

ALLERGENS IN HYMENOPTERA VENOM. IX. SPECIES SPECIFICITY TO POLISTES (PAPER WASP) VENOMS

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Sera from 62 patients allergic to Polistes venom were used to study the allergenic relationships among four individual species of Polistes by both direct and inhibition RAST. Both direct and inhibition results showed that the four species' venoms were closely related. The species within the same subgenus were more closely related than those in different subgenera. The Polistes species were more closely related to each other than various yellow jacket (Vespula) species are to each other. The commercial Polistes venom mix appears to contain all of the relevant allergens.

ALTHOUGH MUCH HAS BEEN WRITTEN on the subject of insect venom allergy in recent years, little has been published concerning allergic reactions to the venom of the genus *Polistes*, the paper wasps. In part this may reflect the geographic distribution of the centers that have performed many of the studies of venom allergy, which are concentrated in the northeastern quadrant of the United States. *Polistes* allergy is most common in the south^{1,2} from Virginia to Florida and west through Texas and Arizona. In the early studies from Maryland^{3,4} and New York⁵ *Polistes* allergy was rare, consistent with recent geographic studies.¹ In 1977 Findlay et al⁶ reported on studies of seven *Polistes* allergic individuals from Texas and demonstrated that *Polistes* venom could release histamine from the leukocytes of allergic individuals. In a recent communication Grant et al⁶ reported upon immunotherapeutic trials of *Polistes* venom in 36 allergic subjects. In these trials a higher rate of systemic reactions upon challenge was reported in treated patients than was found in trials using the other four hymenoptera venoms.^{4,7,8} One suggested explanation for this observation is that the venoms of various species of *Polistes* may be significantly different antigenically and that the concentrations of some of these antigens may be insufficient in the standard commercial treatment mixture which contains *P. annularis*, *exclamans*, *fuscatus* and *metricus*. The most common species of wasp in the Galveston area is reported to be *Polistes apachus*.²

The genus *Polistes* is divided into 11 subgenera by Richards.⁹ The species found in the United States along with their geographic ranges¹⁰ are shown in Table I. The five common species are all in two subgenera, *Polistarchus* and *Fuscopolistes*. *P. apachus*, the only common species not in the standard mix, is closely related to *P. fuscatus* and *metricus*, which are represented in the venom mixture.

In this study venoms from four species of *Polistes*—*exclamans*, *annularis*, *apachus* and *fuscatus*—were examined using sera from 62 individual allergic patients from various regions of the U.S. All of the sera were studied by direct RAST and multiple RAST inhibition experiments were performed with 16 of the sera.

Materials and Methods

Sera—All patient sera were examined by RAST for reactivity with honey bee, yellow jacket, yellow hornet, white faced hornet and mixed *Polistes* venoms. All of those selected were significantly more reactive with *Polistes* than with any other venom. The sera were all from patients who had experienced a systemic or large local reaction to a sting within the previous year, in most cases within two months. Many were known to be skin test positive. The secondary selection criterion was geographic and the northeast, mid-Atlantic, southeast, south central, southwest desert and far west were all represented. The sera studied by RAST inhibition were chosen on the basis of pattern of reactivity measured by direct RAST, sufficient binding to allow reliable inhibition experiments and sufficient serum volume to carry out the studies. All sera were obtained prior to any venom immunotherapy.

Venoms—Mixed *Polistes* species venom and venoms from the four individual species were purchased from

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Vespa Laboratories, Spring Mills, Pennsylvania. The preparation and standardization have been previously described.^{11,12,13} All venom solutions were prepared fresh daily.

RAST and RAST Inhibition—Our techniques for RAST and inhibition of RAST have been described previously in detail.^{11,12,13}

Results

The 62 sera from *Polistes* allergic individuals were tested by direct RAST using mixed *Polistes* species

Table I. Classification of *Polistes* Found in the United States by Subgenera. Those Preceded by * Are Common Species, the Others Are Rare.

