day. In

Trypoxylon rubrocinctum Packard (now *T. collinum* F. Smith ssp. *rubrocinctum* Packard) stung spiders were alive or, at a slightly minor extent, dead; among the living ones, none lived more than fifteen days, less than the best paralyzed prey of *Sceliphron*. Apparently, dead or alive spiders were equally usable by the wasp larva. A spider capture was observed in *Trypoxylon bidentatum* W. Fox: the wasp seized the spider by the top of the cephalothorax, curved her abdomen and stung it in the ventral side of the cephalothorax. The Peckam's observed that, due to the large extension of the ventral ganglionic mass, "a thrust given in almost any part of ventral face of the cephalothorax, or even on either side of the anterior part of its edges, would reach the nervous center" [20: pp. 77, 78, 81-83, 85, 86].

Diodontus (*D. americanus* Packard). Aphid hunters. According to the Peckam's there is no sting, the prey is only picked up and carried off.

> We found that when a wasp secured an aphis she flew with it to another leaf near by, alighting, this time, on the upper surface. She then passed it back from her mandibles to the second pair of legs, and holding it, with them, under the body, she proceeded to make use of the first pair in giving herself a through cleaning [20: p. 101].

The wasp, bringing the aphid forward, squeezes its neck delicately but repeatedly between her mandibles. In most cases the aphides were killed, but in other cases the operation was so light that they so little injured to be able to walk around as soon as they were released. However, only one, out from forty aphides drawn from nest, were alive [20: pp. 101, 105-106].

The whole process may be observed more conveniently, as many times as one wants, placing the wasp in a bottle together with a leaf upon which aphides are present [20: p. 101].

> The tiny wasp would pounce upon an aphis, and holding it with the first legs would squeeze its neck gently between the mandibles, rolling it over and over. After a few moments she would pass the aphis back to the second pair of legs and rest for a short time, usually taking this opportunity to wash her face....In the open, *Diodontus* often alights on one leaf and malaxes her victim and then flies to another and another, repeating the process several time before she finally flies off to her nest [20: p. 102].

Sometimes the captured aphids served not as food for the larvae but for the wasp. In these cases there was no malaxation and the aphis was held in any position, sucked of all its juices and thrown away [20: p. 105].

The Peckam's acknowledge that Fabre "is unquestionably the most accurate of observers", but this does not mean that "all of his inferences must be accepted". In particular they question the point, on which Fabre insisted so many times, regarding the way of stinging that should have been perfectly developed from the origin, remaining otherwise useless for the wasp. In fact the Peckam's note that, to be true, "there is not a single species in which the sting is given with invariable accuracy...they scarcely sting twice alike". This observed variability matches that regarding the state of the prey of the same wasp, some of which may result killed at once, others survive from one days to six weeks or eventually recover. This result seems to indicate to the Peckam's that the primary target of the wasp is not that to paralyze her prey, but only that to subdue it. To this aim different methods may be employed: killing, paralyzing or simply capturing it. The habit to sting in the ganglia simply represents the most advanced degree of this process which has developed, when necessary, to overcome the difficulty to deal with a dangerous prey (as in case of spiders) or the difficulties inherent to its transport (as for grasshoppers). This different view clearly opens the possibility to explain the nowadays situation through a natural selection mechanism that Fabre's perspective explicitly precluded [20: pp. 102, 222, 225-226].

Even the fact of stinging in the nervous centers is not a matter of experimental finding. It is simply an inference based on the observation that very often the prey are not killed but merely paralyzed, remaining motionless for weeks or even recovering. In fact, according to the Peckam's, one third of the solitary wasps kill their prey. Moreover, they question the fact that the real target of the wasp should be that to induce paralysis instead of killing: in many cases the period required by the larva to feed is short enough to make it possible the use of either paralyzed or dead prey [20: pp. 222-224].

The "inference" about the anatomical position of the stings could not be resolved in more positive terms at the Peckams's times and will remain object of experimental investigations for the subsequent century.

Recent findings I: Steiner's review and Steiner's work on *Liris*

The works concerning wasps' stinging behaviour written in the 20th century will be not summarized here, since they have already been reviewed by Steiner [2] until 1986, in a fairly exhaustive way (including a huge amount of bibliography). Instead, one will illustrate only a few specific points mainly related to the work of Steiner himself, whose studies represent the most detailed and comprehensive contribute to the stinging behaviour of some selected species.

Among the Sphecid wasps one may consider a first group of wasps hunting molecrickets (*Larra*: Larrini), cockroaches (Ampulicinae) and crickets (*Liris*: Larrini), which are all stung in the throat (together with other possible stings in different parts of the thorax). In the first case one observes a temporary partial paralysis followed by complete recovery (no deactivation); in the second case the paralysis is substituted by a condition of passivity and ineffective resistance which does not hinder the animal to feed (partial deactivation); in the third case, after recovery from paralysis (a few minutes), one observes permanent deactivation, without feeding: "The crickets have been transformed into passive "reflex machines"; they can stand on their feet and jump or walk if prodded but do not try to escape, feed or groom" [2: pp. 115-117, 122].

Cockroach hunters may be found even in the Sphecinae and in the Larrinae: in the tribe Sceliphronini one finds a group of cockroach hunters and even a few *Tachysphex* species (Larrini) make the same. However, for the prey of these hunters the situation is more variable than before, varying from complete to incomplete paralysis with subsequent recovery. The stings position is unknown [2: pp. 117-118].

Even some cricket-hunters may be found in the Sphecinae. Remember, for example, the case of the Fabre's "Yellow-winged Sphex" (*Sphex flavipennis*), whose first sting, as reported above, is in the neck. However, the real cricket specialist is represented by the genus *Chlorion*, studied by Hingston (1925-1926, quoted by [2: p. 121]). She stings the thorax two to five times, in an irregular fashion which perhaps does not include venom inoculation, then the neck. After this last stroke the prey becomes completely motionless. This sequence of stings, according to the terminology employed by Steiner, should represent a fairly good representation of the C4SP pattern (four stings: a first sting in the neck, followed by a sting in each of the thorax segment), but in a reversed order. A few subsequent abdominal stings were described and a recovered but in some way weakened cricket three minutes later in the burrow [2: pp. 120-122].

The study of Steiner [48] of the stinging behaviour of *Liris nigra* (Van der Linden) (= *Notogonia pompiliformis* (Panzer)) is a true masterpiece, which constitutes a landmark in this kind of studies and deserves to be exposed in some detail. It was the result of many years of study on the stinging activity of this wasp made in laboratory on bred animals and of the analysis of thousands of stings [48: pp. 4-5].

A first point concerns the external localisation of the stings: "only the thoracic and cephalotorax regions of the cricket [larvae of *Gryllulus* *domesticus* Linnaeus; *L. nigra* in general paralyzes immature only, since the adults, although attacked, are too large for this species] shows the marks due to the stings, and only their ventral or latero-ventral parts" [48: pp. 7, 9; 59]. And, more precisely: "All marks of stings are located on the soft and flexible intersegmentary membranes, excluding the chitinuous regions ...crowding, at a certain extent, in the folds, furrows of the soft membranes...or around the different sculptures or tegumental "accidents"" [48: pp. 9-10]. Steiner subdivided the whole "sting region" in twelve "sting areas", located on the soft thoracic-cephalic membranes. Due to their symmetrical distribution with respect to the longitudinal axis, only six of them will be considered. Within each of these areas the density of stings is not uniform, but concentrated in the vicinity of a "characteristic point" around which the sting marks decrease according to a "centrifugal" gradient [48: pp. 10-11].

The stings dispersion around their characteristic points varies as a consequence of the shape and the accidents of the corresponding sting area: stretching in the direction of an elongated membrane, stopping against cuticular reliefs, following the membrane furrows or extending largely and freely around a region of membrane wide and uniform. It is particularly restricted when the characteristic point is situated in a part of the cricket cuticle better localized with respect to some morphological markings, as for examples sculptures, tubercules a.s.o., which allow the wasp to sting with improved precision. However, it depends even on the prey size, increasing with it and interesting even sectors which are unaffected in the smaller sizes. Two possible explanations have given to this fact. One, larger and more powerful prey may better react to the wasp aggression, reducing the degree of the stinging precision. In fact, stings dispersion decreases with subsequent strokes, since even prey resistance progressively decreases. In addition, with the largest crickets the wasp may experiment some difficulties to reach the median ventral line, so increasing the degree of lateral dispersion [48: pp. 15, 18, 20, 21].

Another possible source of variation should be attributed to individual differences: some particular individual wasps frequently sting the cricket more or less constantly in peripherical sectors which are not normally used [48: p. 21].

The second point regards the direction of the stings which, with a good approximation, is indicated by the orientation of the wasp abdominal extremity in a photographic frame. It varies mainly in the antero-posterior direction, while the fairly constant angle which it makes with the horizontal plane is small and may be disregarded. In each stinging area the sting maintains a direction sensibly constant, with a small dispersion around a value that may be considered as the "characteristic direction" of the area. It is worthwhile to observe that analogously to what happened for the soft membranes, which were unable to explain the localization of the "characteristic points", one may not invoke the presence of "postural reflexes" in order to obtain a quite automatical constancy in the direction of penetration of the dart in the stinging areas. Even if they certainly will play an important role in realizing and maintaining it, as a relief or any other accident in the tegument may better delimit the dispersion area around a "characteristic point". In fact, the abdominal extremity of *Liris* assumes a direction very close to that characteristic of the area, in spite of the many different positions which the body of the wasp may assume during her fight with the cricket. Moreover, one finds that the sting direction may vary abruptly (still maintaining a bilateral symmetry) moving from one to an other of two close (which should imply similar postural reflexes) "characteristic points", according to the different "characteristic directions" of the correspondent areas [48: p. 22, 23, 26].

For what concerns the internal localization of the stings, it is necessary to follow the "characteristic directions" starting from the "characteristic points". One finds that all of them converge on a ganglion: the first two (Ia, Ib), beginning from the rear of the cricket, on the metathoracic one; the two intermediate (IIa, IIb) on the mesathoracic; the fifth (III) on the prothoracic; the sixth (IV) on the subesophageal one. The structure which governs the direction of the stings is therefore that of the four ganglia where they converge, rather than the six "stings areas" and correspondent "characteristic points", which in fact mask the true four-element sub lying structure. According to this view, one observes only four kind of different paralysing effects according to the different stings: those in the area Ia or Ib, affect only the hind legs pair; those in IIa or IIb, the intermediate pair; that in III, the front legs; that of IV, the buccal parts. In every case the sting results in the immediate immobility of the respective appendices, and only of them. The complete paralysis of the cricket is therefore the result of the addition of elementary paralyses, separated in space and time [48: pp. 26-28].

Owing to the chitinuous shield which prevents the ganglia from being directly reached, it is necessary to sting them laterally. The only reason why the stings Ia and Ib, IIa and IIb converge on the same ganglia may be due to the presence of the bases of the two posterior pairs of legs, which divide the areas where the sting can be inserted [48: pp. 28-29].

For what concerns their temporal sequence, in "normal" conditions the wasp gives four paralysing stings, rarely more, still less frequently a minor number, according to the following ways:

- fundamental formula (Ia + III + IIb + IV) or formula *a*

The prey, pursued by the *Liris,* is boarded usually from behind; the wasp tries to get on the cricket back but, due to its violent opposition, rarely succeeds in doing so and what obtains is only to grasp at it, seizing a leg between her mandibles, in general the hindest one, with the additional help of her legs which may clasp, for example, those of the prey. The cricket, always running away, carries the sphecid behind itself (unless the frequent autotomy of the posterior leg has been able to free it from its persecutor), while the wasp tries to thread her abdominal extremity behind the base of the seized hind leg, on the soft articular membrane or sting area Ia (this position will be called posture *Ia*...); when she succeeds, she unsheathes her sting giving the first stroke...Immediately the posterior legs of the prey become motionless after a few last tremors...it is this first sting which may be exerted in the most variable positions, depending on the randomness of the fight with the cricket still in possession of its whole vitality; in spite of that, the *direction* of introduction of the sting, characteristic of the area *Ia* is almost invariably maintained *by the abdominal extremity* of the *Liris*... Notwithstanding the paralysis of the posterior legs, the cricket is still able to move or at least to toss about, using its anterior and intermediate unharmed legs. The sphecid (which has not to face with an opposition as strong as that of the beginning), is now able to rise on the back of her victim and places herself slantwise, at the same time lowering her abdomen toward the latero-ventral side of the cricket which is explored by mean of the abdominal extremity...which eventually stops when reaches a position between the bases of the first and second pairs of legs. Firstly, the abdominal extremity is pointed forward in the direction of the base of the foreleg, which receives a sting from behind (in the sting area III...): the two anterior legs are immediately immobilized, stopping each possible progression of the cricket which may nevertheless to shake its middle legs, still trying in vain to escape; then the abdominal *tip* of *Liris* pivots toward the *rear* of the cricket (while the rest of the abdomen remains in its position between the anterior and middle legs...), i.e. in the direction of the base of middle legs which receives then a sting from *forward* (on the sting area IIb), abolishing the movements of the middle legs; the cricket is still able to shake its head in every direction, opening and closing the mandibles, but a last sting in the neck in the area IV gets rid of this last possibility of movement....The immobility of the cricket is nearly total (some movements of the antennae, abdomen et cerci may persist) and lasts a few minutes, a time which is in general sufficient to the wasp to lay the egg and to end nidification. After this

> delay the cricket recovers progressively the use of its different paralysed appendices. It may happen that the wasp skips one of the stings; in such case the prey maintains the use of the correspondent appendices... [48: pp. 32-34].

