

Detection of IgE Antibodies to a Wide Range of Insect Species in Subjects with Suspected Inhalant Allergies to Insects

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Abstract. Sera obtained from subjects diagnosed on the basis of case history and skin tests as having inhalant allergies to insects, were tested for the presence of IgE antibodies to antigens from the house fly *Musca domestica*, a blowfly *Calliphora stygia*, the common clothes moth *Tineola bisselliella*, warehouse moth *Ephesia cautella*, cockroach *Blattella germanica*, carpet beetle *Anthrenus verbasci* and silverfish *Ctenolepisma longicauda*. Approximately one-third of the sera reacted with extracts from all seven species, over half the sera reacted with four of the extracts and only 3 sera proved negative to all of the extracts. Twenty-six of the sera also contained IgE antibodies that reacted with the house dust mite *Dermatophagoides farinae*. Tests with a further eleven species of flies from the order Diptera and with extracts of the grain borer *Rhyzopertha dominica*, locust *Chortoicetes terminifera* and Bogong moth *Agrotis infusa* revealed strong IgE antibody responses to all of the species. Electrophoretic studies revealed up to 15 IgE-binding components in extracts from 11 different insect species including 6 species of flies. Some IgE-binding insect components with similar electrophoretic mobilities indicated the possible presence of common allergens in extracts from different species. In particular, blots of each of the fly extracts showed a dense IgE-binding component of MW approximately 37,000 daltons. 'Pan allergy' to insects may occur in subjects who have been sensitized to one or a few insects and allergenic similarities may extend to at least some other noninsect members of the phylum Arthropoda.

Introduction

It is becoming increasingly evident that exposure to insects in man's immediate environment can lead to some people becoming sensitized to insect antigens and this, in turn, can lead to inhalant allergies including severe respiratory disease [Feinberg et al., 1956; Perlman, 1958; Panzani and Layton, 1966; Gad El Rab and Kay, 1980; Bellas, 1982]. Insect species from at least 12 different orders have been implicated in the promotion of inhalant allergy in man [Bellas, 1982, 1986] but, as yet, little information is available on the worldwide incidence and distribution of insect allergies, the types of allergies encountered, the extent of cross-reactivities between insect allergens and the nature of these allergens. In addition, the relative importance of different species of insects as a source of inhalant allergy in different geographical locations is, as yet, nowhere near as well defined as, for example, the various pollen species.

In an effort to assess the spectra of insect-reactive IgE antibody responses in subjects with diagnosed inhalant allergies to insects, we tested the sera of 41 subjects with water-soluble extracts prepared from a wide range of insect species. Results detailing the unexpectedly wide range of reactions detected are presented together with a possible explanation of the findings and a discussion of the implications these results have for patients allergically sensitized to insects.

Materials and Methods

Insects

The following species or insect materials used, and their sources, were: sheep blowfly (*Lucilia cuprina*), *Ephesia cautella*, *Agrotis infusa*, *Rhyzopertha dominica* and *Ctenolepisma longicauda* from CSIRO, Division of Entomology, Canberra; *Calliphora stygia*, *Calliphora augur* and *Parasarcophaga* spp. caught in traps in the field and supplied by G.W. Crompton and Dr. W.G. Vogt; *Da-*

Table I. Taxonomic relationships between insects tested

Order	Family	Genus and species	Common name
Blattodea	Blattellidae	<i>Blattella germanica</i> (Linnaeus)	German cockroach
Coleoptera	Dermestidae	<i>Anthrenus verbasci</i> (Linnaeus) *	variegated carpet beetle
	Bostrychidae	<i>Rhyzopertha dominica</i> (Fabricius) *	lesser grain borer
Diptera	Calliphoridae	<i>Calliphora augur</i> (Fabricius) • ✓	lesser brown blowfly
		<i>Calliphora stygia</i> (Fabricius) • ✓	eastern golden-haired blowfly
		<i>Chrysomya bezziana</i> Villeneuve • ✓	oriental screw-worm fly
		<i>Chrysomya rufifacies</i> (Macquart) • ✓	hairy maggot blowfly
		<i>Lucilia cuprina</i> (Wiedemann)	Australian sheep blowfly
	Sarcophagidae	<i>Parasarcophaga</i> spp. • ✓	flesh fly
	Muscidae	<i>Musca domestica</i> Linnaeus	house fly
		<i>Musca vetustissima</i> Walker • ✓	bush fly
	Tephritidae	<i>Dacus tryoni</i> (Froggatt) • ✓	Queensland fruit fly
	Chironomidae	<i>Chironomus duplex</i> Walker • ✓	bloodworm
	Culicidae	<i>Aedes aegypti</i> (Linnaeus)	mosquito
		<i>Aedes communis</i>	mosquito
		<i>Culex fatigans</i> Wiedemann • ✓	mosquito
Lepidoptera	Noctuidae	<i>Agrotis infusa</i> (Biosduval) • ✓	Bogong moth
	Pyalidae	<i>Ephestia cautella</i> (Walker) •	tropical warehouse moth
	Tineidae	<i>Tineola bisselliella</i> (Hummel)	common clothes moth
Orthoptera	Acrididae	<i>Chortoicetes terminifera</i> (Walker) •	Australian plague locust
Thysanura	Lepismatidae	<i>Ctenolepisma longicaudata</i> Escherich •	silverfish