Species	Range	Subspecies
<i>Polistarchus</i>		
* <i>annularis</i>	CT to FL west to SD and TX	3
* <i>canadensis</i>	So. AZ	
* <i>exclamans</i>	NJ to FL west to NE CO TX; AZ CA	
* <i>instabilis</i>	So. TX	
* <i>kaibabensis</i>	AZ So. UT	
<i>Fuscopolistes</i>		
* <i>apachus</i>	KS TX CO CA	2
* <i>carolina</i>	PA to FL west to KS TX	
* <i>dorsalis</i>	SE. SW	
* <i>flavus</i>	So. CA NV	
* <i>fuscatus</i>	USA	5
* <i>metricus</i>	NY to FL west to NE OK TX	
* <i>perplexus</i>	MD to GA west to IL KS OK TX	
* <i>palisotius</i>	So. FL TX; So. CA	
<i>Palisotius</i>		
major	So. FL TX; So. CA	2
<i>Epicnemius</i>		
pacificus	So. TX	
<i>Onerarius</i>		
carnifex	So. TX AZ	
Unknown		
comanchus	TX AZ	2

venom and the venoms from *P. exclamans*, *annularis*, *apachus* and *fuscatus* in a single experiment using one lot of reagents. The non-specific binding was determined for each antigen using sera from non-allergic individuals and pooled cord serum; this value was subtracted from all results. The results for all 62 sera are presented in Table II along with the state of residence of each subject. Of the 61 positive sera, 44 were most reactive with either *P. exclamans* and/or *annularis*, eight with *apachus* and/or *fuscatus* and nine with species in both subgenera. The patterns of reactivity were examined as a function of geography; the only statistically significant association found was that *exclamans/annularis* reactivity was more common in the east and that *apachus/fuscatus* reactivity was more common in the west (including Texas).

The direct RAST results for each venom were compared to those for the other venoms by linear regression analysis. The correlation coefficients are given in Table III. The highest correlation was 0.95 between *P. exclamans* and *annularis*, both species in the same subgenus. The two species in the other subgenus studied, *P. apachus* and *fuscatus*, gave a coefficient of 0.91. The correlation coefficients between species in different subgenera were all lower, ranging from 0.76 to 0.87. These are all highly significant and are higher than those reported for most yellow jacket (*Vespa*) species.¹¹ This suggests that although the *Polistes* species are morphologically quite distinct,¹² the venoms are closely related immunologically.

Sixteen representative sera were selected for RAST inhibition studies. A summary of the studies performed and results are given in Table IV. The combinations to be studied were chosen from the direct RAST results to

Table II. RAST to Venoms from Individual Species of Paper Wasps (*Polistes*) in Percent Binding.