The fundamental formula includes therefore four stings but only three different postures, since during stings III and IIb the wasp maintains the same position with respect to the cricket body [48: p. 34]. It may be subject to some variations. Sometimes the wasp, as already said, may be able to get on the cricket back (what "typically" happens after the sting Ia, with an already weakened cricket). In such case she assumes a different posture (Ib, IIa): instead of placing her abdominal extremity behind the hind leg, she positions it between the middle and the posterior ones. The first sting is then given in the area Ib, before the posterior leg. However, even so both the posterior legs are paralyzed, due to the convergence of the "characteristic directions" of both stings Ia and Ib on the same ganglion. The remainder of the sting pattern will be the same of the previous one, and the new formula will be:

- Ib + III + IIb + IV [48: p. 35].
  But still other variations are possible. For example, still starting from posture (Ib, IIa), a second sting is given in IIa, or sometimes simply sketched:
- Ib + IIa + III + IIb + IV. In such case the sting IIb will not produce any effect, since the middle legs have already been paralysed by IIa. But it may even happen, even if very rarely, that in the last variant the sting IIb is only sketched. Then one obtains again a four-stings formula (instead of the previous 5 stings sequence):
- Ib + IIa + III + IV [48: pp. 36-37].

In *normal* conditions holds the rule that every ganglion will receive one and only one sting, with the only exception of the variant of the five-stings pattern: Ib + IIa + III + IIb + IV. A second rule regards the postures, which give rise to a double stings when the wasp abdomen is positioned between two consecutive legs ("two-stings posture"). In the typical case represented by the fundamental formula both rules are respected. Instead, in the variants, one of the rule will be violated. Either that concerning the number of stings received by each ganglion or because a two-stings posture gives rise to one single sting [48: pp. 37, 39].

Until here one has considered a "normal" situation. But some difficulties may arise, which interrupt the "normal" development of the sequence, like the fight with the prey or the presence of other individuals or mechanical obstacles. In such cases different solutions may be adopted.

A.- *Liris* "skips" one given sting (rarely), passing directly to the subsequent one; then

a) once the obstacle is removed, she may go back to the omitted sting (the most frequent case of the two);

b) the omitted sting is definitively forgotten.

B.- *Liris* stops (more common case). She does not give the subsequent stings trying obstinately to give the "blocked" sting.

a) If the obstacle is removed after a certain delay:

aa) *Liris* takes up again her paralysing activity, starting from the blocked sting;

bb) *Liris* takes up again her paralysing activity, starting from the beginning (more rarely), so that a few sting will be given twice.

b) If the obstacle is permanent or the area to be stung is artificially suppressed:

aa) the vain efforts to execute the blocked sting vanish after a period which is in general rather long, the sting is omitted and the wasp goes on with her sequence;

bb) the wasp abandons the "abnormal" cricket and begins a new complete stinging sequence on a new one; however, the preceding event will leave a trace, since it is rare that the wasp will perform in a typical way the sting that had been blocked: she will "insist" a long time in executing it or will give it many times, either consecutively or returning back to it after subsequent steps in the cycle [48: pp. 40-41].

In some cases, variations in the stinging sequence may be observed independently from external conditions, as at the beginning or end of the reproductive period, due to some internal factors or to undetermined or fortuitous reasons. It is even frequent to observe an inversion in the stings sequence, particularly of stings III and IIb, probably related to the facility to exchange them in the two-stings posture (III, IIb) [48: p. 41].

For what concerns the cricket, its "normal" behaviour in respect of *Liris* attack consists in a flight reaction. In the case of a different response, especially in the case of absence or weakness of flight or antagonistic reactions, the wasp may respond either with a stings sequence of the "normal" type, or with a paralysing sequence of a special kind (c-type, see above) or even shifting to a different section of her nesting cycle, for example prey transport or malaxation [48: pp. 42-43].

In much less frequent cases the cricket responds in a completely different way with a “freezing reflex”, consisting in a rigid posture in which the abdomen and the hind legs are straightened on high, while the other legs are strictly adherent to the body and the antennae completely folded back. This position is much more apt to avoid the attack of the wasp which in most cases, after a few turns around the cricket, went away without stinging it. Similar and still less frequent, is a specular reflex: the cricket assumes a position with the head, instead of the abdomen, straightened on high. Even in the few cases when this posture was observed, the *Liris* went away without giving any sting [48: pp. 44-45].

According to Steiner, in the “freezing” posture the cricket weakens the wasp stinging response. However, this effect should not be attributed to the immobility, since motionless crickets (paralyzed crickets or even exuviae) still elicit the stinging response or at least the beginning of it. One may not reduce the stimulus to an elementary external condition, like a smell or a specifical chemical substance, but one should think to a more complex factor, to a “significative stimulating situation”. One must observe that the kind of prey is very specific, since it was impossible to induce the wasp to sting insects others than crickets, even if closely related to them, unless they were imbued with the smell of *G. domesticus* or covered with its spoils. But, even so, one observed only a few attacks not followed by stings [48: pp. 45, 48].

However, also individual factors may play an important role. A few wasps, for example, seemed to be unable to unable to carry on a correct stinging sequence and inclined to rubber already paralysed cricket from other ones, developing a cleptoparasitism which is probably latent in all *Liris*, even in those (the most part) which master their paralysing activity [48: p. 46].

Even the size of the cricket is important, since the largest ones were able to repel the wasp’s attack. In this way one obtains an automatic control of the prey dimension, since too large prey are useless. In fact, if one prevents the cricket from defending itself, leaving it to be paralysed, the wasp will not be able to transport it to her nest [48: p. 46].

Steiner explored even the ontogenesis of the sting pattern, observing how it developed at the beginning of the hunting period. He found three forms of *incomplete* pattern (formulas of the kind *b*): Ia; Ia + III; Ia + III + IIb. These forms followed each other in this transitory period, eventually leading to the complete formula Ia + III + IIb + IV, matching the parallel increase of the hunting motivation of the wasp and of her sensibility to the presence of the cricket. The lacking stings may sometimes be sketched without unsheathing of the sting. However the stinging sequences, in spite of being shortened, show the same order of the normal one, which is simply interrupted before being completed. The wasp in general abandons the cricket subject to incomplete stings pattern, not even malaxating the base of the anterior legs it as it is usual at the end of the normal case. In particularly good conditions the transitory period may be very short and difficult to detect or even completely absent, the wasp beginning immediately with the normal sequence [48: pp. 49-53].

If the sequences of the b-type, in spite of being incomplete in the number of stings, maintain the same structures of the a-type (typical), the same is not true for the c-type (atypical formulas): IIb; III; III + IIb; III + IIb + IV. In this case the basic sting, which is never lacking, is IIb (less commonly III), instead of Ia which, at the contrary, is the last to appear when the atypical sequence matches the typical one. The c-sequences appeared when the cricket did not show a "normal" reaction to the wasp, but a decreased one. This in general happened when it had already received the paralysing stings before. In fact the prey, a few minutes after being stung, progressively recovered its capability of moving and jumping. Even if, as already stated before, it will maintain a passive behaviour, able to respond to external stimulation but lacking of spontaneous movements as feeding (deactivation). In any case after a period of about one week the cricket resumed an increasing torpor which will lead it to die [48: pp. 54-56].

When a wasp, during the hunting period (characterized by the fact that she is not yet in possession of a prey) encounters a half recovered cricket (for example previously abandoned by the same or by another wasp), she may avoid to sting, maybe malaxating it if there is no response to the contact (what corresponds to the usual situation in the malaxation). Otherwise, she will begin to assume the posture corresponding to sting Ia; if the cricket does not respond vigorously to this contact, by opposition or by escaping, the wasp will leave this posture passing to (III-IIb), which will lead to sting IIb, that will result the first given to the cricket. Then she will provide subsequent stings in accord with the opposition of the cricket: no other sting, in case of weak reaction; or III + IIb, III + IIb + IV in case of increasing responses, however always lacking of the flight response. Only when this is present the sting Ia will be given, making the c-formula converge to the normal one [48: pp. 58-59, 62, 66].

C-sequences may even be observed in the subsequent phases when the wasp is already in possession of the cricket, as during the transport, when the prey oppose some resistance. This may happen if the prey has been badly paralyzed, or the transport phase is abnormally long or for accidental "exchanges" of crickets in different stages of their recovery period. But even due to factors independent from the cricket, as to obstacles to the transport due to the presence of roots or afterward an attempt of robbery of the prey by part of a different *Liris* individual. In these cases the wasp will equally make use of the atypical c-sequences, with the difference that the preliminary Ia posture will be omitted, but with a lower level of response, what greatly increases the proportion of the stinging sequences reduced to only IIb or more rarely III. In other words, the threshold necessary to elicit the stinging response will rise as soon as one moves further away from the more sensible hunting phase. Then, at the closing phase, the wasp becomes completely insensible to the presence of the prey which is treated as mere building material [48: pp. 59-60].

According to Steiner, one may explain the wasp stinging response (which may be generalized to other kinds of responses of her biological cycle) in terms of a reaction to two different kinds of “situations”: that of “opposition to the manipulation of *Liris* on the cricket” and that of “subtraction of the prey = escape of the cricket”. In fact, in her stinging sequence the wasp does not make any distinction between active or passive movements of the prey. It is equally possible to observe the stinging reaction either in case of escape of the cricket or its accidental loss or when the observers try to take it (even using cricket recently dead). This may explain a fact well known to the observers, who often reported how wasps may be induced to sting their already paralyzed prey in many different circumstances [48: pp. 62-63].

In some cases only the stinging Ia is observed: this happens when the cricket performs only an *escape reaction*, independently from the *opposing reaction*, as it may happen when it has been paralyzed only in the first two pairs of legs, maintaining the movement of the posterior ones, the “jumping legs” which may enable it to jump away. To summarize the different possible stinging responses [48: pp. 63-64]:

- cricket escape + opposition = a-paralysis
- opposition (only) = c-paralysis (without Ia sting)
- escape (only) = sting Ia (only or sometimes included in a complete sequence).

Trying to investigate the reasons of the different sting sequences, Steiner stressed the importance of the change in the internal factors which govern the different phases of the wasp biological cycle. For example, the Ia posture, which is characteristically at the beginning of the normal (a) stinging pattern, is only sketched or completely absent in the c-sequences (depending if the wasp is still or not within her hunting period) when she begins with the posture III, IIb. But this is even the posture assumed before egg laying, while the stinging areas IIb and III approximately match those of oviposition and malaxation. In particular, the “characteristic point” of the sting area IIb closely corresponds to that where the cephalic head of the egg will be located. In addition, a non-escaping cricket is more similar, as a stimulus, to a an egg-laying than to a hunting situation. The postures Ia and III-IIb are spontaneously assumed in the two corresponding situation (hunting and egg-laying), before any response by part of the cricket. Hence, they are unrelated to any external stimulus. Instead, the shift to the basic posture III-IIb in the c-stinging sequence should be related to a kind of "attraction" by part of the future phases of the biological cycle [48: pp. 67-69; 49]. Reciprocally, the sketched Ia posture in the c-sequences observed during the hunting phase, finds its explanation in the effect, still present at a certain extent, of the normal sequence scheme competing with the contemporary effect of the future oviposition phase.

One reason to insist so much in this part of Steiner's work regards his methodological implications. In nature it is rather difficult to observe hunting wasps in the moment when they catch the prey and sting it. What one does in many, if not in most cases, is to observe a re-stinging of the prey subtracted, and then given back, to the wasp in her way back to the nest. In general close to it, where it is easier to detect her. Or maybe substituting the prey with that subtracted to another wasp, or with an intact and artificially immobilized one to prevent its escape. The implicit assumption is obviously that the wasp stinging behaviour of the experimental situation will be the same of the natural one. The above analyses of Steiner show, on the contrary, that this may not be true, and the caution necessary in the use of re-stinging results.