cus tryoni, taken from a laboratory culture and supplied by Dr. P.H. Smith; *Chironomus duplex*, supplied by Dr. J. Martin, Department of Genetics, University of Melbourne; *Musca domestica*, supplied by Dr. E. Shipp, University of NSW; *Musca vetustissima*, supplied by J. Walker, Canberra; *Tineola bisselliella* and *Anthrenus verbasci* from CSIRO, Division of Textile Industry, Belmont, Vic.; *Chortoicetes terminifera* adults and faeces from the Biological and Chemical Research Institute, NSW Department of Agriculture, Rydalmere; *Chrysomya bezziana* from the Australian Screw-worm Fly Unit, Laloki, Papua, New Guinea; *Blattella germanica* collected locally from a Sydney house; *Aedes aegypti*, *Aedes communis* and *Culex fatigans* from the School of Public Health and Tropical Medicine, University of Sydney.

A complete list of the genera and species studied, their taxonomic relationships and common names are set out in table I.

Preparation of Insect Extracts and Radioallergosorbent Test (RAST)

These were carried out as previously described [Kaufman et al., 1986]. All insect extracts used were whole body extracts except for a locust faecal extract which was used in addition to locust whole body extract. RASTs for IgE antibodies to house dust mite (*Dermatophagoides farinae*), ryegrass (*Lolium perenne*) pollen, Timothy grass (*Phleum pratense*) pollen, the fungi *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria tenuis*, the foods codfish and pea-

nut and the epithelial preparations from dog and cat were carried out with Pharmacia allergen discs.

RAST results were scored in the following way: RAST negative = radioactive uptakes of ^{125}I -anti-human IgE < 2.5 times the cord serum value; equivocal = 2.5–3.5 times cord serum value; positive = > 3.5 times cord serum value.

Subjects and Sera

Sera were obtained from 41 subjects (31 male and 10 female) diagnosed as having inhalant allergies to insects on the basis of case histories and/or skin test reactions to insect extracts. One subject (J) had no apparent history of insect allergy but developed asthma and angio-oedema after eating snails, crustaceans or other non-fish seafoods. Subject J was, however, skin test positive to insect extracts (see below). Ages ranged from 5 to 66 years. Fifteen subjects were less than 20 years of age while only 4 were less than 10 years old. Thirty-six of the subjects were asthmatic. Other symptoms encountered in some patients were allergic rhinitis, spasmodic cough, angio-oedema and urticaria.

Human cord serum was obtained from the Department of Obstetrics, Royal North Shore Hospital, and used as an IgE-'free' control. Sera from 10 non-allergic ('normal') subjects were obtained from hospital employees and sera from 10 grass pollen and/or mite-allergic subjects who were not allergic to any insect were provided by the Allergy Clinic, Royal North Shore Hospital.

