

ALLERGENS IN HYMENOPTERA VENOM. VIII. IMMUNOLOGIC COMPARISON OF VENOMS FROM SIX SPECIES OF VESPULA (YELLOW JACKETS)

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Venoms from six species of ground dwelling yellow jackets of the genus Vespula were studied by direct and inhibition of RAST. Significant differences were found among the various species. Reactivity of patients showed significant correlation with insect distribution with the exception of some patients from the far west. The immunologic relationships observed among the venoms correlated well with the current phylogeny, which is based upon morphologic, behavioral and ecologic studies. The correlation with isoenzyme studies that have recently been reported was less complete. Individual patients exhibit highly significant differences in their reactivity to the various species, in many cases comparable to the differences seen in reactivity to yellow jackets and aerial hornets (Dolichovespula).

IN A RECENT STUDY from our laboratory it was demonstrated that there were significant immunologic differences between the venoms of the two species of ground dwelling yellow jackets, *Vespula maculifrons* and *Vespula squamosa*.¹ That study suggested that there were further differences in the reactivities of human IgE antibodies from individuals clinically allergic to yellow jacket stings that may have resulted from species differences in venoms. In the present study the reactivity of the 39 sera from patients allergic to yellow jacket venom used in the previous study was examined with venom from six species of yellow jackets. The venoms used were from *Vespula vulgaris*, *V. maculifrons*, *V. flavopilosa*, *V. pennsylvanica*, *V. squamosa* and *V. sulphurea*. Significant immunological differences among the species were found and these differences correlate with the morphological, behavioral and ecological characteristics of the species.

Materials and Methods

Sera—The sera and the patient population are identical to those previously described.¹

Radioallergosorbent Test—The methods for direct and inhibition RAST are as previously described by our laboratory.^{1,2,3}

Venoms—The venom from the individual species was purchased from Vespa Laboratories, Spring Mills, Pennsylvania. The very rare *V. sulphurea* venom was a generous gift from Vespa Laboratories Inc. The method of preparation and standardization are as previously described.^{1,3}

Results and Discussion

Each of the six venoms was coupled to CNBr activated paper discs under standard conditions. The 39 positive sera plus negative control sera were all tested by direct RAST against the six venoms in a single experiment. The results are given in Table I. All of the 39 sera were significantly positive to at least four of the six venoms. Only three sera, all from the southeast, were most reactive with *V. squamosa*. However, 21 sera had much weaker reactions with *V. squamosa* and *sulphurea* than with the other species' venoms. Significant differences in reactivity appeared to be present for all six venoms. The average RAST score was highest for *V. flavopilosa*, then *vulgaris*, *maculifrons*, *pennsylvanica*, *squamosa* and *sulphurea*. This reflects the geographic distribution of patients and insects. The results of RAST to *V. maculifrons* and *V. squamosa* from the present study were compared to those reported in the previous study.¹ For each venom the correlation coefficient for the two experiments using

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Table I. RAST to Six Species of Yellow Jackets in Percentage Binding after Subtraction of Non-specific Binding.