The last part of Steiner's work examines the effects on the *Liris* stinging pattern exerted by some modifications operated in the cricket morphology. One will review them very briefly.

Every specific act of *Liris* is in general preceded by an orientation phase. This holds for stinging as well as for other activities. For example, before paralysation the wasp will direct toward the posterior part of the cricket; before transport toward the head, to seize its antennae. The stimuli responsible to the orientation are in general different from those which produce the corresponding specific action. In spite of being strongly linked in natural conditions, the two kinds of stimuli may be experimentally separated: if one eliminates the portion of the cricket bearing a sting area, the orientation response will still be present without being followed by any stinging [48: pp. 83, 86].

Steiner determined a certain number of stimuli which govern the orientation phase, driving *Liris* to the first posture (Ia) which precedes the first sting: direction of prey movement; cricket shape, in particular its anterior-posterior polarity; influence of the prey posterior legs movement; influence of direct contact with the prey; influence of the dorsal-ventral polarity. The results obtained by a series of conflictual experimental situations – for example, moving the cricket in the direction of the tail the stimulus induced by the prey shape becomes contrary to that due to its movement – allow to compare the relative importance of the different stimuli: cricket displacement direction < shape < movement of posterior legs, all of them being stimuli which act when the wasp is a at a certain distance from the prey.

Instead, when she arrives in contact with the cricket the new stimulus due to the immediate tactile perception is strongest than the previous ones, and may modify or even invert their effects [48: pp. 87-89, 105-106].

Through a series of experiments of inversion and transposition of different segments of the cricket body, Steiner [48: pp. 90-107] showed that the wasp stings only if she finds the right sting area on the prey surface. If, for example, the sting areas III, IIb and IV are replaced by the ventral segment (where no sting area is present), no sting will be elicited. The same if the right sting area has been replaced by a different one. If, for example, the sting area IV is replaced by an additional area III, the stings Ia, IIb, III will be given normally; subsequently, the wasp will reach the position which, in normal situations, should be that of posture IV. But now her abdominal tip will meet sting area III, so that the sting will not be given. Considering now the case in which all sting areas are present, but one among them, for example area IV, has been displaced with respect to its natural position. After the first three stings the wasp will assume posture IV (with respect to sting area III), but no suitable sting area will be found. Then she will move the abdomen randomly on the cricket surface until its tip casually will contact sting area IV, and the sting in most cases will be delivered. One observes again the predominance of the tactile reaction with respect to the orientation stimuli (consisting in the posture assumed by the wasp in relation to the cricket regions stung before or by the shape or other directional indicators present on the prey surface): the lack of the tactile stimulus does not allow the sting which, on the contrary, may be elicited by the tactile stimulus alone, even in absence of any previous orientation [48: pp. 97, 100-102].

On the base of his experiments, Steiner excluded the possibility that the stinging behaviour, as any other behaviour of *Liris* biological cycle, may be explained uniquely through a chain of reflexes such that each of them acts automatically as stimulus for the subsequent one. Otherwise one could not explain, for example, the reason why the wasp does not sting a wrong sting area, provided that it is located in the right position with respect to the previous one. On the contrary, according to Steiner the wasp must possess a pre-existent scheme of the cricket which allows to sting only the right areas. This scheme does not involve necessarily the whole body of the prey, but only the region relevant to the concerned phase. Even in the case of a c-stinging sequence which lacks of the sting in area IV, a decapited cricket works as well as an intact one: the wasp, after the last sting, may even begin its transport, provided that a pair of “antennae” are applicated to the decapited end [48: pp. 95, 110].

Considering the paralysing phase of *Liris* in the frame of whole her biological cycle, one observes that in many cases the lack of a characteristic area does not hinder the fulfilment of the corresponding act: a cricket deprived of antennae may be transported by a leg; deprived of anterior legs may be malaxated at the base of middle or even posterior legs; or the wasp may lay her egg if the prey is deprived of more anterior segments. However, the possibility of use *substitution areas* is not the same for the different activities of the wasp. In fact, it goes from a *maximum* for the transport to a *minimum* in the case of stinging, where no substitution is effectively possible (except a few rare and controversial cases). In the middle one finds oviposition and malaxation. The need to cross a threshold level explains the necessity of a certain delay and of the performance of void attempts, awaiting the moment when the degree of excitation is increased to a value such to allow the substitution. However, to any substitution area a certain value of *threshold* should be attributed in relation to the level of excitation of the wasp. In the case of the sting areas this threshold is so high that it will be never practically possible to reach it. The reason of this may well be due to their close relations with the nervous ganglia, what makes useless any other surface of the prey body [48: pp. 108-110].

The reason why the stings are given in the right position, from the beginning of the wasp adult life [48: p. 114], without any previous knowledge of the cricket body, remains very obscure. Even if one may not completely exclude that

> ...the larval life of *Liris* may have played a role in the formation of these structures of behaviour...Nevertheless in the case of the paralysing stings one may not reject the hypothesis of a *direct* detection of the nervous ganglia, what clearly would remove every innate and preformed character to the reaction; but this seems to us inconsistent with some data, as the attempt to give sting IV to decapitated crickets or the precision in the orienting postures before the sting, *since the first paralysation* (then without learning).
>
> If these different influences are ruled out, everything occurs to all appearance as if the “specific” behaviour of the adult Wasp, in relation to the cricket, was settled in function of a *true “innate anatomical knowledge” (visual, tactile, etc.) of the cricket...*” [48: p. 116].

The last words of this quotation are clearly reminescent of the Fabre's concept of "an inborn knowledge of the internal anatomy of the prey" (see above).

Recent finding II: Steiner's later works and 21st century's contributions

Steiner extended the kind of work made on *Liris* (not only *L. nigra*, but also *Liris argentata* (Palisot de Beavois) and *Liris aequalis* (W. Fox), which all show very similar sting patterns) to different genus of sphecid and vespid wasps, as *Oxybelus*, *Prionyx*, *Tachysphex*, *Euodynerus*, *Podalonia*, *Larra* [49, 50, 51, 52, 53, 2: p. 124]. In the following, one will review some of his results.

Comparing the stinging patterns of different wasps to the correspondent prey nervous system structures, Steiner found additional support to what already stated in the case of *Liris*. In the flies, which are the prey of *Oxybelus*, the nervous system shows a remarkable level of concentration since all thoracic ganglia are fused in one single mass; moreover, many muscids lack of a distinct subesophageal ganglion, which is fused with the supraesophageal structures to form a single unit. Flies also lack of the powerful jaws present in the Orthoptera, which do not need to be neutralized. For these reasons, one understands that one single sting-pattern in the *nervous* thoracic mass may be sufficient, as one effectively observes. The fact that a different fly-hunter, *Crabro latipes* F. Smith, belonging to the same subfamily (Crabroninae) but to a different tribe, in addition to the thoracic sting sometimes delivers an occasional sting in the neck, could be interpreted as the presence of a "vestigial" sting in the way to be eliminated from the sequence [50, 54].

Even among species which share the same number of stings, one may observe a remarkable *adaptation* of the sting pattern to the ganglia position. This has been observed comparing the sting pattern relative to three Orthoptera-hunting wasps: *Liris* (hunting crickets), *Tachysphex* spp. and *Prionyx parkeri* Bohart and Menke (hunting short-horned grasshoppers). All of them show a C4SP pattern (see above), but with some differences in the order and position of the different stings: *Liris* and *Tachysphex* follow a posterior-anterior direction starting from the metathoracic segment. In contrast, *Prionyx* follows a reverse order, starting from the throat to terminate in the mesothoracic segment which will receive two stings. As in the case of *Liris*, the sting in the throat has an immediate *local* effect consisting in the elimination of any movement in the mouthparts of the prey and of its defensive regurgitation, and a general *deactivation* effect which in crickets becomes apparent only after partial recovery from temporary paralysis: "If stinging is interrupted after the first sting both crickets [stung by *Liris*] and grasshoppers [stung by *Prionyx*] have lost one defence system (hind-legs vs. mouth-defences) but grasshoppers have also been "deactivated" and hind-leg defences have become depressed and inefficient, although not fully eliminated" [49].

The prey of all three wasp species show a subesophageal and three distinct thoracic ganglia, but with some differences, in the grasshoppers with respect to the crickets, due to a migration of the three thoracic ganglia toward the head, as it is shown by the correspondent increase in the length of the nerves roots of the different legs. According to Steiner, the basic stinging pattern remains unchanged even in fairly systematically distant wasps (*Liris*, *Tachysphex* = Larrinae; *Prionyx* = Sphecinae), since it is determined by the prey internal nervous structure which remains essentially the same, and should represent a typical situation of *convergent* evolution. In contrast, the order of the sting sequence or of the body segments involved may vary, depending on the divergent evolution of systematically distant wasps. However, one may not discard the possibility of second order adaptations. *Liris* is a frail wasp which needs to give a first stroke in the rear position, in order to master her cricket by paralysing its powerful hind legs. This is not the case for *Prionyx*, which overpowers her grasshopper "embracing" it, by mean of her strong and spinose legs, in an antiparallel posture, i.e. with the head directed toward the prey tail. In *P. parkeri* the sting in the mesothoracic segment (which receives two stings) rather than the metathoracic one, should be due to its minor distance from the third thoracic ganglion. In fact, one observes a parallel *cephalization* of both c.n.s's and stinging patterns. This is shown even by an accurate examination of the stinging pattern of some common *Tachysphex* grasshopper-hunting species like *Tachysphex tarsatus* (Say) which show an intermediate situation, since the hindleg-paralysing sting is still on the third thoracic segment but in a position more frontal than in *Liris* (and in a non identified *Tachysphex* species it has shifted to the middle-legs as in *Prionyx*). In some cases the sting order may be modified under the influence of strong adaptation necessities, as in the mantis-killing *Tachysphex* species (see above) which give priority to the paralysis of the front raptorial legs, in contrast to grasshoppers-hunting *Tachysphex* species which firstly paralyze hind legs. A shift between long- and short-horned grasshoppers, and a parallel cephalization of both prey nervous systems and wasp stinging pattern, may be observed even within the subtribe Prionyxina, passing from *Palmodes carbo* Bohart and Menke to *P. parkeri* [49, 55].

The study of the stinging patterns of *Prionyx* gives the same results obtained for *Liris*: the analysis of the external positions and directions of the stings (including the abrupt change passing from the first sting directed toward the subesophageal ganglion, to the second one directed toward the first thoracic ganglion) makes it very plausible that their internal targets are the thoracic and the subesophageal ganglia. The presence of soft membranes is a necessary but not a sufficient condition to elicit the sting: for example, the last sting is located on a very reduced and inaccessible membrane when compared with the other ones; yet the total number of stings recorded in a certain number of cumulative observations is very similar for all of them. The thing is still more evident in the case of *Oxybelus uniglumis* (Linnaeus), which delivers only one sting, while the number of soft membranes is certainly much higher. In this case the sting, which is given at the base and rear of one foreleg, remains considerably constant both within and between *Oxybelus* species and even in *C. latipes* (which equally hunts on flies), showing that probably this point is primarily a stinging site widespread among Crabroninae, becoming only secondarily a transporting site as one observes in many *Oxybelus* species (Oxybelini) [50, 49].

The whole of Steiner's works validates with strong evidence the exactness of Fabre's opinion that the wasp stinging behaviour is strictly correlated to the nervous anatomy of the prey. In Steiner's terminology it will be named *locomotor ganglia hypothesis*: "sting number and distribution are regulated only by *ganglia involved in locomotion, escape and defence*". It closely matches not only the results relative to *Liris*, *Tachysphex*, *Prionyx (*and other wasps: *Sphex ichneumoneus* (Linnaeus), *P. carbo*, *Isodontia* sp.), where stings concern only thoracic ganglia (locomotion, escape through hind legs and defence system) and subesophageal ganglion (mouth defence, which may interfere with subsequent stinging or prey transport), but even those relative to *O. uniglumis* (only one sting directed toward the center of the single mass of fused thoracic ganglia that governs the whole locomotion of the fly, whose buccal apparatus is, on the other hand, completely harmless) and *Podalonia luctuosa* (F. Smith). The last species, differently from the others, deliver even abdominal stings. However this is only an apparent exception, since her prey is constituted of large caterpillars which use also abdominal segments for locomotion. The case of *Pryonyx* is a particular meaningful test that shows, due to cephalization of the prey neural system, as stings location is determined by the internal rather than the by the external anatomy of the prey (two stings on the same mesothoracic segment). What could not be excluded on the base of other cases for the one-to-one correspondence between internal and external features [50, 49, 51].