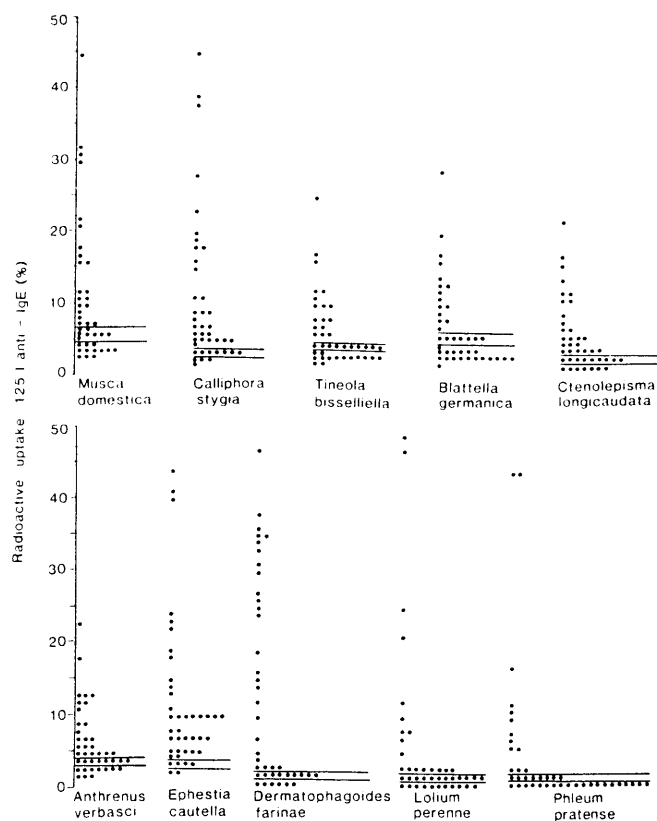


Fig. 1. Side-by-side comparison of RAST findings when sera from 41 insect-allergic subjects were tested for reactivities with insect, house dust mite and pollen extracts. The lower of the two horizontal lines drawn near the bottom of each column marks the division between negative (below) and equivocal (above) reactions. The upper horizontal line in each column marks the division between equivocal (below) and positive (above) reactions (see 'Materials and Methods').

Skin Tests

Skin tests were carried out intradermally with 0.02 ml of a 1–10,000 dilution of skin testing extract. A test was considered positive when there was at least a 5-mm wheal together with a 1-cm flare. Skin reactions were evaluated after 15–20 min and compared with a control of phenol-saline. Insect extracts used for skin testing were a mixture of household insects consisting of extracts of *M. domestica* (household fly), *Aedes communis* (mosquito), *Periplaneta brunea* (cockroach), *Formica rufa*, and *Spodoptera exigua* (lesser armyworm). Individual extracts from *M. domestica*, *P. brunea*, *S. exigua* and *Agriope* spp. (spider) were also tested. All extracts were from Berkeley Biologicals, Berkeley, Calif.

Gel Electrophoresis

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) was carried out as previously described [Tovey and

Baldo, 1984; Ford et al., 1986] using 9–27% gradient acrylamide gels. High and low MW markers (Bio-Rad or Pharmacia) were included in each electrophoretic separation. Except for the MW markers, protein extracts were not treated with mercaptoethanol prior to electrophoresis.

Electrophoretic Transfer of Proteins to Nitrocellulose (NC)

This was as previously described [Tovey and Baldo, 1984; Ford et al., 1986] except for the time allowed for transfer which was extended to 120 min.

Probing of NC Membrane Strips and Autoradiography

NC strips (5 mm in width) obtained after SDS-PAGE were incubated with sera (diluted 1 in 8 with 0.1% Tween-20/0.1% sodium azide in PBS), and then with ^{125} I-anti-IgE (50,000–80,000 cpm/strip) overnight. After extensive washing (30 min) with 0.05% Tween-20 in PBS, the NC strips were air-dried at room temperature and loaded into a Kodak X-Omatic cassette (KP68375) together with photographic film (Fuji Rx-safety). The cassette was then stored at -70°C for periods of 24–72 h. Fetal cord serum and sera from non-allergic individuals were used as controls.

Protein Staining

PAGs were stained with Coomassie brilliant blue G250 (CBB; Sigma Chemical Co.) using the enhancing method described by Neuhoff and Stamm [1984]. NC transfers were stained with amido black.

Results

Initial Screen of Sera against Seven Different Insect Species

Sera from the 41 subjects diagnosed as having probable inhalant allergies to insects were tested for the presence of insect-reactive IgE antibodies. Only 1 of the 41 sera gave clear negative reactions with all 7 species while 2 other sera were classed as negative or equivocal reactors with each of the seven different insect species studied. Positive radioactive uptakes of ^{125} I-anti-human IgE (that is, > 3.5 times the cord/normal serum uptake) ranged as high as 40–50% with discs coupled to proteins from some insect species (fig. 1). The number of positive reacting sera ranged from a high of 35 with *E. cautella* discs down to 15 with *B. germanica* discs. Tests for *B. germanica* and *T. bisselliella*-reactive IgE antibodies showed the highest number of clear negative reactions with 19 and 14 subjects, respectively. The highest individual RAST uptakes were recorded with discs prepared from extracts of the two Diptera species and *E. cautella*. By contrast, positive results obtained with *C. longicaudata* and *A. verbasci* discs were clustered at lower RAST uptake values.