Serum	State	vulgaris	flavopilosa	maculifrons	pensylvanica	squamosa	sulphurea
2597	NY	34.3	28.9	34.4	31.0	19.2	13.6
2694	NY	44.1	28.3	32.5	37.7	36.1	25.2
2737	CA	11.5	13.4	20.2	5.1	4.5	0.7
2762	CA	23.9	37.1	29.3	23.1	23.3	26.6
2788	CA	34.9	46.6	31.8	37.3	25.5	39.9
2794	CA	18.8	16.1	13.1	11.8	9.7	10.8
2874	NY	14.8	22.3	15.7	9.5	10.5	8.7
2883	NY	11.2	19.0	21.5	5.8	0.1	0.8
2962	CA	24.4	39.1	26.5	18.4	7.2	8.4
3016	NY	8.2	11.6	22.0	5.3	7.7	4.5
3117	NY	27.6	38.8	19.3	12.8	1.9	6.7
3351	NY	25.1	29.6	18.7	22.7	13.7	11.8
3507	NC	16.8	35.7	21.7	24.5	17.7	25.0
3526	CT	32.3	47.2	28.3	32.2	34.0	40.8
3644	NC	41.5	44.8	36.0	35.6	35.3	39.2
3684	AL	24.6	21.3	13.8	23.0	38.4	21.2
3711	PA	43.7	43.5	31.1	39.1	3.6	4.6
3900	CA	20.5	9.6	16.8	26.1	9.5	5.0
3926	NY	43.6	37.3	25.6	27.7	20.0	17.7
4092	FL	20.8	26.8	27.1	17.4	32.6	25.6
4127	NC	9.7	13.1	10.0	6.1	9.7	10.0
4428	NY	14.5	16.8	22.5	6.7	3.1	1.4
4661	NY	24.8	21.4	21.5	14.8	3.4	13.6
4662	NY	5.7	3.4	3.4	5.2	5.3	1.9
4670	NY	18.7	21.4	19.5	14.2	5.1	2.7
5673	CA	23.4	29.7	18.7	23.3	22.1	26.2
5675	CA	21.4	30.9	17.9	8.9	16.6	21.5
5747	NY	37.5	30.0	31.6	24.6	25.9	18.8
5861	MD	26.3	29.1	21.5	23.8	31.3	26.9
5865	CA	38.4	33.6	28.4	34.4	25.8	23.2
5875	PA	18.6	26.9	11.2	13.6	1.5	1.2
5934	CA	11.7	34.0	36.8	7.4	9.5	3.4
6129	CA	16.1	10.7	15.1	23.0	14.6	6.6
6250	PA	28.3	29.4	20.7	31.4	29.3	22.8
6426	CA	29.2	36.5	28.0	15.2	13.4	11.6
6603	CA	14.6	14.1	9.2	14.5	10.8	10.0
6642	NC	13.5	12.0	8.5	9.6	2.9	3.1
6738	CA	38.6	37.5	37.4	34.6	25.3	21.7
6797	PA	8.6	17.6	8.3	9.5	5.9	8.4

39 positive sera was 0.91. Different lots of labelled antibody and discs were used in each experiment.

The RAST results for each venom were compared to those for the other five by linear regression analysis. The correlation coefficients are given in Table II. All are significant at p less than 0.01 using the F distribution test. The highest correlations were obtained for the pairs *V. vulgaris* and *pensylvanica* and *V. squamosa* and *sulphurea*. The two species *V. squamosa* and *sulphurea* form one group and the four species *V. vulgaris*, *maculifrons*, *flavopilosa* and *pensylvanica* another group. *Vespa pensylvanica* appeared somewhat less related to *V. flavopilosa* and *maculifrons* and more related to *V. squamosa* and *sulphurea* than the other members of its group. The results were also subjected to multiple linear regression analysis. The correlation coefficients were not significantly improved but a significant association between *V. maculifrons* and *V. flavopilosa* became apparent.

The immunological relationships between the various species venoms was further explored by RAST inhibition studies using individual patient sera. Some of the results are presented in Table III. The binding of IgE antibodies from serum 4092, which is primarily reactive with *V. squamosa*, to *V. squamosa* was inhibited at a lower

Table II. Correlation Coefficients Between RAST Results to Yellow Jacket Species.

	flavopilosa	maculifrons	pensylvanica	squamosa	sulphurea
vulgaris	0.74	0.70	0.87	0.59	0.59
flavopilosa		0.73	0.64	0.46	0.64
maculifrons			0.60	0.46	0.47
pensylvanica				0.68	0.68
squamosa					0.87

concentration of the closely related *V. sulphurea* than the less related *V. maculifrons*. Serum 6129 is most reactive with *V. pensylvanica* by direct RAST; this specificity is confirmed by inhibition which shows that *pensylvanica* venom is five to eight times more potent an inhibitor of binding to *V. pensylvanica* venom than the three related venoms are. A similar situation is seen for serum 4661, which binds most strongly to *V. vulgaris*. In this case *vulgaris* venom is a three times more potent inhibitor of binding to *V. flavopilosa* venom than *flavopilosa* venom is.