However, in some cases a reduction in the number of stings may be observed. Leaving apart the incomplete and atypical patterns as those observed in *Liris* (see above) but recorded even in *Prionyx* [49], this may be observed in the Eumenine wasp *Euodynerus foraminatus* (Saussure), whose prey are particularly small, weak and easy to overcome. They result very imperfectly paralysed as already observed by Fabre (see above), but without any loss of precision for the stings which simply skip some ganglia; however unable to escape, since too tightly packed to move anyway or owing to the "deactivating" effect of the throat sting, which may be of particular importance in case of temporary or (as in this case) very imperfect paralysis. An extreme case of reduction is observed when no sting is given, as in case of *Microbembex*, which preys dying or dead insects, or of the aphid-hunter *Diodontus* (see above) [50, 49, 51, 52].

A last point regards venom action. We remember as Ferton expressed doubts regarding the possibility that the venom could reach directly the ganglion, at least in the case of *P. subfuscatus* (see above). Steiner observed that the stinging pattern of *P. subfuscatus* is probably similar to that of *P. parkeri*. If so, Ferton, who described only two stings, has omitted the first one in the throat and probably an additional one in the thorax. Possible mistakes in number and positions of stings are very easy when one works without the help of video-photo techniques and without subsequent check of stings marks on the prey body. Alternatively, Ferton may have observed incomplete sting patterns in non optimal conditions. In any case, the lack of a one to one correspondence among the number of ganglia and those of the sting and the doubt that the sting could reach a ganglion located too far away should have been the main reasons to attribute the paralysis only to the diffusion of the venom. In fact, if the spread of the venom may play some role, it may not explain the instantly paralyzing effect, so important to prevent prey-escape. One should exclude also nervous peripherical effects, since otherwise the fourth sting given to *P. parkeri*, located at the base of the middle legs, would affect them instead then the rear pair [49].

An interesting problem regards the possible relations between stinging and carrying systems in *Oxybelus*. Already many early observers, starting from Fabre (quoted by Ashmead in [20: p. 73]; see also [56]) had reported that many species of this genus carry the prey impaled on the sting (abdominal transport), which in most cases is not taken out after the paralysing stroke. In some other cases, instead, the prey is brought with the legs (*O. emarginatus* Say: [57]; *O. trispinosus* Fabricius: [58] and Polidori et al., unpublished data). In still other species, however, one assists to both kinds of transport: the prey frequently is impaled on the sting only after reaching the nest (*O. bipunctatus* Olivier: [47: p. 366]). It should be of a certain interest to determine the stinging position in species of *Oxybelus* using pedal transport, to check if it is the same of those which, as *O. uniglumis*, carry the fly on their sting. The method of stinging of the latter has been examined in some detail: the fly is stung on the ground, sometimes in flight; the wasp curls her abdomen around the anterior part of the prey close to the head, with her body axis quite transversal to that of the fly, slightly tilted forward; the sting is given in the ventral side, always in the same position behind a foreleg, right or left, while one wing of the prey is hold with the claws; after being stung, the abdomen unfolds (as already observed by Ferton, see above) and the fly changes its position from dorsal up to ventral up, while its longitudinal axis makes an open angle with that of the wasp; the stung side of the fly results uplifted with respect to the other one; instant and almost complete paralysis follows, all reactions disappearing within few days [57]. For what concerns the sting apparatus morphology, it has been found a reduction of the lancets length in two species showing abdominal transport (*O. argentatus* Curtis, O. *haemorrhoidalis*), so as to uncover the indented structures that could help transport on the sting (Polidori et al., unpublished data).

One will examine only briefly two other works in which Steiner analyzed very accurately the different ways how the prey of *L. nigra*, *P. parkeri*, and some *Tachysphex* interact with the host wasps and try to avoid their attacks. Many other details may be found in the original works [59,55], In the case of *Liris*, one should distinguish between early and full hunting phases. In the first situation, to an increasing vigour of the wasp attacks correspond different gradual behavioural responses of the cricket (*G. domesticus*): raising abdomen, raising abdomen and body in a tilted position, body swaying. At this stage of the intensity scale threshold for escape is nearly reached, and one will observe: kicking with hind legs (less frequent than any other response), jumping and running away. Probability of subsequent pursuit is, however, still relatively low. In the full hunting phase, instead, responses lack of any “gradation” either for what concerns the cricket or the wasp. The basic sequence, which involves detection by scent, sight or both, is now followed by attack (pouncing) of prey which escapes, by jumping or running, is pursued by the wasp and in general captured and stung. In contrast with the early hunting phase, the behaviour of the cricket is now unpredictable. It may jump away or stop suddenly, enter a burrow or change direction, what frequently disrupts wasp pursuit. Then the wasp stops instantly, standing upright in a particular head up posture, the fore legs maximally stretched, the vibrating antennae held in a special manner, making rapid movements of the body and often of the head in quick succession; or she may perform jerky zig-zag displacements. In this period of fast visual scanning of the surrounding, the wasp attacks any moving object. However, no stinging of any insect other than crickets has ever been observed. Final capture of the prey results quite tumultuous and difficult. The wasp uses mandibles and legs to grasp parts of the struggling prey, very often one of the posterior legs (see above). Before being stung, the cricket may try to enter a burrow or to kick away the wasp with one of the hindlegs. Another possibility consists in the autotomy of the seized (hind) leg, observed frequently in captivity but seemingly widespread even in field conditions. Still one other situation arises when the pursued cricket suddenly stops on the top of a small obstacle assuming one of different possible "freezing" postures. In most instances the wasp does not pounce on it; in case she does, in general no stinging or only abortive attempts of stinging follow (see above) [48: pp. 44-45; 59].

Many anti-predatory strategies have been recorded even in grasshoppers preyed by *P. parkeri* [55], like jumping and flying away, kicking, freezing, biting, assuming intimidating postures or by regurgitation of a repellent fluid. When the prey has already been seized by the wasp, it makes frantic efforts to kick or push away the enemy with the tarsi of its powerful hind legs, which appear to be precisely directed at the points grasped by the wasp. To prevent wasp initial sting posture, it may rise its long folded hind legs beyond the vertical, headwards. Instead, hind leg autotomy observed in crickets was never observed. If the wasp is able to overcome all difficulties, as she frequently does (even dashing at flying grasshoppers and stinging them in mid-air), she will deliver four successive stings, as described above [55].

In the case of *P. parkeri* and *Tachysphex*, a statistical analysis allowed only to assess a slight, non significant advantage in avoiding or reducing stings number in prey where defensive actions were recorded, even if some of them, like that consisting in jumping and flying away or perhaps freezing (that seems to be, however, less efficient in avoiding wasp attack than in the case of *L. nigra*) and mouth regurgitation, appear to be helpful. However, such investigations are made particularly difficult by the wide fluctuations in responsiveness of the hunting waps [55].

In a subsequent work, Steiner [51] was involved in the study of the stinging pattern of the Nearctic species *P. luctuosa*, one of the few sphecid wasps genus which, together with *Ammophila*, hunt caterpillars. In particular, *Podalonia* prey consist mainly in cutworms of the moth family Noctuidae, which burrow into the soil during daytime [51; 47: p. 143]. Even in this case, as in those examined before by the same author, data were obtained by offering the prey in cages held in laboratory, to overcome the difficulty to obtain them afield. This contrasts to natural conditions in which the caterpillars are found at the base of plants or within their subterranean nests. In spite of this unusual condition caterpillars were readily accepted by the wasps, and results were considered as valuable [51].

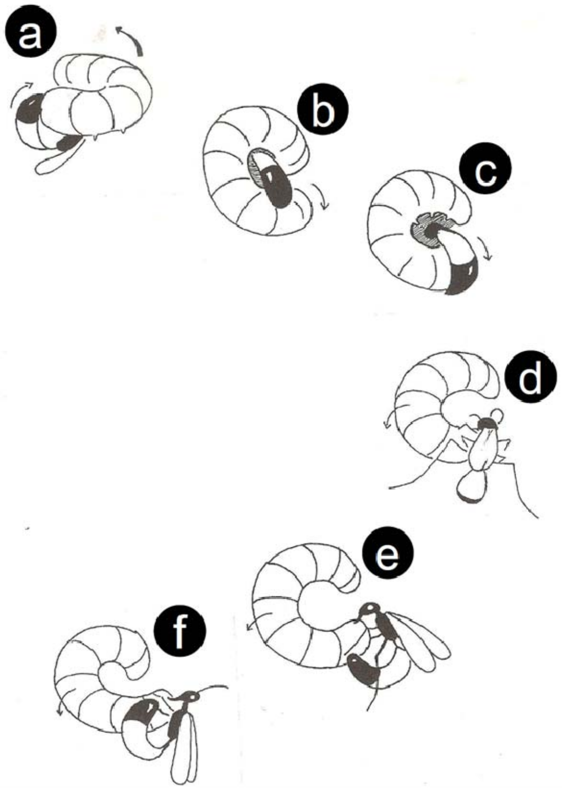

Figure 1. A *Podalonia affinis* (W. Kirby) stings a cutworm dug out from its subterranean shelter (from a video tape recorded sequence redrawn by S. Agostini): a-f, different positions assumed by the cutworm and the wasp during the fight; arrows indicate the direction of rotation of the cutworm around its longitudinal and transversal axes.

Defensive reactions of the cutworm consist in spraying of repelling fluid and vigorous coiling in alternation with flicking and twisting [51; see also 18-II: p. 25]. In Fig. 1 is represented in a half-schematic way a few (a-f) of the different positions taken from the cutworm and the wasp during the stinging phase, sketched from a video recorded sequence in natural conditions: a rare event which one had the chance to observe from the initial excavation of the prey (Andrietti, unpublished data).

The hunting behaviour of the Palearctic species *Podalonia hirsuta* (Scopoli) had already been object of study by part of different authors, starting from Fabre (*Ammophila hirsuta* = *P. hirsuta*). In particular, Fabre firstly described digging behaviour during hunting, which is uncommon to observe in other sphecid wasps [18-II: p. 24; 51]. He also described the two sequences of 4 + 6 stings, in a forward and backward direction respectively, interrupted by the "triumph" dance (see above and [51]). More recently (from 1966 to 1982: see [51] for detailed references) Fulcrand, Gervet and Truc made a careful investigation of the stinging pattern of this species.

The stinging pattern observed in *P. luctuosa* appears to be similar to that of the other examined species. It consists, at least in experimental controlled situations, in ten stings on the ventral side: a first sequence (type I) is delivered from T_3 (third thoracic segment) to H (head segment), which matches the one described previously; a second sequence (type II) follows in a reverse direction, from A_1 (first abdominal segment) to A_6, where sting marks abruptly stop. In Steiner's terminology the complete pattern will be represented by the formula C4SP + C6SP. In both cases the wasp faces the caterpillar head segment, proceeding in opposite directions [51]. However, it may happen that she mistakes the tail for the head of the cutworm, giving a few "erroneous" stings on the abdominal segments, either proceeding in the tail direction (type I "error": [51]) or in the opposite one (type II "error": [51]; see also [18-IV: p. 251]). These kinds of errors are quite common in caterpillar-hunters wasps [51; see also Fabre, above, concerning eumenine wasps hunting Chrysomelidae larvae].

In conclusion, the sting pattern of *P. luctuosa* reflects the caterpillar nervous organization which lacks of the any significant concentration. All segments are stung, from the head to the sixth thoracic one where the last fused ganglia are located [51]. In *P. luctuosa* sting areas are wider compared to the previous considered wasps (*Liris*, *Prionyx*, *Tachysphex*, *Oxybelus*) which sting sclerotized prey. In fact, no constraint due to the presence of hard membranes is present in caterpillars which are uniformly soft. However, characteristic points coordinates are well identified and they may be mapped by mean of a grid over imposed to the ventral surface of the cutworm. They appear to be located, in the thoracic and abdominal segments, respectively behind and before the closest ganglion. This matches the direction of the sting which, even if rather difficult to assess with precision, seems to be forwards in the C4SP sequence, backwards in the C6SP one, even if the change does not appear to be so abrupt and rigidly fixed as in the previously studied wasps. A part the higher number of stings, the *Podalonia* pattern is characterized by stings and sequences repetitions (2.5 repetitions on the average for the complete sequence) and, in general, by a larger amount of variability than the already mentioned wasp-prey systems. Final paralysis depends now more on the cumulative effect of numerous stings, each of ones appearing now to be less precisely localized and with mainly a local and segmental effect. Moreover, the decreasing response of the prey renders the last (abdominal) stings less important and less frequently repeated, in contrast with the other systems in which all stings are approximately equally important [51].