Serum	State	Mixed	exclamans	apachus	fuscatus	annularis	Serum	State	Mixed	exclamans	apachus	fuscatus	annularis
3747	AZ	16.2	17.3	10.5	10.0	19.1	5755	CA	20.3	16.8	22.2	20.7	17.0
3800	NC	6.7	6.5	6.2	4.5	5.6	5768	NC	15.0	18.5	6.9	7.3	20.3
3801	NC	18.2	13.6	12.7	6.6	12.7	5862	MD	0.2	0.6	0.4	0.5	0.7
3802	NC	15.0	12.9	9.7	9.3	13.7	5926	VA	2.0	1.4	2.1	1.8	1.4
3840	FL	11.3	12.6	8.5	5.4	13.9	5937	NC	12.3	12.0	10.6	4.5	14.7
3841	FL	27.1	14.3	19.4	17.1	10.6	5938	NC	10.8	11.8	7.8	8.7	12.1
3913	CA	3.5	15.2	9.5	11.8	9.5	5944	NC	9.3	12.4	9.5	9.3	11.0
3990	CA	4.5	2.8	4.3	6.7	4.6	6010	NC	7.8	9.0	4.7	4.4	10.4
4023	CA	2.8	2.6	2.0	2.5	3.9	6016	NY	30.1	30.3	9.5	15.8	19.2
4037	FL	3.7	5.1	2.9	2.6	9.4	6033	NC	0.5	1.0	0.3	0.4	1.0
4088	FL	4.7	2.3	1.3	1.3	2.9	6074	NC	28.0	38.6	31.9	19.1	42.7
4164	FL	8.0	6.1	6.0	6.7	8.8	6080	VA	16.9	20.6	22.1	11.0	20.3
4227	FL	6.1	7.5	5.7	5.6	8.3	6128	NC	9.0	7.5	5.2	6.4	7.7
4796	NC	4.9	3.7	3.3	2.9	5.2	6243	NC	1.9	1.7	1.0	0.7	2.0
4856	IN	3.9	3.4	3.0	4.1	4.1	6500	VA	1.8	0.9	4.9	11.6	6.7
4895	NC	0.2	0.3	0.0	0.0	0.0	6527	VA	9.4	7.6	2.1	2.1	5.2
4998	NC	6.4	6.7	5.2	3.9	6.3	6629	TX	2.4	2.2	2.0	1.9	3.6
5162	AL	2.0	3.9	1.1	1.7	3.5	6638	TX	1.8	2.8	1.5	1.3	2.7
5339	NC	9.3	9.1	3.5	5.5	7.8	6639	TX	2.6	1.5	1.6	1.9	3.4
5387	AL	6.9	6.5	3.5	3.4	8.3	6642	NC	12.9	8.7	5.9	6.3	10.2
5411	NC	4.5	6.6	3.3	2.3	7.8	6683	FL	26.5	22.4	27.9	28.1	17.8
5436	NC	30.0	31.8	25.1	24.1	25.4	6736	AZ	0.6	0.7	0.4	0.3	1.1
5442	VA	4.4	7.4	3.6	3.2	7.7	6737	TX	8.5	6.4	8.6	9.9	6.5
5451	NC	3.7	2.7	2.6	2.4	3.6	6750	CA	1.5	1.3	5.4	5.1	1.1
5519	VA	17.9	13.8	8.8	4.8	12.3	6844	TX	0.1	0.2	0.2	0.1	0.2
5542	NC	3.6	4.0	1.2	1.2	4.4	6929	VA	21.8	17.5	16.5	11.8	16.4
5551	NC	3.8	4.2	3.8	3.2	3.8	7203	CT	16.9	11.3	12.5	9.7	10.3
5589	NC	5.7	5.5	3.7	3.6	6.3	7249	TX	2.5	2.8	1.9	1.5	2.7
5652	PA	4.4	2.7	1.5	1.6	3.1	7281	NC	2.5	1.9	0.9	0.6	3.3
5656	NC	9.0	8.9	2.4	1.2	8.0	7326	NC	0.5	0.1	0.2	0.0	0.5
5694	NC	2.4	5.6	2.0	1.8	5.7							

explore the differences between the two venoms representing each of the two subgenera and between the two subgenera. The inhibition experiments using insoluble mixed species venoms were performed because of the apparent close relation among the species venoms. In almost all cases it was possible to obtain more than 75% inhibition of binding in the range of concentrations tested from 0.1 to 100 micrograms per tube. In one case, serum 3913, the inhibition concentrations required were two orders of magnitude lower. If one compares the concentrations required for 50% inhibition, in almost all cases the differences for the various venoms are relatively small, less than a factor of three. This further suggests that the venoms are closely related. Four examples of inhibition experiments are illustrated.

The RAST inhibition curves for serum 6929 are illustrated in Figure 1. All three venoms tested as inhibitors are capable of inhibiting more than 90% of the binding to mixed species. The 50% inhibition concentrations are 0.9 micrograms for *P. exclamans*, 1.3 for annularis and

0.8 for apachus. This serum comes from Virginia, outside the range of *P. apachus* but where the related species fuscatus and metricus are common. This serum shows almost total cross-reactivity among the species.

Serum 3800, illustrated in Figure 2, was obtained from an allergic individual resident in North Carolina. By direct RAST this serum was about equally reactive with all four species. The inhibition experiments show that all four venoms are potent inhibitors at the 50% point but that while *P. fuscatus* venom can give almost complete inhibition of binding to either fuscatus or mixed venoms, *P. exclamans* venom can only give 70% inhibition of binding to *P. fuscatus* and about 82% to mixed species. This suggests that *P. fuscatus* venom contains an allergen or allergens not found in *P. exclamans* venom.

