

specimen. This behavior begins with a sudden increase in speed of the pursuing fish and appears after either aggressive or random contact, or when the presence of the opponent is clearly felt.

20. Mutual chasing: rapid revolutions on the aquarium floor with each fish trying to bite the other's tail fin.

21. Flight: retreat initiating with an abrupt increase in speed of the fleeing fish, after an attack or random contact.

22. Avoidance: displacement away from the opponent, varying from a swerve to a reversal of direction without pursuit by the other fish.

23. Twirling: the fish swims along a wall of the aquarium in an elliptical trajectory, with the major axis of the ellipse usually vertical. Contact with the wall is maintained with the belly during ascent and with the back during descent.

24. Ascent: rise from the bottom of the aquarium to the upper levels, usually in connection with flight and/or avoidance, less frequently with pursuit.

25. Back-bending: the fish lies immobile with the antero-ventral part of its body in contact with the aquarium floor and the remainder arched so that the caudal axis is often vertical. This is usually seen during pauses between particularly prolonged and violent encounters (fig. 2).

26. Upwards posture: prolonged pause in a vertical position, with the head towards the water surface and the belly touching the aquarium wall. The fish maintains this position by slow movements of its tail, occasionally sinking passively to the floor where it remains briefly immobile with the tip of its tail touching the bottom.

27. Jumping: repeated attempts to jump out of the aquarium, sometimes as a sequel to upwards posture.

Of the above patterns all but three (23, 26 and 27) were shown – with varying frequency – by both the α and ω of each pair, and thus can be defined as 'common' displays whose appearance could be related to the onset of aggressivity rather than to the attainment of a dominant or subordinate status. Instead, twirling (23), upwards posture (26) and jumping (27) were shown almost exclusively (23, 26) or exclusively (27) by the ω . This, plus the fact that they were the sequel to avoidance or flight rather than to strictly aggressive events, probably indicates that these are typical of a subordinate status.

Of particular interest are the patterns of gaping (17) and back-bending (25). In fact, their role in the aggressive behavior of *Uegitglanis* is not completely clear. One hypothesis – which we deem worthy of thorough investigation in any case – is that these might be related to the emission of

mechanical (gaping) and chemical (back-bending) signals. If confirmed, the biological and behavioral significance of the 2 patterns would be explained. Otherwise, these may have been inherited unmodified, as behavioral rudiments, from the epigeal ancestor, in which the 2 patterns probably operated as visual displays.

The temporal sequence of the various patterns and their relative frequency in each partner is shown in figure 3.

Conclusions and discussion. The complexity and extreme variety of the aggressive behavior patterns used by *Uegitglanis* is obvious both from their description and from a careful study of figure 3. The behavioral repertoire of this species includes all the patterns observed in the epigeal siluriforms *Ictalurus natalis* Lesueur and *I. nebulosus* Lesueur⁸. In contrast to other species which show a similar adaptation to the hypogean biotope but have a reduced behavioral repertoire⁹⁻¹², the pronounced morpho-physiological regression of *Uegitglanis* – so clearly adapted to the hypogean environment³ – has not been paralleled by a regression in behavior. This, in fact, seems to have conserved all the aggressive behavior patterns of the epigeal Siluriformes. A probable explanation of this phenomenon is that, during the process of regressive evolution undergone by *Uegitglanis*, the biological significance and adaptive value of their aggressive behavior has remained unaltered.

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Sting alarm pheromone of the honeybee: the recruiting effect of an artificial blend of volatile compounds of the worker sting (*Apis mellifica* L., Hymenoptera, Apidae)¹

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Summary. Eight volatile compounds present in the sting of the honeybee worker were incorporated in different hexane solutions and their recruiting effect was evaluated by a bioassay at beehive entrances. The efficiency of the whole blend was higher than that of pure isoamyl acetate.

Sting alarm pheromone of the honeybee worker, released from the setaceous membrane of disturbed bees during alarm behavior² provokes the recruitment of the other workers ready to attack the intruder. The effect can be readily induced by freshly excised stings crushed at beehive entrances². Isoamyl acetate has been identified as a major and active compound of the worker bee sting secretion³,

but its efficiency at hive entrances is lower than that of the whole sting extracts⁴, indicating the presence of other compounds in the sting alarm pheromone.

Recently, other volatile compounds have been identified in the worker sting⁵, but neither their presence on the setaceous membrane nor their role in the elicitation of the alarm response has been reported.