On the other hand, the relative scarcity of characteristic features increases the dimension of the stinging areas on the caterpillar segments and the arising of possible sting errors. With the only exception of the head capsule which, correlatively, exhibits a highly restricted distribution of stings; conversely, they are more widespread on A_1 and A_2, devoid of appendages. The same variability is observed in the egg-position, in contrast to the greater site specificity reported for the other studied wasp-prey systems [51].

In other works specifically devoted to investigate the effect of the reactivity of the prey to the stinging pattern of *P. hirsuta*, Truc and Gervet [60, 61] found that: 1) in the normal (control) case the prey initially elicited mostly a type 1 attack (C4SP), with a trend to shift toward type 2 (2 – 8 stings delivered on metathorax and abdomen) when it became progressively paralyzed; 2), in an experimental case made of permanently reactive caterpillars (since they were systematically replaced after the first attack), the onset of types 2 was delayed, even if they gradually increased in proportion, notwithstanding the state of the prey was stable; 3), in a third group of already paralyzed prey, the first attacks were made either of types 1 or 2 in equal proportion, with a tendency to alternate one to the other. These findings may be explained on the base of two tendencies, the first linked to the position in the stinging cycle sequence, the second to the state of the prey. If in case 1) they match together, in cases 2) and 3) they conflict giving rise to anomalous situations [61].

In *Ammophila sabulosa* (Linnaeus) the hunted prey were found to belong to the two caterpillars families Noctuidae and Geometridae, in roughly equal proportion.

Differently from *Podalonia* species, these prey are not found in the soil but on the leaf of some trees, were they are traced in a peculiar way: wasps walk on the ground with the raised abdomen, searching very carefully for caterpillar faeces; once found them, they fly off exploring a conical three dimensional volume at increasing heights; if they find the prey, this is knocked onto the ground, where it is stung; otherwise, the wasps resume their exploring on the ground. Sting marks on the caterpillar body were found on the throat and in the first eight segment, leaving apart segments A_6 and A_7. In spite of the number of stung segments which are approximately the same (10 in *P. luctuosa* against 9 in *A. sabulosa*); the fact that the first stung segments (T_3 or throat, respectively) are those more frequently stung; the increase followed by a decrease of sting frequencies from A_1 to A_5; the abrupt stop after A_5 (A_6 in *P. luctuosa*) which allows to maintain defecation of the caterpillar; and finally, the fact that the wasp faces the head of the caterpillar, one should note some differences in the sting patterns of the two wasps. The two different positions of the first sting could be related to different evasive manoeuvres of the prey: the cutworms hunted by *P. luctuosa* make vigorous coiling around a "hinge" approximately located at the level of T_3, which should be firstly neutralized; if this would not happen for *Ammophila* prey (what should be checked), the sting pattern could begin at one end of the caterpillar and specifically at the throat, which contains higher centers which control mouthpieces movements. So the whole sting pattern proceeds regularly backwards and it has no need to be partitioned in two different sequences. Instead of a C4SP + C6SP one has now a single C9SP. After stinging, females sometimes used their mandibles to massage the prey's body, maybe to permit an adequate spreading of poison since no feeding or fluid exuding from the caterpillar was observed. Since the mark of the sting is approximately midway between two consecutive ganglia, and no information is available regarding the sting direction, there is no way to decide about the possibility that ganglia may be reached or not by the sting tip. One has observed a (weak) positive correlation between stings number and prey size [62].

In an evolutionary perspective, the use of Lepidoptera larvae is probably a character derived from a previous situation of Orthoptera-hunters, as in most sphecine wasp. If a C4SP scheme or even a reduced one may be still sufficient for small and weak caterpillars packed in tight cells as in the case of eumenine wasps (see above and below), this is not the case for powerful caterpillars as those hunted by *Podalonia* and *Ammophila*. This could have brought to a secondary specialization with an additional C6SP pattern, and the discontinuity between the two sequences (in *Podalonia* but not in *Ammophila*, what should be verified) could be a sign of their different origin.

In other hunting caterpillars solitary wasps, the number of stings appear to be reduced. The sting pattern of *E. foraminatus*, which feeds on small Lepidoptera larvae, was the object of a detailed study [52]. According to Steiner, in the Eumeninae the C4SP pattern is often reduced to the first and last step of the normal sequence (in the throat and the third thoracic ganglion: C2SP), sometimes up to four stings, instead than the regular 10. This modification should be due to the weakness of the Eumeninae prey, constituted mostly by lepidopterian caterpillars but even by larvae of weevils and chrysomelids beetles. Instead, T_1 and T_2 receive stings with a much lower frequency. A few additional stings may be found irregularly distributed in the abdominal segments which, in contrast with the regular thoracic ones, are irregularly scattered and not necessarily located close to the median line. However, the number of these irregular stings is low (three cases out of 23), and does not justify the claim of a fundamental imprecision and lack of constancy of this species, which fundamentally follows a 2-stings pattern rule. In fact stings are clearly clustered, not uniformly or randomly distributed. Stinging directions were not systematically investigated, due to difficulties of observation, but they were in general directed toward the medial ventral line of the prey [52, 51, 2: pp. 129-130, 134]. Even Cooper (1953, quoted by [2]: p. 130) reported that *Ancistrocerus antilope* (Panzer) received three times more stings in the throat and the third thoracic ganglion than in all other areas combined.

In contrast with the prey of *Podalonia* and *Ammophila* (which may show coiling and mouth regurgitation), eumenine wasps prey apparently lack of any mean of defence, except to suddenly drop the ground after having been extracted from within their rolled leaves. In all observed cases sting H always preceded sting T_3, even if initially in a motionless prey either head or tail were investigated; this did not happen in moving prey, meaning that direction of escape, as in the case of *Liris*, is an important visual orienting clue. The wasp grasps the caterpillar dorsally with her mandibles facing its tail in an antiparallel posture, stings the throat area and then proceeds slightly tailwards to sting the metathoracic area; sometimes she repeats the same procedure even several times. After stinging is terminated, the wasp pulls or stretches the prey with the mandibles and/or compress and chews the tail or the head, presumably to lick some body fluid as already described by Fabre for a different eumenine wasp (see above). Differently from what observed in other wasps (see above), abortive or reduced sting patterns were rarely observed even in the case of using already stung prey, probably because they are quite as reactive as the non stung ones [52].

In any case many reports from the literature mention three thoracic stings in several Eumenines, as reported by Fabre for *Odynerus* (see above). So it is possible that in some cases the C2SP pattern may be substituted by a C3SP (Steiner 1983a, p. 19; 1986, Tav. VI, pp. 131-133), maybe even C4SP. In fact, Fabre underlines that *O. nidulator* (*S. murarius*), when giving the three stings in the thorax, "insists" particularly under the neck, what maybe could mean a first stroke in the throat (H), almost inseparable from T_1 by direct observation alone [18-IV: p. 204; 52].

Recently, Budrienè and Budrys re-examined the sting patterns of a few Eumeninae in a series of interesting works [15, 16, 17]. Their results do not match completely those of Steiner, since the sting pattern appears to be much less stereotyped. In the case of *Symmorphus allobrogus* (Saussure), which hunts leaf beetles (Chrysomelidae) larvae, they found 6-9 stings (average 8.7) distributed over 4-6 (average 5.1) prey segments. The highest number of stings was given to the first five segments: the throat, the three thoracic and the first abdominal. Since the locomotion of the leaf beetle larvae is mainly due to thoracic legs, their CNS is weakly concentrated, and they use abdominal dorsal glands for defence, their sting pattern agrees with the "locomotor ganglia" hypothesis without the particular reduction in number reported by Steiner. In fact, there is a negative correlation between the mobility of the prey and both the presence and the number of the stings in the three thoracic segments and the first two abdominal ones. Surprisingly, this same correlation becomes positive when made with respect to the throat. This last effect could be attributed, according to the authors, to the fact that the stings in this position, directed to the subesophageal ganglion, should produce a (transitory) deactivation of the prey (see above) which may reduce the number of subsequent stings. In such case, after recovery the prey will result more mobile [15]. In fact, this explanation does not match the absence of any correlation between the stings in the throat and those in the thoracic ganglia, the most important in controlling prey mobility.

In *S. allobrogus* the effective C5SP pattern appears more flexible compared to that considered by Steiner. However, one should note that in this work the position of the stings was determined uniquely from the traces left on the body of the larva [15], without any control to establish the biological cycle of the wasp and her hunting condition in order to avoid incomplete or atypical sequences (see above). This may increases the variability of observed sting schemes [57]. Neither the effective presence of inactivation after stinging the throat has ever been really checked. On the other hand, when comparing the different patterns between *E. foraminatus* and *S. allobrogus* (C2SP and C5SP, respectively), one should also take into account the possible diversity in the organization of the CNS of the two kinds of prey (caterpillars and leaf beetles larvae).

In a second work Budrienè & Budrys [16] considered the stinging pattern in ten species of Eumeninae belonging to three different genera: *Ancistrocerus*, *Symmorphus* and *Discoelius*. The studied species of the first and third genus hunt caterpillars; those of *Symmorphus* hunt leaf beetles larvae, except *S. debilitatus* (Saussure) which hunts caterpillars. The statistical analysis revealed that in most cases the number of the sting traces (whose averages varied from a minimum of 3.9 in *A. nigricornis* (Curtis) to a maximum of 51 in *S. murarius*), and their distribution among the 13 body segments of the prey (including the throat) is a species specific characteristic. Moreover, in all the ten cases one found a very significant difference between the observed patterns of stings distribution and those expected on the base of a C4SP or a C2SP scheme. However, four caterpillar hunting species show a distribution close to C2SP, while the stinging scheme of the three species of *Symmorphus* which hunt Chrysomelidae larvae show a regular pattern consisting in stings in the throat, the three thoracic segments and the first abdominal one (C5SP). The distribution of stings on the prey may be used in phylogenetic studies as a comparative character. In fact, each of the three considered genus show some peculiarities with respect to the other ones: *Ancistrocerus* shows a lower stinging effort in the mesothorax; *Discoelius*, a higher stinging effort in the fourth, third and fifth abdominal segments; *Symmorphus*, a higher stinging effort in the first abdominal segment. Since these differences were determined independently from the kind of prey, the authors find more plausible that the stinging pattern is a phylogenetically inherited feature rather than being mainly an adaptative character to the physiology and nervous anatomy of the prey as supposed by Steiner. A strict subordination to the taxonomy of the species seems to receive additional support from the fact that the difference between the stinging patterns of the less related caterpillar hunting genera *Ancistrocerus* and *Discoelius* is larger than that between the related genera (hunting different prey) *Ancistrocerus* and *Symmorphus*. In any case, adaptation to a particular kind of prey may play an important role as well: hunting leaf beetles wasps deliver more stings to the mesothorax and the first abdominal segments [2: p. 129; 16]. For what concerns a possible relation between the number of stings and the prey dimension, an analysis performed on eight of the above mentioned species has shown only a (weak) positive correlation, while the relative sting effort, i.e. the number of stings per prey weight unit, was negative with respect to the prey weight while the total stinging effort per offspring positively depended on the number of prey specimens stored in a brood cell [17].

Comparing the results relative to the stinging pattern of different eumenine wasps, one has the clear impression of a high level of variability even between closely related species [2: Table VI, pp. 131-133] or even intraspecifically [63, 64]. In some cases these differences may not be attributed to unreliable observations made in the field since, as in the case of Steiner's and Budrienè & Budrys's findings, they were both obtained by examining the sting traces left on the larva body.

Modern times and the future: Integrating neuroanatomy, electrophysiology, ethology and biochemistry

In spite of the mentioned evidences, firstly presented by Fabre's and subsequently reinforced by Steiner's works, a definitive proof of the correctness of the "locomotor ganglia" hypothesis could arise only on an histological base. However, a simple histological investigation of the nervous system of paralyzed insects may lead to different results, as it appears from the works of Nielsen (1932) and Hartzell (1935) (quoted by [27]). Piek (1978, quoted in [2: p. 143 and Fig. 11, p. 144]) was probably the first to identify, by mean of an autoradiographic method, the site of the venom injection by part of a *Mellinus arvensis* Linnaeus inside the compound thoracic ganglion of a fly.

Gnatzy and Otto [65] used a different method, cutting a "window" into the metathoracic sternite of *Acheta domesticus* (Linnaeus) (one of the crickets hunted by *L. nigra*) and directly observing the penetration of the wasp sting into the metathoracic ganglion, without stopping in the hemolymph space. This result may be considered as an unequivocal proof that the venom, to act, must be inserted inside the ganglion. In fact, if it is applied artificially to the vicinity or surface of a thoracic ganglion, it fails to affect the CNS or the behaviour of the cricket: its molecules are too large to cross the perilemma membrane around the ganglion.