This conclusion is reinforced by results obtained for serum 5755, shown in Figure 3. In this case *P. exclamans* venom cannot completely inhibit binding to *P. fuscatus* and apachus. Venoms from *P. apachus* and fuscatus could each totally inhibit binding to the other and to mixed species venoms. The similarity of the curves for *P. apachus* and fuscatus is readily apparent.

The inhibition results for one serum, 7203 obtained from a patient who lived in Connecticut, were quite different from those expected from the direct RAST results. The direct RAST indicated a comparable degree of reactivity to all four species. However, inhibition of RAST, illustrated in Figure 4, showed over a thousand-

Table III. Correlation Coefficients of RAST with Venoms from Polistes Species for 62 Sera from Polistes Allergic Individuals.

	exclamanans	annularis	apachus	fuscatus
Mixed species	0.92	0.85	0.87	0.84
exclamanans		0.95	0.87	0.82
annularis			0.95	0.76
apachus				0.91

Table IV. Concentrations of Venom Required for 50% Inhibition of RAST.

Serum	Binding to	Inhibitor	Concentration for 50% inhibition	Serum	Binding to	Inhibitor	Concentration for 50% inhibition
3747	mixed species	exclamanans	2.5 micrograms	5755	mixed species	apachus	1.4
	exclamanans	exclamanans	2.6		fuscatus	fuscatus	2.2
	annularis	annularis	3.7		apachus	exclamanans	1.7
		exclamanans	1.8			apachus	1.0
		annularis	5.0			fuscatus	1.7
3800	mixed species	exclamanans	0.75		fuscatus	exclamanans	2.8
		fuscatus	0.75			apachus	1.4
	exclamanans	fuscatus	1.7			fuscatus	1.9
	fuscatus	exclamanans	1.6	5768	mixed species	exclamanans	0.6
3801	annularis	exclamanans	3.0		exclamanans	exclamanans	0.6
	annularis	apachus	1.6			annularis	0.6
	apachus	exclamanans	0.9		annularis	exclamanans	0.3
	apachus	apachus	1.1	5937	mixed species	apachus	1.0
3802	exclamanans	exclamanans	1.7		exclamanans	exclamanans	1.6
	exclamanans	annularis	2.0			apachus	1.2
	annularis	exclamanans	2.7		apachus	annularis	1.5
	annularis	annularis	3.3			apachus	1.4
3841	mixed species	apachus	1.0	6645	apachus	apachus	3.2
	apachus	apachus	1.9			fuscatus	4.4
	apachus	fuscatus	2.0		fuscatus	apachus	8.5
	fuscatus	apachus	2.8			fuscatus	7.0
3913	mixed species	exclamanans	0.2	6750	mixed species	exclamanans	7.5
	exclamanans	exclamanans	less than 0.01		exclamanans	exclamanans	5.8
3990	mixed species	fuscatus	95		annularis	exclamanans	2.5
	fuscatus	exclamanans	not 50% inhibition			annularis	1.0
	fuscatus	apachus	64	6929	mixed species	exclamanans	1.0
		fuscatus	45			annularis	1.2
4037	mixed species	exclamanans	4.8			apachus	1.3
	annularis	exclamanans	6.0		annularis	exclamanans	1.2
5436	mixed species	exclamanans	1.7	7203	mixed species	exclamanans	greater than 100
		apachus	4.0			annularis	greater than 100
		exclamanans	6.0			apachus	1.2
	apachus	apachus	46		exclamanans	apachus	1.0
					apachus	exclamanans	greater than 100