Table 1. Composition of the different volatile blends

Compounds	n-Butyl acetate	Isoamyl acetate	Isoamyl alcohol	n-Hexyl acetate	2-nonanol	n-Decyl acetate	Benzyl acetate	Benzyl alcohol
Percentages of mean weight in one sting	1%	27% (1 µg)	12%	3%	9%	1%	13%	3%
Mean weight in 500,000 stings	18,500 µg	500,000 µg	222,000 µg	55,500 µg	166,500 µg	18,500 µg	240,500 µg	55,500 µg
Density	0.88	0.87	0.83	0.87	0.82	0.95	1.06	1.04
Volume to dilute in 1 l hexane	21 µl	574 µl	267 µl	63 µl	203 µl	19 µl	226 µl	53 µl
Vapor pressure (760 mm Hg)	118.0°C	142.0°C	130.0°C	-	213.5°C	299.5°C	213.5°C	204.7°C
Composition of the fractions		Isoamyl acetate solution						
		Light fraction						
					Whole blend		Heavy fraction	

Table 2. Evaluation of the different recruiting effects under field conditions

Material	Fresh sting	Empty control	Sting extract in 2 µl hexane	Control with 2 µl hexane	Whole blend 2 µl	Control with 2 µl hexane	Light fraction	Control with 2 µl hexane	Heavy fraction	Control with 2 µl hexane
Colony A	188	59	134	57	216	48	146	56	81	49
Colony B	91	16	96	48	89	37	80	46	49	43
A + B	279	75	280	105	305	85	226	105	130	83

Table 3. Comparison of the recruiting effects: number of workers recruited by the whole blend and by the isoamyl acetate solution

Colony	Material Whole blend		Isoamyl acetate	
	1st test	2nd test (reversed position)	1st test	2nd test (reversed position)
1	13	8	1	5
2	12	14	4	10
3	14	0	1	6
4	10	0	0	6
5	27	39	11	47
6	16	19	12	10
7	5	2	2	1
8	1	1	1	0
9	3	4	2	0
10	9	0	6	1
Σ		197 (61%)	323	126 (39%)

In this study, the alarm response of the workers to various volatile compounds present in the sting alarm pheromone was determined by a bioassay.

Materials and methods. Our blends were prepared according to the amounts of these compounds in a sting as reported by Boch-Schaerer⁶ and by Blum et al.⁵ (table 1, line 1: n-butyl acetate 1%, percentage of mean weights in 1 sting). The weights of substances in 500,000 stings (table 1, line 2) and the density of each substance (table 1, line 3) give the volume to dilute in 1 l of hexane so that each 2 µl of the solution contain the same amounts of compounds as found in a single worker bee randomly collected on a comb (table 1, line 4). Three blends were composed as follows: a) a whole blend (n-butyl acetate, isoamyl acetate, isoamyl alcohol, n-hexyl acetate, 2 nonanol, n-decyl acetate, benzyl acetate, benzyl alcohol), b) a 'light' fraction (n-butyl acetate, isoamyl acetate, isoamyl alcohol, n-hexyl acetate), c) a 'heavy' fraction (2 nonanol, n-decyl acetate, benzyl acetate, benzyl alcohol) and an isoamyl acetate solution. Moreover, whole sting extracts (1 sting/2 µl hexane) were prepared with stings rapidly

dissected from bees collected at random on the combs. The experiments were carried out with slightly aggressive Italian colonies (*Apis mellifica* L. *ligustica* Spinn). The worker bee population of the colonies was similar with bees on 8 combs and 5 combs of sealed brood in the brood chamber (brood chamber in Dadant hive: vol. 54 l, inside size 32×37,5×45 cm with 10 combs) and a super with bees working on 4 combs (super of Dadant hive: vol. 26 l, inside size 15,4×37,5×45 cm, with 9 combs). The samples to be tested (1 freshly removed sting of a bee collected at random, a single sting extract in 2 µl hexane, or 2 µl of the different blends) were applied to a white filter paper disk (5 cm diameter) fixed upon a metal drawing pin on the flight board at the hive entrance.

Bioassay. The number of the recruited bees going out of the hive, visiting the paper disk at least once, was recorded from the moment the sample was offered, during 1 min. Drones and foraging bees coming back into the hive were not included in the counts. Our tests did not deal with secondary alarm behavior.

Results. 1. Evaluation of the different recruiting effects. Bioassays were conducted in June with 2 colonies (A and B) in the morning (7–9 h) and in the evening (19–21 h) during 6 humid days with an average temperature of 15°C. The colonies exhibited low foraging activity and nectar secretion. Six series of 5 tests were carried out; before each test, the number of bees reaching the controls (clean paper disks or disks impregnated with 2 µl hexane) was recorded for 1 min. The results (table 2) show that the recruiting effects of a fresh sting, of a single sting extract in hexane, and of the whole blend were high as compared to the controls and represented approximately the same values. The efficiency of the 'heavy' fraction was lower than that of the 'light' one. However, the responses of colony A were stronger than that of B.