Gnatzy and Otto were also able to correlate the venom injection to different electrophysiological and behavioural data. Extracellularly recorded spontaneous spike frequency from the connective between pro- and mesothoarax ganglia decreased continuously during stinging sequence, reaching its lowest level after it was completed. After 4 – 10 min interneuronal activity raised again slowly. This is the time necessary to recover from the initial total (legs, mouthparts and antennae) and transient paralysis. After this delay, the cricket is again able to respond to stimulations by reflex actions (intersegmental reflexes recover later and with more difficulties that intrasegmental ones), while the spike rate in the connective increases and, in about two hours, reaches the 80% of the original rate. The cricket, now become a "reflex machine" (see above), maintains this "deactivated" state for several days, becoming progressively worse for the reason that it does not feed any more: the motor behaviour is further reduced until the cricket eventually dies [65].

In a second set of experiments, recording was made at the level of the connective between SEG (subesophageal ganglion) and T_1, while a burst of action potentials was elicited by mean of tactile stimulation. These were abolished after a sting in T_1 or in SEG (by mean of a suitable apparatus which allows *Liris* to sting only at given positions of the cricket), to reappear a few minutes later. Moreover, stings in T_3 and T_1 only did not impair the reflexes of the middle legs. On the base of their results, Gnatzy and Otto concluded that the immediate action of the *Liris* venom paralysis likely consists in the inhibition of spike generations in neurons of the stung ganglia [65].

In subsequent works Gnatzy and co-workers established that, during total paralysis, venom blocks synaptic transmission and action potential generation. The first effect is probably localized at the presynaptic side, and is due, at least partially, to a block of inward calcium currents. The second is due to a block of voltage-gated sodium inward currents of the corresponding neurons. Even if the effect of the poison is restricted to the stung ganglion, it does not selectively affects leg motoneurons but any other neuron, like inter- and neurosecretory neurons. On the other hand, sensory information from legs mechanoreceptors of the stung ganglion may be recorded during the whole period of total paralysis. For what concerns suppression of spontaneous behaviour, instead, it is necessary that the venom is injected inside the SEG [66, 67, 68].

The neuroethological researches of Gnatzy extended also to other aspects of the *Liris*-cricket interaction. They are quite interesting and merit to be examined, since they appear as a physiological correlate to the ethological investigations of Steiner [59]. Crickets (*A. domesticus*) respond to the approach of *L. nigra* with conspicuous behavioural reactions, like head-stand (raising abdomen), stilt-stand or defensive kicks, which seem to belong, at least partially, to the threatening postures repertoire of the males mating behaviour. Gnatzy has shown, through lesioning experiments, that defensive reactions (head-stand and even stilt-stand) are neither elicited by visual nor by substrate vibrations, but by the air movement generated by the predator approach. Reception is localised in the filiform hairs of the cerci. Neurograms were recorded from the axons of giant interneurons in the terminal abdominal ganglia of the crickets, which receive their inputs from the cercal filiform hairs and by adjacent campaniform sensilla. Results showed that they responded to air currents generated by flying wasp females when they were at a distance of 15-20 cm. Filiform hairs are especially sensitive in the range of velocities and wing beat frequencies of the flying wasps (50 cm/s and 150 Hz). Instead, if the wasp approaches the cricket from behind hunting “on foot”, no response is given up to the distance of 1-3 cm. These results may help to explain the method adopted by *Liris*, which never flies but only runs during hunting. In such situation, moreover, she holds her wings folded motionless over her back, in contrast to other solitary wasps as, for example, the Pompilidae. However, hunting females run very fast, at a speed not very lower than that of the flight. Even if this increases the intensity of the produced signals, it reduces the time available to the detected prey to react, and enables the wasp to explore a larger amount of territory [67]. Instead, Steiner [59] gives a different account of *Liris* hunting method

> The wasp walks slowly, head down with the antennae tapping the soil. Information picked up may be chemical or chemotactile. The wasp, like a hunting dog, follows with great precision the scent trails left by crickets and investigates dropping.

In any case, prey recognition by part of *Liris* is due to visual (from a distance of up to 15 cm) and chemical cues, detected by mean of a kind of sensilla which are commonly found on the antennae of several insect orders: the sensilla basiconica. In the present case they are distributed in large number only on the upper side of the six distal flagellomera of the females, only a few of them being present in male flagella. The prey is attacked only after their contact with the prey body. Only they are involved in prey recognition, as it is apparent in video analyses and in ablation experiments. Instead, the other types of contact chemosensitive hairs present in the antennal flagella of the females are unimportant. Their structure presents a perforated oval plate at the tip of the sensillum hair shaft, differing either from typical olfactory sensilla (whole hair shaft perforated) or typical contact chemoreceptors (only one pore). However, the need of contact with the prey supports the hypothesis that one is dealing with contact receptors [67].

An important role for prey recognition should be attributed to an attractive substance present on the prey cuticular surface, which may be transferred to filter paper or to another cricket species (*Gryllus bimaculatus* De Geer) normally not accepted as a prey. In crickets, contact chemical cues are used in sex recognition when acoustic communication is not possible, and may have been secondarily used by predators to localize their prey [67].

Recently, in a series of correlated papers [69, 70, 9, 12, 71, 72, 73, 11, 8, 13], Libersat and colleagues examined the site of injection of the venom, the electrophysiological and the pharmacological effects in the CNS of the common cockroach *Periplaneta americana* Linnaeus, when stung by the wasp *Ampulex compressa* (Fabricius).

After grabbing the cockroach at the pronotum or at the base of the wing, a first brief sting (10-20 s) given into the first thoracic ganglion through the soft membrane between the front leg and the prothorax induces a few minutes of paralysis of the forelegs, which recover in a few minutes. Within this delay, the coackroach is unable to use its front legs to fight off the wasp, what facilitates the second and more important sting, much more precise and time-consuming, in the head [72, 8, 74].

The authors made use of a combination of liquid scintillation and light microscopy autoradiography to investigate the precise localization of the venom. By mean of ^{14}C radiolabeled amino acids injected in the wasp and subsequent measure of radioactivity in the stung (by the wasp) prey, they found that radioactivity was mostly localized in the first thoracic ganglion rather than in the other thoracic ganglia or non-neuronal tissue. To prove that the venom is injected directed in the first thoracic ganglion rather than to diffuse from outside into the nervous tissue, radiolabeled amino acids were experimentally injected into the thorax of the cockroach outside of its ventral nerve cord: most of the radioactive signal was recorded in the surrounding thoracic non-neuronal tissue, significantly less in the thoracic ganglia [72].

After the second sting, the radioactivity accumulates in the head ganglia of stung cockroaches (more in the brain than in the subesophageal ganglion), remaining very low in the surrounding tissues. More precisely, radiolabeled venom was found posterior to the central complex and around the mushroom bodies of the brain and around the central part of the subesophageal ganglion. This seems to show that the wasp stings both into the SEG, which lies underneath the stinging site in the neck, and separately into the brain which lies 2 mm deep in the head capsule. Both of them are considered higher neuronal centers which send descending tonic signals to thoracic motor centers to modulate the beginning of locomotion, so providing their role in the motivation to initiate or maintain walking-related behaviours. According to Libersat, this precise stereotaxic injection should require the presence of sense organ on the sting tip. In fact, receptors have effectively been identified on the tip of the sting (or ovipositor) of different Terebrantia [75, 76, 77] and social wasps and bees [78], but in solitary wasps this information is known only for the sting sheaths [27, 67]. These last ones may allow detecting the precise position where to insert the sting on the cuticle surface, not inside the prey body. When labeled amino acids were experimentally injected in the head cavity, most of the radioactivity was found in the surrounding non-neuronal tissues and significantly less in the head ganglia. One may conclude that venom does not diffuse into the head ganglia from the stung site, but is directly inserted inside the brain by the sting tip [11, 8, 74].

The biochemical basis of the transient paralysis of the cockroach forelegs is probably to find in a central synaptic block due to the enhancement of chloride conductance by part of a GABA receptor channel present on the cell body of some (as the fast costal) thoracic motoneurons whose motor output is under cholinergic control. In fact it has been shown that *Ampulex* venom contains high level of GABA together with two agonistic substances – taurine and β-alanine – which have the similar effect to activate the conductance of a chloride channel and that additional to prolongate the action of GABA, probably inhibiting its reuptake from the synaptic cleft. The three amino substances present in *Ampulex* venom, together with GABA receptors, have been found even in the venom of *Vespa* and of the spider wasp *Anoplius samariensis* Pallas, but their physiological roles remain to be demonstrated [79, 7].

If the effect of the thoracic sting is to induce (1) transient paralysis of the front legs, that of the subsequent sting in the head produces (2) intense grooming, and (3) long-term hypokinesia [8]. The stung prey "first grooms extensively, after which it becomes sluggish and is not responsive to various stimuli. The wasp grabs one of the antennae of the cockroach, which follows docilely to a suitable oviposition location..." [11]. The ethology of host manipulation has been studied by Keasar et al. [80]. At the end of the grooming phase, the wasps cut the antennae of the prey (which are constituted by females more readily than males). The cutting region is localized through sliding the mandibles several times over each antenna, and results close to where a maximal variation between consecutive segments length is perceived, which may be considered as a point of discontinuity. The wasp now feeds on the hemolymph from the cut end, which is located in a position which is optimal for an easy flow. Then the cockroach, kept by one of the stumps, is led to the nest with the cockroach proceeding forwards, the wasp backwards facing the prey. Learning does not appear to play any significant role in subsequent behavioural sequences, which appear to be primarily genetically determined [80, 74].

These unique effects (2-3) suggest that a high center of the insect CNS may be involved in the action of the venom, possibly the brain. Moreover, one has shown that the *A. compressa's* venom has no effect on the cockroach neuromuscular junctions [8, 11].

The evoked grooming (2) lasts about 30 min nonstop following the sting and may not be evoked in any other way, neither stress nor attack or mechanical stimulation or venum injection in a location other than the head, apparently activating a specific neural network controlling all the components of a normal grooming behaviour which involves the coordinated movements of many different appendages (antennae, mouthparts, legs). The responsible of these effects, which are evocated only if venom is injected into the head, has been identified as a dopamine-like substance which could stimulate dopamine receptors present in the cockroach SEG, where a group of dopaminergic neurons (some with axons branching extensively in the SEG, others connecting to the brain or thorax) have been identified by mean of immunohistochemical studies. In fact, injection of DA or DA-receptor agonists induces excessive grooming, similar to venom-induced one, while injection of DA-receptor antagonist prior to sting markedly reduces it. However, a stung and hypokinetic cockroach does not show any grooming neither if stung a second time or injected by DA. This suggests the presence, in the venom, either of a DA-like substance inducing grooming and of a dopamine-blocking component which subsequently blocks it and could be even responsible for eliciting hypokinesia [8, 69, 74, 12, 71].

However, the primary function of the second sting is that to produce the long-lasting lethargic effect (3), which fully develops at the end of the extensive grooming, lasts for 2-5 weeks and may be defined as a "long lasting change in the threshold for initiation of various locomotory behaviours". Its action regards spontaneous activity and specific motor behaviours, leaving others like grooming, righting or flying unaffected. The most striking effect concerns the escape behaviour, which is no longer elicited in stung cockroaches by wind stimuli directed to the cerci or tactile stimuli to the antennae or anal plates. Tactile (from cuticle or antennae) and wind sensory information all seem to converge on the same pool of thoracic interneurons (thoracic premotor circuitry) which, either directly or via other interneurons, excite the motoneurons involved in fast leg movements. Since experimental results have shown that the sting does not affect sensory descending or ascending (GI, giant interneurons) pathways, the authors propose that the ultimate effect of the venom is exerted at the level of the thoracic escape circuitry. Given that thoracic interneurons receive a comparable synaptic input from GI in control and stung animals, it is suggested that the venom acts at the level of descending neural inputs from the head ganglia, whose importance has been demonstrated [81] through their removal, which induces a reduction of leg movement and fast motoneurons activity as in stung cockroaches. Their effect may possibly be exerted through dopamine-like facilitating neuromodulatory neurons on the connections between pre-motor internerneurons and (fast) motoneurons of the thoracic circuitry or maybe directly on the motoneurons synapses. This appears to be a more parsimonious way to prevent escape, rather than to block all sensory inputs coming from antennae, cerci and cuticular surface. In addition, it explains the specificity of the inhibition, since stung animals maintain activity in slow motoneurons and do not produce rapid movements. However, fast motoneurons may be recruited during behaviours others than escape, as righting, swimming or flight. Recent studies have shown that in stung cockroaches (as well as in cockroaches lacking the SEG) the activity of identified neurons in the thorax (DUM neurons) which secrete octopamine (OA) and control the excitability of specific thoracic premotor neurons is compromised, probably through a modulation of calcium currents. This result appears presumably due to a removal of descending neuromodulatory inputs from the head ganglia. Since the postsynaptic activity on thoracic (OA secreting) DUM neurons of stung cockroaches was found comparable to that of control ones, the input of the descending pathways should not directly control these neurons, rather other interneurons which synapse on them. OA seems to play a role even at the level of cerebral ganglia, since injection of an OA-receptor antagonist into the brain of an unstung cockroach significantly reduces walking activity. On the contrary, octopamine partially restores walking in stung wasps. Clusters of OA-immunoreactive neurons were identified in the SEG; at least some of them provide dense innervation in the ellipsoid body of the central complex and in the protocerebral bridge, which are regions involved in the control of locomotion. In conclusion, according to the last findings it appears plausible that the wasp manipulates octopaminergic circuits in cockroach cerebral ganglia that, in turn, influence octopaminergic circuits in the thoracic ganglia to induce hypokinesia [8, 74].

