

Heterogeneous chemical profiles of *Vespa velutina nigrithorax* alarm pheromone



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Context

Invasive species are permanently modifying the distribution and diversity of native species worldwide. Due to its invasive potential, the yellow-legged hornet, *Vespa velutina nigrithorax* represents a high-concern species under both economical and ecological perspectives. Native to Southeast Asia, *V. v. nigrithorax*, has since spread throughout Europe, causing significant harm to insects, specifically honeybee.

Materials & Methods

44 hornet colonies were collected in France. To quantify the chemical compounds in hornet females, we dissected:

- 75 **workers**,
- 40 **gynes** (mated female with no nest),
- 30 **foundresses** (mated female with a small nest and some eggs),
- 30 **queens** (mated female with workers).

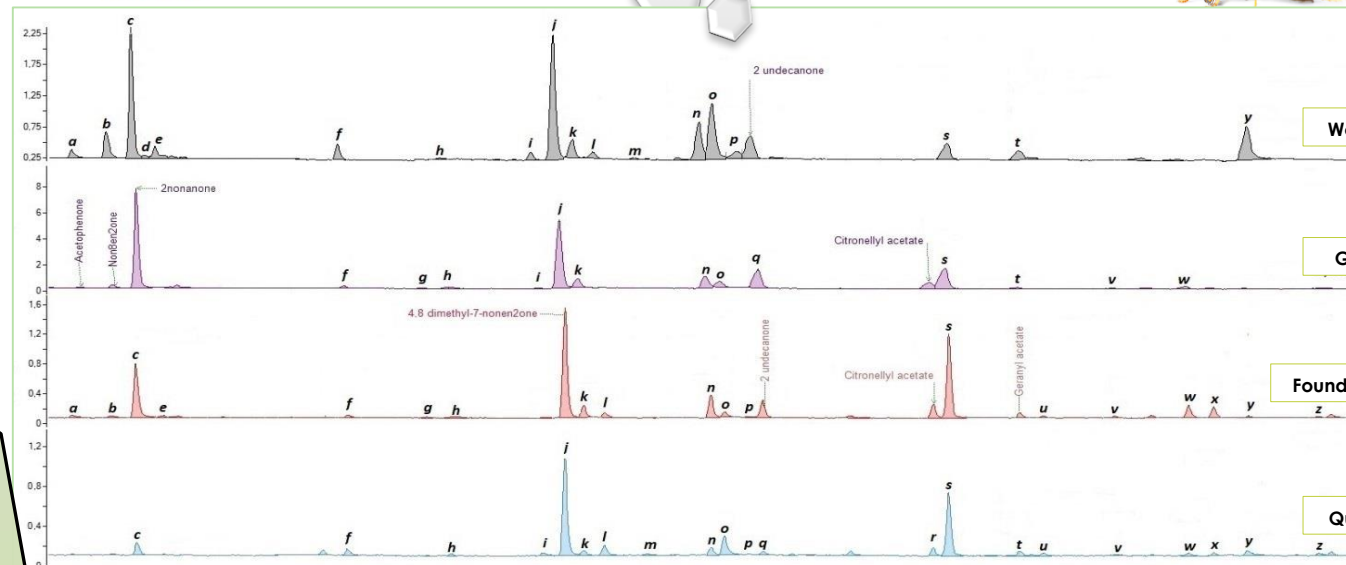
Within each type of females,

a set of 5 venom sacs was placed in a vial and then perforated in one ml of heptane. The vials were vortexed at 300 rpm for 1 min.

Two microliters of extract were analyzed using a gas chromatograph (Agilent Technologies 7890B) coupled to a mass spectrometer (Agilent Technologies 7000C GC/MS Triple Quad) with an HP-5 capillary column (30 m x 0.32 mm x 0.25 µm); helium was

the carrier gas (flow rate=1.2 ml/min). The temperature was set to ramp up from 50°C to 200°C at a rate of 8°C/min and then from 200°C to 315°C at a rate of 5°C/min; the cycle finished with a 5-min hold at 315°C. The samples were analyzed using an Agilent 7890B gas chromatograph coupled with an Agilent 7000C mass spectrometer.

Our goal was to clarify the composition of the species' alarm pheromone and to assess differences in chemical profiles among queens, foundresses, gynes, and workers.



Workers

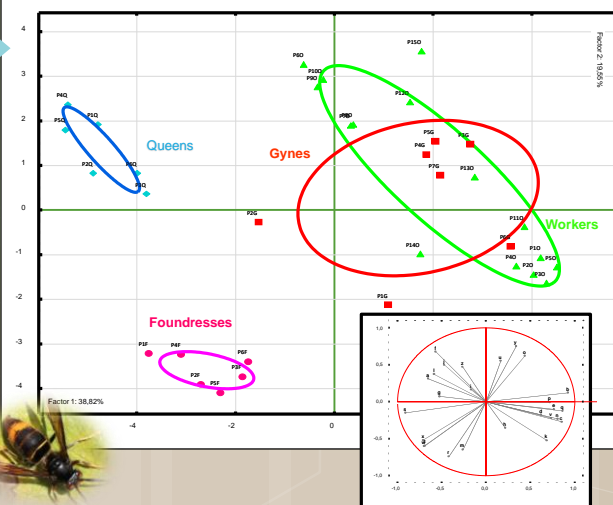
Gynes

Foundresses

Queens

Results : 26 compounds were present in the profiles of the gynes and foundresses, but three were absent from the workers' profiles, and one was absent from the queens' profiles.