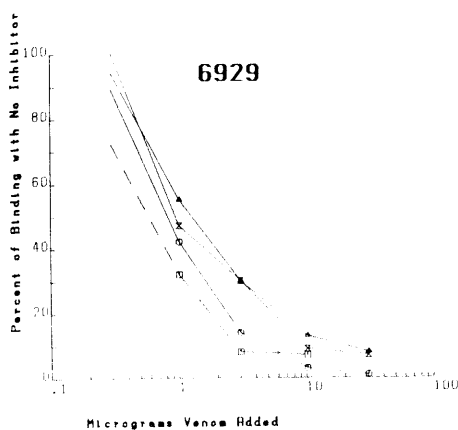


Figure 1. Inhibition of RAST for serum 6929. The solid lines are inhibition of RAST to *Polistes* mixed species venoms and the dashed line inhibition of RAST to *P. annularis*. The inhibitors are indicated by the symbols X and $\square$ *P. exclamans*, Δ *P. annularis* and $\circ$ *P. apachus*.

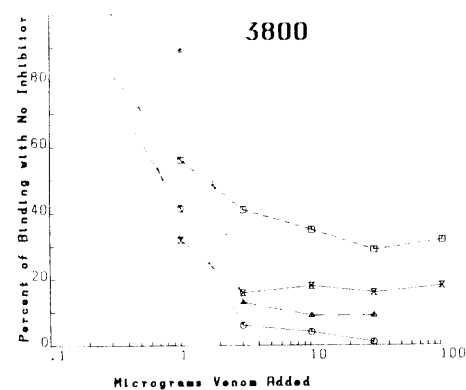


Figure 2. Inhibition of RAST for serum 3800. The solid lines are inhibition of RAST to mixed species, long dashes to *P. exclamans* and short dashes to *P. fuscatus*. The symbols for the inhibitor venoms are X and $\square$ *P. exclamans* and $\circ$ and Δ *P. fuscatus*.

fold difference between the potencies of *P. apachus* and *P. exclamans* to inhibit binding. *P. apachus* was a potent and complete inhibitor of binding to mixed species venoms and to *P. exclamans* venom, while *P. exclamans* and *annularis* gave only about 20% inhibition of binding to mixed venoms at the maximal concentration tested, 100 micrograms. There was an insufficient amount of this serum to obtain any more curves, so it was not possible to measure the homologous inhibition of *P. apachus*.

The results obtained with this serum indicate reactivity with *Fuscopolistes* and a weak cross reactivity with *Polistarchus*. No other serum tested was found to be like 7203.

The specificities of the IgE antibodies against *Polistes* venoms of the 16 sera that were examined by both direct and inhibition RAST are compared in Table V. In general the agreement is good but two major discrepancies were found—that in serum 7203, discussed above, and serum 5937, which showed *fuscopolistes* reactivity on inhibition to a much greater extent than was seen by direct RAST.

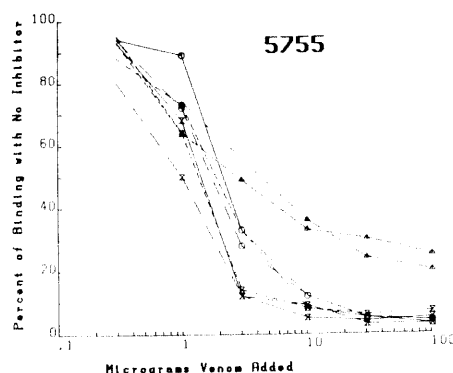


Figure 3. Inhibition of RAST for serum 5755. The solid lines are inhibition of binding to mixed species, the long dashed lines inhibition of binding to *P. apachus* and the short dashed lines inhibition of binding to *P. fuscatus*. The inhibitor venoms are represented by the symbols X for *P. apachus*, $\circ$ for *fuscatus* and Δ for *exclamans*.

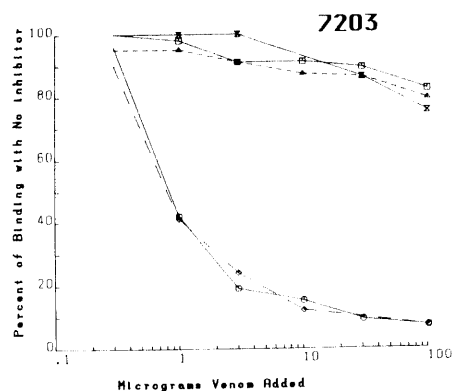


Figure 4. Inhibition of RAST for serum 7203. The solid lines are inhibition of binding to mixed species venoms, the long dashed line *P. exclamans* and the short dashed line *P. apachus*. The inhibitor symbols are X and Δ *P. exclamans*, $\circ$ and $\diamond$ *P. apachus* and $\square$ *P. annularis*.