Many other works have been devoted to the pharmacology of the substances able to induce prey paralysis present in solitary wasps venom. They will not be reviewed here, since they are too far from the ethological focus of the present work. Some of them may be found in Piek [82] and in the references of more recent works [79, 7].

A last point, which is worthwhile to mention, concerns the state of the paralyzed prey. In particular two hypotheses may be considered: 1), paralysis is only a mean to immobilize the prey and/or to prevent escape, without any effect on its metabolic rate; 2), paralysis reduces the metabolic rate of the prey, so extending its life span at the interior of the nest. Probably the answer may vary according to different cases. In a rather old work Nielsen (1935, quoted by Roces and Gnatzy [83]) made an accurate study regarding the metabolic rates of caterpillars (Geometridae) and spiders (*Epeira cornuta* (Clerc)) paralysed by *Ammophila campestris* Latreille and *Episyron rufipes* Linnaeus. He did not find any difference in long term metabolic records between paralysed and food-deprived animals, suggesting mechanism 1). Instead Roces and Gnatzy [83], working on *L. nigra*, found that paralysis significantly reduces both the metabolic rate and the mortality of her prey, the house cricket (*A. domesticus*). Since the control sample was constituted by food and water deprived animals which were unable to move, the change in the metabolic rate of the hosts was due to a direct effect of the wasp venom, not to a decreased locomotory activity [83].

Analogously, Haspel et al. [10] found that the sting of *A. compressa* not only renders the cockroach prey helplessly submissive but also changes its metabolism, measured by a decreased oxygen consumption. This occurred even after pharmacologically induced paralysis or after severing the neck connectives. However, neither of these two groups of treated cockroaches survived more than six days, while 90% of stung cockroaches survived at least this period. In addition, cockroaches with severed neck connectives lost significantly more body mass, mainly due to dehydratation. One may conclude that the metabolic manipulation caused by the sting in the brain operates in a subtler way than simply removing descending inputs from the head ganglia, since it leaves some physiological processes, such as water retention, intact.

Once again one finds a confirmation of what established by Fabre for the Orthoptera-hunting “Sphex languedocien” (*P. occitanicus*), whose prey, *Ephippiger*, shows to live much longer when paralysed than when alive and without food

> What seemed as though as it should be a cause of death was really a cause of life.
>
> However paradoxical it may seem at first sight, this result is exceedingly simple. When untouched, the insect exerts itself and consequently uses up its reserves. When paralysed, it has merely the feeble, internal movements which are inseparable from any organism; and its substance is economized in proportion to the weakness of the action displayed. In the first case, the animal machine is at work and wears itself out; in the second it is at rest and saves itself. There being no nourishment now to repair the waste, the moving insect spends its nutritive reserves in four days and dies; the motionless insect does not spend them and lives eighteen days. Life is a continuous dissolution, the physiologists tell us; and the Sphex's victims give us the neatest possible demonstration of the fact [18-I: p. 187].

Prey selection and the "locomotory ganglia hypothesis": A new frontier of investigation

According to Steiner's "locomotory ganglia hypothesis", the stinging pattern of solitary wasps (number and position of stings) should match the nervous organization of the host. Under the hypothesis of a fixed stinging pattern for any given species, this would limit the range of the possible prey to those which share a common neuroanatomy. As a matter of fact, most solitary wasps hunt a restricted prey spectrum, in some cases only one single species (as *P. triangulum*) or only very related ones (as *Liris*). But this is not always true. Excluding extreme cases like *Microbembex*, which preys any sort of dead or dying insects but without stinging them, there are some genus which prey insects belonging to different taxa. One of them is represented by *Cerceris*, which may hunt beetles of different families but even halictids and other bees (see above). If in most cases the diet of one single *Cerceris* species is restricted to a single taxon (in general a family of beetles, like weevils or buprestids or chrysomelids), sometimes only to a single or few species (as *Cerceris tuberculata* (Villers), see [18-I: p. 67]), there are a few species more generalist. In a recent investigation which regards the prey spectrum of the beetle-hunting *Cerceris rubida* Jurine (Fig. 2), one has found that more than 95% of the hunted specimens belonged to three families: Chrysomelidae (50%; presence in the environment 39%), Curculionidae (32%; 17%), Phalacridae (13%; 7%).

Among the other three hunted families, one found also a small amount of Scolytidae (1.3%; 0%) which, as the weevils, present fused thoracic ganglia

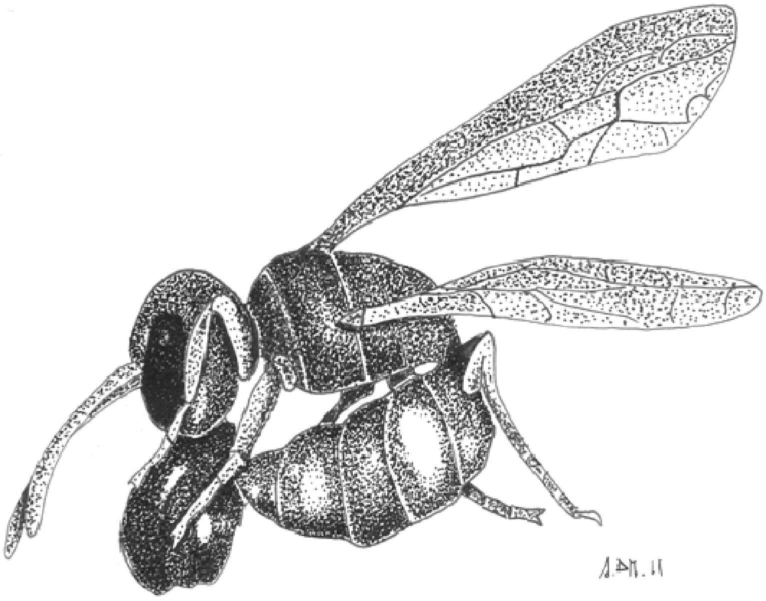

Figure 2. A female *Cerceris rubida* while stinging her chrysomelid prey. Drawing by Silvia Di Martino, from a picture taken on field by C. Polidori.

(see above) [84]. One could think to the poliphagy of *C. rubida* as representing a transition phase between the more common weevils (or buprestids) hunting species and the chrysomelids-hunting ones. Assuming, in absence of any data, that the stinging pattern of *C. rubida* consists of only one sting, as it is common for other beetles-hunting *Cerceris* species [2: Table V, pp. 113-114], it would be more suitable, on the whole, to paralyse weevils than chrysomelids. In fact, the first ones show in general a more advanced level of fusion of thoracic ganglia (second and third in quite all subfamilies, and partial fusion of first and second in a few of them) than the second ones [85, 86]. Effectively, the ratio given by (percentage of hunted prey)/(percentage of available prey) has been found higher in weevils (1.86) than in leaf beetles (1.27).

In spite of her 'generalist' attitude with regard to different beetles subfamilies, *C. rubida* shows "taxonomical" specialization at the level of single prey species which may not be explained uniquely in terms of other well known factors responsible of prey selection as ecology, size, shape, age, sex, escape behaviour, etc., and, of course, availability [84]. To explore the possibility that this taxon-biased selection could be related to differences in prey nervous organization, one should dispose of precise neuroanatomical descriptions of all preyed species. Unfortunately, we may dispose only of data concerning the nerve cord structure of a few species of different leaf beetles and weevils subfamilies, not necessarily those hunted by *C. rubida*. For what concerns Chrysomelidae, the most frequently hunted genus is

Chaetocnema (5 species which, in total, represent the 47.6% of the total hunted specimens from all prey families; scarce among specimens collected in the environment); the second more frequent genus is *Cryptocephalus* (4.5%; 5.0%) [84]. The literature does not provide any data for *Chaetocnema*, even if in other species belonging to the same subfamily (Halticinae) one may find a moderate concentration of the thoracic ganglia, mainly of the meso- and the metathotacic ones which, in some cases, may be connate or fused [85]. However, to the same subfamily belongs *Phyllotreta*, a genus which results abundant in the environment (more than 40%) and completely absent in wasp hunting records [84]. In the case of *Cryptocephalus*, whose hunting frequency agrees with its environmental frequency [84], the investigated species show all thoracic ganglia contiguous with each other [85]. For weevils, the more hunted genus is *Protapion* (23.8% hunted; 15.8% in the environment) [84]. For the species of the same subfamily (Apioninae) which have been investigated for what regards the anatomy of the nerve cord, one reports fusion between the second and the third thoracic ganglia, and in some cases a partial fusion between the first and the second. However, these are features which are common to most weevil subfamilies [86]. The subfamily (Entiminae) to which belongs *Sitona*, a genus which is well represented in the environment (7.2%) but is very infrequently hunted (0.2%), does not show meaningful differences, since the investigated species show fusion of the second and third thoracic ganglia [84, 86]. On the base of the above considerations, it does not seem possible to attribute to the nerve cord morphology, at least at the level of the gross anatomical analysis considered here, a major role in determining the high discrimination in prey selection among species which are systematically very close (in some cases belonging to the same genus as in *Chaetocnema*: [84]), and probably share a similar nervous organization. However, a more reliable indication could be obtained only through a neuroanatomical study devoted to the prey specifically hunted by *C. rubida*.

Conclusions

Looking back to the old literature outlined at the beginning of the present work, one sees how many of the problems which will be matter of discussion up to nowadays were already present from the beginning. This is true in particular for the controversial ideas regarding the way how stings induce paralysis in the prey. The initial position adopted by Fabre in the last decades of the 19th century fell soon in disfavour with respect to that of Ferton and followers, which remained dominant (even if not universally accepted) for large part of the 20th century. However, the first researches of Steiner on *Liris* revitalized Fabre's opinion, rebaptizing it under the term of "locomotory ganglia hypothesis" and were reconfirmed by his subsequent works.

The arguments of Steiner appear quite convincing, at least in the case of *Liris* [48] for which the point and the direction of insertion of the sting have been accurately recorded. The same may be said for *Oxybelus* [50] and, even if at a minor extent, for *Prionyx* [49]. Still more convincing are the results of Piek for *Musca domestica* Linnaeus stung by the sphecid wasp *M. arvensis*, based on autoradiographic methods (see above); or those of Gnatzy and Otto for *A. domesticus* stung by *L. nigra* (see above); or those of Libersat and colleagues regarding the two stings received by *P. americana* by part of *A. compressa*, the first localized in the first thoracic ganglion, the second in two precise and separate locations of the head ganglia (brain and SEG) (see above). However, a part these few cases, anatomical evidence is lacking for the large majority of the predator-prey systems.

On the other hand, observational data, in spite of their abundance [2: Tables I-V] are in general too imprecise and contradictory to allow unambiguous answers. Moreover, in many cases they were obtained in situations different from the "standard" ones, for example in re-stung cases, which may give rise to different responses (see above). It is probable that many, if not most of the old data concerning stings location, are affected by errors. This may explain the large differences in the results coming from different authors. Take, to consider only one case among the many possible ones, *O. uniglumis*: according to very old accounts (Sickmann, 1883, Chevalier, 1926, Adlerz, 1903, all quoted in [2: Tab. V, p. 106]) the unique sting is located in the neck. Instead, according to Steiner [50], and based on the marks of sting and 150 cases (no number of observations was indicated by previous authors, presumably very low or maybe only a single one), the sting is behind one foreleg base (see above). One has no doubt about the exactness of Steiner's finding even if, without making use of the traces left by the sting, or at least some accurate video or photographical techniques, the posture assumed by the wasp during stinging may give the false impression that the prey is effectively stung in the neck [57: Figs. 1 and 4]. Among the other species (cfr. [2: Tav. V, p. 105]), *O. bipunctatus* has been reported to sting in the middle of the prey thorax [87] and *O. haemorrhoidalis* between the head and T_1 [23]. Another discrepancy, which concerns the sting pattern in *Prionyx*, has already been discussed above.