Chemical profiles of the venom glands revealed 38 compounds with chain lengths ranging from 8 to 12 carbons. K-means cluster analysis distinguishes 4 groups.



Name:	Queens	Foundresses	Workers	Gynes
Acetophenon	a 1,7 ± 1	0,4 ± 0,3	0,2 ± 0,2	0,008 ± 0,001
8-Nonen-2-one	b 0,4 ± 0,3	0,8 ± 0,2	3,7 ± 0,9	3,2 ± 0,5
Nonan-2-one	c 3,4 ± 0,9	16,4 ± 2,8	18,2 ± 3,2	14,2 ± 3,7
Nonan-2-ol	d 0,3 ± 0,3	0,4 ± 0,2	1,6 ± 0,8	0,8 ± 0,9
Isoamyl isovalerate	e 0,1 ± 0,1	0,8 ± 0,2	2,0 ± 0,9	2,5 ± 0,8
X-, X'-cyclohexanol	f 3 ± 0,4	0,7 ± 0,1	1,3 ± 0,9	0,8 ± 0,7
Isoamyl senecioate	g 0,6 ± 0,5	0,2 ± 0,1		0,1 ± 0,1
X-C12:1	h 0,4 ± 0,4	0,4 ± 0,1	0,7 ± 0,9	0,8 ± 1,1
Unknown	i 1,4 ± 0,3	0,2 ± 0,1	0,8 ± 0,6	0,5 ± 0,5
4,8-diMe-7-nonen-2-one	j 28,3 ± 5,7	21,8 ± 2,9	23,3 ± 5,3	24,6 ± 3,1
2-Nonanol, 2-acetate	k 1 ± 0,6	3,7 ± 0,5	3,4 ± 1,1	4 ± 0,8
β-Citronellol, methyl ether	l 4,5 ± 2,1	1,0 ± 0,4	1,2 ± 0,8	1,7 ± 0,8
2-Phenethyl acetate	m 0,4 ± 0,2	0,4 ± 0,2	0,2 ± 0,3	0,2 ± 0,3
X-Undecen-2-one	n 2,6 ± 0,2	5,5 ± 0,4	9,3 ± 2,9	5,8 ± 1,4
X'-Undecen-6-one	o 6,7 ± 1,8	2,3 ± 0,7	8,5 ± 2	11,5 ± 2,0
X''-Undecen-2-one	p 0,2 ± 0,3	0,2 ± 0,1	1,2 ± 0,9	1,5 ± 0,5
Undecan-2-one	q 2,4 ± 1	6,1 ± 1	7,7 ± 1,6	7,7 ± 2,0
Citronellol acetate	r 1,6 ± 0,8	5,9 ± 2	0,5 ± 0,5	0,7 ± 0,6
Unknown	s 23 ± 5	15,8 ± 2,2	5,2 ± 1,6	7,2 ± 2,6
Geraniol acetate	t 1,2 ± 0,5	1,7 ± 0,4	0,1 ± 0,1	0,1 ± 0,2
Decane-2,9-dione	u 0,2 ± 0,2	0,1 ± 0,2	0,8 ± 0,6	0,6 ± 0,4
Unknown	v 0,2 ± 0,2	0,5 ± 0,1	1,2 ± 0,7	1 ± 0,3
Isopentyl benzoate	w 1,3 ± 0,1	2,4 ± 1		0,3 ± 0,4
3-Me-3-butenyl benzoate	x 1,2 ± 0,3	2,0 ± 0,8	0,2 ± 0,2	0,4 ± 0,6
X-Undecene-2,10-dione	y 2 ± 0,6	0,8 ± 0,4	4,5 ± 2,1	4,7 ± 1,8
Undecane-2,10-dione	z 1,6 ± 0,5	0,5 ± 0,3	1,3 ± 0,8	0,4 ± 0,5

Chemical composition of the venom glands of *V. v. nigrithorax* for queens, foundresses, gynes, and workers (% ± se).

Chemical profiles workers, gynes, foundresses and queens (based on four pools of venom glands), the letters refer to the compound names (Table).

Conclusions :

- ✓ Marked separation among **foundresses**, **queens** and **gynes**. This result indicates that the chemical signature of a female's venom gland changes across life-history stages
- ✓ Gynes are closer to workers : gynes' profiles later shift
- ✓ Contrary to previous studies, the major compound observed, in this study, in all castes was 4,8-diMe-7-nonen-2-one (21-28 %)

Complex composition of the alarm pheromone of queens, foundresses, gynes, and workers in *Vespa velutina nigrithorax* Berville L, Lucas C, Haouzi M, Khalil A, Gévar J, Bagnères A-G, Darrouzet E