Table V. Comparison of Specificities of IgE Antibodies in Sera from Patients allergic to Polistes Measured by Direct RAST and by Inhibition of RAST Using the Concentration Giving 50% Inhibition.

Serum	State	Specificity by RAST	Specificity by inhibition
3747	AZ	exclamans-annularis	exclamans
3800	NC	all	exclamans-fuscatus
3801	NC	exclamans-apachus-annularis	exclamans-apachus
3802	NC	annularis-exclamans	exclamans-annularis
3841	FL	apachus-fuscatus	apachus-fuscatus
3913	CA	exclamans	exclamans
3990	CA	fuscatus	fuscatus
4037	FL	annularis	exclamans-annularis
5436	NC	exclamans	exclamans
5755	CA	apachus	apachus
5768	NC	annularis-exclamans	exclamans-annularis
5937	NC	annularis-exclamans	apachus-annularis-exclamans
6645	FL	fuscatus-apachus	apachus-fuscatus
6750	CA	annularis-exclamans	exclamans
6929	VA	exclamans-apachus-annularis	exclamans-apachus
7203	CT	apachus-exclamans	apachus

Discussion

Of the 62 sera from *Polistes* reactive individuals tested by RAST with mixed venoms and the four species studied, *P. exclamans*, *annularis*, *apachus* and *fuscatus*, 61 were clearly positive using twice non-specific binding as a criterion. In no case that exhibited more than 1% binding to any venom was the serum positive to only a single species. The mixed species venom was only slightly less sensitive than the individual venoms. In many sera the reactivity followed the phylogeny of *Polistes*, *P. exclamans* and *annularis* in the subgenus *Polistarchus* and *P. apachus* and *fuscatus* in *Fuscopolistes*. Correlation studies of the direct RAST results confirmed that the species within the subgenera were more related than between the subgenera. Rast inhibition studies further confirmed that there was a great deal of cross-reactivity among the species. The inhibition studies indicated also that *Fuscopolistes* venoms contain an allergen or allergens not found in *Polistarchus* venoms. One serum that appeared to be highly cross-reactive by direct RAST was shown to be *Fuscopolistes* specific with a weak cross reaction to *Polistarchus* by inhibition experiments.

The differences observed among the *Polistes* species appear to be much smaller than we have previously reported among the species of the vespid genera *Vespula*^{12,13,14} and *Dolichovespula*.¹² The correlation coefficients for direct RAST with *Vespula* species range from 0.46 to 0.87 while those found in this study for *Polistes* range from 0.76 to 0.95. This indicates that the *Polistes* species are more closely related to each other than the yellow jacket species are. This is confirmed by inhibition studies.

One common *Polistes* species was not studied, *P. metricus*. *P. metricus* belongs to the subgenus *Fuscopolistes* and is quite similar to *P. fuscatus*. The other species listed in Table I are all relatively rare in the United States.

One of the reasons for undertaking this study was the

report of an excessive number of treatment failures to *Polistes* immunotherapy⁶ and suggestions of difficulty in diagnosis. The immunotherapy study was carried out in the Galveston, Texas, area, where the most common *Polistes* is reported to be *P. apachus*.² The seven patients we studied from Texas were all residents in the greater Houston area, near Galveston. They did not show a significantly greater reaction with *P. apachus*. In the studies reported in this paper *P. apachus* was found to be very similar to *P. fuscatus* in allergenic reactivity. The standard diagnostic and therapeutic mix contains *P. exclamans*, *annularis*, *fuscatus* and *metricus*. These studies indicate that this mix should be sufficient in almost all cases. The problems with immunotherapy may be related to insufficient dose as has been reported with patients treated with only yellow jacket venom.¹⁷

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