Apart the difficulty to localize the exact position of the sting, accidental elements may obscure the typical sting pattern, depending either on the state of the wasp in relation to her biological cycle, or on the state of the prey. This is one of the reasons why fortuitous field observations may give variable responses. To avoid this difficulty, one should make use of experimentally controlled situations: prey (discarding those with deficient or absent responses) should be offered to wasps which are in the right phase of their nesting site [48: p. 8; 49], and a large number of data should be collected. If this method has the advantage to produce more regular results and to better identify the average typical sting behaviour, ruling out abnormal or irregular cases, it has also the difficulty to imply long-term studies, and the necessity to rear the wasps as a way to have a better control of their nesting cycle [57, 49, 51]. This is not always possible or easy to do. Moreover, in many cases the wasps will not always accept experimentally provided prey. Another point concerns the knowledge of the nervous anatomy of the prey, which often is scarce or not available.

However, even among the wasps studied by Steiner according to his criteria of investigation (analysis of sting traces and, whenever possible, sting directions in reared wasps), there are some for which the "locomotory ganglia hypothesis" is far to be proved: in *P. luctuosa* there is only a vague match between the sting direction and the position of the ganglia (see above); still more uncertain is the situation for the eumenine wasps which, in addition, present a reduced number of stings which does not match a correspondent reduction in the number of ganglionic masses [52]. The same may be said for *A. sabulosa*, in which sting marks are about midway between consecutive ganglia (see above).

A part these dubious situations, there is at least one case represented by the bee wolf *P. triangulum* in which the venom is injected in the hemolymph of the prey, a fact which was firstly acknowledged by Rathmayer [27]. He found that the sting delivered by the wasp was always given, either in experimental (prey and wasp maintained in small containers) or natural conditions, in the unsclerotized membrane on the ventral side of the bee behind the first pair of legs. One has already observed (see above) that this result does not match with those provided by Fabre. However, Tinbergen in a series of repeated observations confirmed Fabre's findings, reporting that the bee was stung "under the chin" [88: p. 50, text and figure], at least in experimental situations. After the sting, which has a duration of about 30 s [27], the bee is turned ventral up and malaxated by the wasp which squeezes the bee's abdomen, as observed by Fabre (see above) and confirmed by Rathmayer [27].

Of particular importance for the present discussion was the histological analysis performed by Rathmayer who, in ten out of twelve cases, found that the sting did not reach the first thoracic ganglion. In the other two cases the perilemma of the ganglion appeared to be damaged, but probably the wasp's sting had broken off and so reached the ganglion. Additional considerations showed that the venom could not consist in a neurotoxin which blocks the ganglionar centers, since it does not produce a sudden paralysis but takes effect gradually. In fact, the nerve cells of the different muscles of the same leg are positioned very close to each other in the same thoracic ganglia and should be reached by the venom at the same time. Yet observations proved that the coxae and femur were paralyzed when the tibia and basitarsus of the same leg were still movable [27].

One must conclude that the venom has a peripherical effect, firstly spreading around the puncture and then reaching the musculature by mean of the hemolymph. This explains the progressive paralysis of the leg segments. Muscles which control movements of the coxae and femora are directly bathed by the hemolimph, which is responsible for a quick transport of the poison, while the muscles of the tibiae are, instead, located within the femora. It takes a considerable time for the venom to reach them through the action of the accessory pulsating organs located at the basis of the leg. Still more to affect basitarsi muscles, which are located inside the tibiae [27].

Another point examined by Rathmayer concerns the site of the sting, to understand if its position behind the fore legs is made necessary for its closeness to the thoracic ganglia, as claimed by the "locomotory ganglia" hypothesis, or only because it is one of the few sites which can be pierced by the sting. New stinging spots were predetermined, by experimentally cutting windows in the chitin in different positions on the bee body. The results showed that the delay in the onset of the paralysis varied according to the distance from the sting [27].

In conclusion, the venom does not affect the ganglia but produces paralysis when it reaches the hemolimph. Even in the case of a bee stung in the head, the effect is that to *paralyze* antennae and proboscis leaving other parts unaffected, without showing any disturb in the movements coordination as it would be in the case of any action on the CNS. A further proof came from the histology, which showed no changes in the brain, SEG and thoracic ganglia, except the first one in the immediate vicinity of the puncture in which alterations in the glial cells and neuropilem, not in the ganglion cells, were visible. Only 24 hours later these modifications spread out to the other thoracic ganglia, but nerve cells were almost never affected. Not until three days after, shrinkage in the nervous structures was noticeable. It is possible that after peripherical paralysis has set in, the venom disturbs the metabolic functions of the glial cells and only indirectly the neurons [27].

If the effective position of the sting has no more importance to induce the paralysis, but is only determined by the possibility to find a suitably soft membrane, it could vary at a certain extent. This could explain the diversity of reports concerning its effective location, rather than to attribute it to mistakes of very valuable observers.

The neurotoxins (philanthotoxins), reaching their sites of action represented by the neuromuscular junctions exert a diversity of effects on synaptic transmission processes, basically interfering presynaptically with the release of the excitatory transmitter and blocking the postsynaptic glutamate receptors [89, 8]. This allows to understand the reason why one single sting is sufficient to paralyse the bee, in spite of the fact that it does not own a fused thoracic gangliar mass as is the case for the flies, which equally receive only one sting by *O. uniglumis* (see above), or for spiders stung by pompilid wasps, which in general receive only one or few stings in the mouth and/or thorax/base of legs (see above and [2: Tab. IV, pp. 84-90]). The alternative explanation suggested by Steiner [49, 51] is so ruled out. It consisted in the possibility that in "higher" insects, as the bees, a single sting not too far from the SEG, whose functions of coordination and control are become particularly important, may be sufficient for a complete block of locomotion. In addition *Philanthus* venom lacks of specificity, since it exerts its effect in all insect orders, including closely related Sphecidae, to the extent to suggest its use as a new class of pesticides [27, 90].

One should stress the need for further researches, mainly on a histological level, to individuate the possible presence of wasp radiolabeled venom inside the ganglia of the host. The researches should be planned for the most important genus of solitary wasps as *Cerceris*, *Bembix*, *Sceliphron*, pompilid wasps, etc., not yet investigated in this way up to now and still awaiting for such an analysis, for which we dispose of not always reliable and often controversial data [2: Table V, pp. 94-114]. Take, for example, the case of *Cerceris arenaria* (Linnaeus). According to different authors and due to the extreme sclerotization of the weevil prey, stings may be delivered only between the head and the thorax, between T_1 and T_2 or between the thorax and the abdomen [50; 2: Table V, p. 113] (Fig. 3). In this case, however, we dispose even of a more reliable result given by Piek [82: p. 612 and Fig. 10 p. 613] who observed discolouration through the ventral side of the soft membrane between head and thorax of the weevil prey, providing a SEM picture of it. Even a search for possible receptors on the sting tip of solitary wasps should be of interest, to confirm the possibility to detect the right position where to inject the venom.

In addition, the ethology of the stung prey should be re-examined to discover other cases of "manipulation of host behaviour". Leaving apart the challenge that these findings pose to their explanations in terms of natural selection theory, they show that "some of the parasites know more about the brain than all neuroscientists combined" [74], so stressing the importance of their study. The phenomenon is not confined to wasps, appearing quite widespread among phylogenetically distant parasites like fungi, trematods, nematods, Nematomorpha (Gordioidea), Acanthocephala, and is probably much more common than we actually know. In insects, this ability may be found in Terebrantia. The most striking situation has been described by Eberhard [91] and regards the ichneumonid wasp *Hymenoepimecis*, which induces a change in the orb web construction of her prey, a spider which, after recovering from the sting, resumes apparently normal activities and builds normal orb webs, while the wasp's egg hatches and the larva grows by sucking the prey's hemolymph. Instead, one or two weeks later the larva induces the spider to build a web especially designed to support the wasp cocoon. Its construction is highly stereotyped, consisting of many repetitions that are almost identical to the early stages of one subroutine of normal orb weaving, the other components of which are repressed [91].

A different case reported concerns the braconid wasp *Perilitus coccinellae* (Schrank), which preys on ladybirds. Emerging from the beetles, the parasitic larva weaves a cocoon around itself, entangling with some threads the prey (still alive) as well. In the case the cocoon is removed “the beetle runs around furiously in search of it; having found the cocoon, the beetle sits on it and tries again to get its legs entangled in the loose silken tissues surrounding it” [92: p. 102].

Among Aculeata, the number of stings and/or the complexity of hunting strategies are often correlated to the host/prey size and to its behaviour. In the case of pompilid wasps, for example, a first stroke in the mouth incapacitates the fangs of the spider, while *Liri*s gives a first sting in the metathoracic segment to paralyze the strong cricket hind legs. When the prey is of small size or harmless, a single stroke may often be sufficient; otherwise, many of them are needed. In general, the wasps paralyze their prey to drag them into a burrow or a nest. This is not always the case: *Larra anathema* (Rossi), after having dislodged the prey (mole cricket) from its nest, induces a transient paralysis stinging it in the mesothorax, prothorax and below the throat, and ovoposit on it. Resuming from the short-term paralysis, the mole cricket will go back to its shelter with the wasp egg, eliminating the need of a long-term paralysis and sparing the wasp from building her own nest and to transport the prey into it. Also pompilid wasps hunting ctenizid spiders (*Nemesia*) oviposit in the prey’s nest (see above), without any need to build their own one. However, what marks the difference is the fact that *Larra* oviposits *outside* the mole cricket’s nest. This will leave the door open to superparasitism by part of the same or similar species of wasps, as effectively it has been observed in *Larra bicolor* Fabricius. However, in no instance did more than one larva per host survive to pupation. Superparasitism is rather common in Terebrantia and it has been reported also in primitive aculeate wasps as *Tiphia femorata* Fabricius, which equally does not build any nest and, after oviposition, inspects the ventral aspect of the prey to discover strange eggs that, in the case, will be eaten. Instead, *Larra* "kneads" with the mouth the site of the prey where she will oviposit, probably to destroy any strange egg or larva. [92: pp. 119, 124, 125; 93; 74]. Among aculeate wasps which oviposit inside a nest from which the prey may not escape, superparasitism and consequent kneading behaviour are rare, even if non completely forgotten events, since they reappear in cases of intraspecific parasitism: for example, in a population of *A. sabulosa* were often observed wasps which opened the nest of a previous one to remove the egg from the caterpillar, replace it with their own, and then re-close the nest [94].

Instead, "host behaviour manipulation" is a quite rare phenomenon to observe, since its presence (together with the venom induced grooming behaviour) is restricted, as far as one knows, to *A. compressa* (see above) [74].

If the stings at the thoracic level induce paralysis in the corresponding limbs, those delivered in the head (throat) may give rise to a variety of situations which are quite complex to analyze, as one sees comparing the results from different wasps. In the case of different species of *Larra*, which adopt a C3SP (see above), but also a C2SP -scheme according to different species (and authors) [53], after a first temporary paralysis the wasp recovers in a few minutes and resumes normal activities until eaten by the developing host larva. Instead, in the case of *Liris* (C4SP) one has shown, by selective eliminations of the different stings, that only the last one in the throat is the necessary (and sufficient) condition to induce long-term hypokinesia (deactivation) in the cricket (that will not attempt to escape). The different action of the head sting in the two wasps is even shown by the fact that, according to Tsuneki [87], maxillary and labial palpi of the prey stung by *Larra carbonaria erebus* Smith could still move, in contrast to crickets stung by *Liris* wasp which seldom move these appendages. A similar permanent partial deactivation may be observed in *Prionyx* (C4SP in reverse order with respect to *Liris*) [87, 53]. Lastly, in *Ampulex* (C2SP), after the second sting (in the head) one observes grooming, hypokinesia and manipulated induced behaviour (see above). The diversity of responses due to the sting in the head should not wonder if one considers the neural complexity of the head central ganglia with respect to the thoracic ones. This means that the effect of the sting will change in relation to the specific head centers involved. We recall the precise stereotaxy of the venom injection of *Ampulex* in the brain centers and in the SEG (see above).

It appears that even in some cases of behavioural manipulations induced by worms, there is a parasite migration in the head ganglia; in other cases an overproduction of particular proteins or amino acids has been measured in the head or in the CNS of the host, probably induced by the parasite either directly or indirectly via a host genome response [74].

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