The direct RAST results for sera 5675 and 5875 show the strongest binding to *V. flavopilosa*. However, in the case of 5675 *vulgaris* venom is five times as potent an inhibitor and inhibition is complete with both venoms. This indicates that *flavopilosa* venom may contain more

of the relevant antigens. For serum 5875 pensylvanica venom is a more potent inhibitor of binding to flavopilosa, but vulgaris inhibits more completely.

Serum 3526 is not clearly specific for any of the species. Since *V. sulphurea* is a rare species found in the southwest and the patient lives in Connecticut, the potent inhibition of binding to *V. sulphurea* by the eastern species *V. flavopilosa* and the western species *V. pensylvanica* suggest that this individual produces a highly cross-reactive antibody.

Serum 5673 is from a patient in California; it shows significant reactivity with all six venoms. Binding to *V. flavopilosa* was inhibited by small quantities of vulgaris and pensylvanica venoms. Inhibition of binding to *V.*

squamosa required about 10 times more *V. sulphurea* venom; however, even more maculifrons venom was required. The largest amount of inhibitor was required for inhibition of binding to *V. maculifrons* by *V. squamosa*.

In most cases the RAST inhibition results confirm the relationships among the species that were found by analysis of the direct RAST results. The immunological relationships among the six species are diagrammed in Figure 1. Since the immunological relationships parallel the morphological, behavioral and ecological relationships,^{4,5} the subgenera are also included in the figure. The genus *Vespula* is divided by many workers into three subgenera. The first, *Allovespula*, is the so-called "rufa" group of species, *atropilosa*, *consobrina*, *vidua*, *acadica*, *intermedia* and *austriaca*. The insects in this group live in small colonies and are strictly predacious, so they rarely sting man.⁶ The second subgenus, *Paravespula*, is found in both the New and Old Worlds and includes *V. maculifrons*, *flavopilosa*, *vulgaris*, *pensylvanica* and the introduced *germanica*. These are the most common species of yellow jackets and representatives are found throughout the United States.^{6,7} All except *pensylvanica* are found in the East and *V. vulgaris* and *pensylvanica* are found in the West. The third subgenus is *Vespula* and contains only two species, *V. squamosa* found in the southeast, the only yellow jacket with an orange queen, and the rare *V. sulphurea* found in California and occasionally in surrounding states. This taxonomy is based primarily on morphological grounds⁴ but is confirmed by behavioral and ecological evidence.^{5,6} Studies of the isoenzyme banding patterns of the *Paravespula* species show complete correlation with morphologic species.⁴

The immunologic data suggest that *V. vulgaris* and

Table III. Inhibition of the Radioallergosorbent Test with Various Yellow Jacket Species.

Serum	Specificity	Disk-inhibitor	Concentration for 50% inhibition
4092	squamosa	squamosa-sulphurea	2.2 micrograms
		squamosa-maculifrons	8.4
5673		squamosa-sulphurea	13
		squamosa-maculifrons	17
		maculifrons-squamosa	35
		flavopilosa-vulgaris	1.1
		flavopilosa-pensylvanica	0.9
5675	flavopilosa	flavopilosa-flavopilosa	2.4
		flavopilosa-vulgaris	0.5
3526		sulphurea-flavopilosa	0.8
		sulphurea-pensylvanica	0.3
6129	pensylvanica	pensylvanica-vulgaris	13
		pensylvanica-maculifrons	11
		pensylvanica-flavopilosa	17
		pensylvanica-pensylvanica	2.1
4661	vulgaris	flavopilosa-vulgaris	1.6
		flavopilosa-flavopilosa	4.6
5875	flavopilosa	flavopilosa-vulgaris	0.4
		flavopilosa-pensylvanica	0.2