2. Comparison of recruiting effects (table 3). To learn more about the relative contributions of the different components of the honeybee recruiting pheromone, experiments were carried out with 10 colonies, under the same conditions mentioned previously. The flight board was divided in two halves by a vertical partition; on each

side of the partition, a filter paper was deposited, one with the isoamyl acetate solution (2 µl) and the other with the whole blend. The number of bees recruited by each paper disk was recorded for the length of 1 min. Another evaluation was carried out 30 min later with new paper disks and fresh solutions but in reversed positions.

The results obtained with the 323 workers recruited by the 2 solutions were as follows: the whole blend recruited 197 (61%) bees, but only 126 (39%) were recruited by isoamyl acetate (table 3).

Discussion and conclusion. The testing method, carried out with outdoor colonies, has been previously and successfully used for ethological studies with *Apis mellifica* L.^{2,3,7} and with the Asian species of the genus *Apis*⁸. However, in the 1st experiments with colonies A and B, the bees gradually became accustomed to the presence of the filter paper disk and reacted even to a control paper disk or to hexane alone. This lead us to reduce number of tests carried out with the same colony. The compounds identified on the sting⁵ have been detected on the setaceous membrane⁹ which releases the sting alarm pheromone of the worker². Accordingly, an artificial mixture of these compounds was tested; its efficiency had been compared to the stings of bees collected at random on the combs. These stings containing an average of 1 µg of isoamyl acetate were used as reference. Although they are not rich in volatile substances, the stings or their extracts in hexane exert a recruiting effect. The whole blend showed a similar effect and may be considered as an active alarm pheromone. Like freshly excised stings, its recruiting effect is stronger than that of isoamyl acetate alone. Both light and heavy fractions have a recruiting effect showing that the 2 fractions participate and give to the whole blend its full activity. However, this blend seems to be an incomplete alarm pheromone: n-octyl acetate⁵ and other unidentified compounds were not incorporated. All the compounds of the blend and n-octyl acetate have been separately tested in a laboratory test with small groups of young caged honeybee workers¹¹. Two substances (n-decyl acetate and benzyl alcohol) were found inactive; 2 nonanol and benzyl acetate caused the longest reaction times, but

this had never been proved with outdoor colonies. The effect of the blend differs with bees from different colonies. Behavioral differences between colonies A and B can be attributed to internal factors (genetics): aggressivity and sensibility threshold to the pheromone. Like most pheromones of the honeybee (mandibular gland pheromone of the queen, Nasanoff gland pheromone of the worker), the alarm pheromone appears as a blend. Functional connections between the Koschewnikow gland and the setaceous membrane have been established by light microscopy¹²; volatile compounds of the blend have been detected in the Koschewnikow gland. The alarm pheromone is produced by the Koschewnikow gland, stored on the setaceous membrane and released when the guard bees are disturbed². Other stimuli (visual, olfactory) induce the attack of the recruited bees^{2,10}.

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Vasodilatation on preoptic heating in capsaicin-treated rats

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Summary. Rats treated with 50 or 300 mg/kg capsaicin were less tolerant of a warm environment than controls. However, preoptic thermoregulatory impairment, as shown by a decreased vasodilatation on preoptic heating, was not found for the lower dose, suggesting a deficiency in extrahypothalamic thermoreceptive structures.

In rats treated with capsaicin, an irreversible deficit of thermoregulation has been reported; when exposed to heat, the animals develop severe hyperthermia due to an impaired functioning of the thermolytic mechanisms²⁻⁸. Although several lines of evidence indicate that the drug affects the preoptic/anterior hypothalamic (POAH) warmth detectors^{3,4,8-13}, some observations suggest that a deficiency of the extrahypothalamic thermoreceptive structures may also contribute to the thermoregulatory impairments^{3,14-16}. Moreover, in rats treated with capsaicin 2 days after birth only peripheral morphological alterations without damage of the POAH were reported¹⁷⁻¹⁹. To obtain information on the role of the POAH in thermoregulatory impairment by capsaicin, we studied the tail skin vasodila-

tion response, an important thermolytic reaction, to local heating of the POAH.

Methods. Male rats of the CFY strain were used. Capsaicin (32.7 mmol · l⁻¹) was dissolved in saline with the aid of Tween 80²⁰. Three groups of animals were injected s.c. with the drug under light ether anesthesia. Newborn rats received a single injection of 50 mg/kg capsaicin 2 days after birth (group ND-50). Two-month-old rats were treated with either 50 mg/kg (group AD-50) or 300 mg/kg (group AD-300) capsaicin. The latter dose was administered in 6 daily fractions (10, 20, 20, 50, 100 and 100 mg/kg). The vasodilatation experiments were carried out with 3-4-month-old animals.

Before the POAH heating experiments, the rats kept at