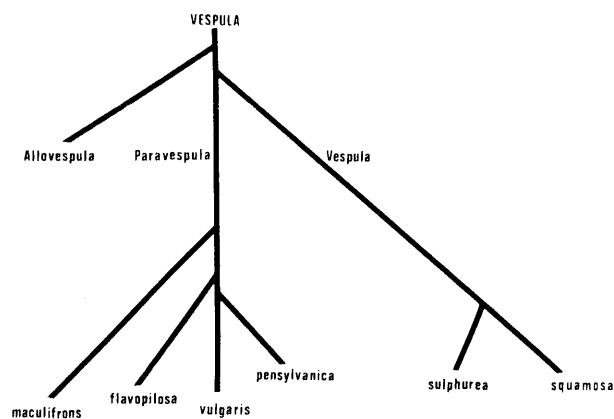


Figure 1. Diagram of the immunological relationships observed among the six species of *Vespula* studied. The three subgenera established by morphologic, behavioral and ecologic criteria are shown midway down the figure.

pennsylvanica are closely related; however, both the hexokinase and malic dehydrogenase isozymes are different for these species.⁴ There are also several significant morphologic differences. The high correlation of reactivities to these two species may be a result of inclusion of a substantial number of patients resident in the far west, where these are both common species. The similarities between maculifrons and flavopilosa and between flavopilosa and vulgaris seen morphologically are confirmed by the isozyme studies; the hexokinase isozymes are different for each species and the malic dehydrogenase isozymes are the same. The immunologic studies also indicate a relatively close relationship among these three species. The two species of the subgenus Vespula, squamosa and sulphurea, are morphologically distinct from Paravespula. It should be noted that V. squamosa is a facultative parasite on V. maculifrons⁵ and probably also on V. flavopilosa.

Jacobson et al¹ have proposed that V. pennsylvanica and V. germanica may form a subgroup of Paravespula based upon morphological grounds. Our data are contradictory on this point. Reactivity to pennsylvanica is very highly related to reactivity to vulgaris but is less related to reactivity to flavopilosa and maculifrons. V. pennsylvanica venom also appears to be more related to squamosa and sulphurea venoms than the other Paravespula venoms are. This study did not include V. germanica, a species introduced from Europe.

The immunologic reactivities of six species of Vespula venom indicate that significant differences exist among venoms from different species. These differences generally parallel the phylogeny established by morphologic, behavioral and ecologic studies and isoenzyme typing. The differences between the subgenera Paravespula and Vespula are substantial and there are individuals who are reactive to venoms from only one subgenus. Most of the sera studied exhibited some degree of species specificity. The patterns of reactivity observed generally correlated with species distribution. Primary reactivity to V. squamosa was found in patients from the southeast. The strongest reactivity of patients from California was more variable. About half were most reactive with the common yellow jackets found in the west, V. vulgaris and pennsylvanica; most of the rest were most reactive with flavo-

pilosa, and two were most reactive with maculifrons, neither of which is found in the west. The most probable explanation suggested by the RAST inhibition data is that the venoms of V. flavopilosa and maculifrons contain larger quantities of some of the allergens common to all of the Paravespula species. Alternatively some of these individuals may have been sensitized in the east, although all experienced their reactions in California. No sera from the east were primarily reactive with the western species V. pennsylvanica.

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"I no sooner come into the library, but I bolt the door to me, excluding lust, avarice and all such vices, whose nurse is idleness, the mother of ignorance and melancholy herself, and in the very lap of eternity, amongst so many divine souls, I take my seat, with so lofty a spirit and sweet content that I pity all our great ones and rich men that know not this happiness."

Robert Burton

The Anatomy of Melancholy