Thirteen, or approximately one-third of the sera examined, reacted with extracts from all seven insects screened while over half of the 41 sera reacted with four or more species (table II).

Tests for IgE antibodies to three other commonly encountered inhalant allergen sources showed that sera from 26 subjects reacted to the house dust mite

D. farinae, 18 reacted to *L. perenne* pollen and only 13 to *P. pratense* pollen. Only 6 subjects showed clear negative reactions to *D. farinae* but 20 subjects were negative to *P. pratense*.

Control sera from normal (non-allergic subjects, from subjects allergic to mites and/or pollens and from cord serum showed background binding with the insect discs, namely radioactive uptakes of from 0.8 to 1.9%.

Table II. Reactions of IgE antibodies in subjects' sera¹ with extracts from seven different insect species²

	Number of insect species						
	0	1	2	3	4	5	6
Number sera reacting ³	3	7	3	6	3	3	3

¹ Results of RASTs with all 41 sera examined.

² Insect species listed in 'Results' section and table III.

³ A positive reaction was taken as a radioactive uptake of ¹²⁵I-anti-human IgE which was > 3.5 × the uptake obtained with human cord serum (see 'Materials and Methods').

Further Investigations with Sera that Reacted Strongly with Insect Extracts

Tests for IgE Antibodies to Other Insects, Particularly Flies. Thirteen of the 41 sera that reacted strongly with all, or nearly all, of the seven insect extracts (table III) were selected for further RAST studies. RASTs were performed with more Diptera species and another species from the order Lepidoptera since the reactions observed when testing the 41 sera with *M. domestica*, *C. stygia* and *E. cautella* were so strong (table III). Nine of the sera selected (F, H, 4, 18, 19, 21, 28, 111 and 126) reacted strongly with all seven of the initial panel of insects. All but one of the selected 13 sera produced clear positive reactions with *D. fari-*

Table III. RAST reactions of IgE antibodies in the sera of 13 of the most strongly insect-reactive subjects¹ with extracts from seven insect species and three common aeroallergens

Serum from subjects	Radioactive uptake (%) of ¹²⁵ I-anti-human IgE ² with discs coupled to components from extracts of									
	<i>Musca domestica</i> ³	<i>Calliphora stygia</i>	<i>Ephestia cautella</i>	<i>Tineola bisselliella</i>	<i>Blattella germanica</i>	<i>Anthrenus verbasci</i>	<i>Ctenolepisma longicaudata</i>	<i>Dermatophagoides farinae</i>	<i>Lolium perenne</i>	<i>Phleum pratense</i>
C	37.7	37.6	38.9	3.8	2.6	3.1	2.8	29.5	1.2	0.8
D	6.7	8.2	6.4	5.1	4.0	5.7	4.2	30.9	1.3	0.6
F	15.2	10.3	9.8	10.0	9.6	12.2	11.6	4.2	2.3	2.1
H	16.2	19.2	25.0	9.0	12.0	7.9	5.7	11.9	2.9	6.9
I	11.0	17.9	9.9	6.0	4.3	2.0	2.0	24.2	1.3	0.4
J	44.6	44.3	41.0	3.5	2.6	1.7	2.2	38.6	0.6	0.5
4	34.3	27.2	43.9	25.0	28.9	22.6	21.8	13.9	41.8	43.9
18	15.1	10.5	9.1	11.1	15.6	13.0	11.2	35.1	1.8	0.7
19	21.7	18.0	17.7	11.7	16.5	12.4	15.8	37.6	9.9	9.8
21	20.6	22.8	13.2	10.3	12.9	10.1	10.6	32.4	7.2	8.0
28	11.5	15.9	22.3	6.1	13.8	8.8	8.0	1.2	2.3	1.0
111	35.2	38.2	9.5	17.0	12.4	11.5	13.7	46.8	1.8	0.9
126	16.5	18.2	17.5	14.3	20.0	17.4	16.4	25.9	2.3	1.7
Normal	1.5	1.1	1.8	0.9	1.3	1.3	1.2	0.5	0.7	0.4
Cord	1.9	1.0	1.0	1.3	1.7	1.1	0.9	0.5	0.4	0.3

¹ Thirteen subjects from the 41 tested (see fig. 1) with extracts from the above 3 insects and three common inhalant allergen sources.

² Directly proportional to the amount of IgE antibodies that react with the allergen discs.

³ For common names, see table I.



nae. Radioactive uptakes with mite discs of 24% or more were recorded for 9 of the 13 sera. Reactions with the pollens were far less obvious and high RAST uptakes were obtained in only 3 cases with *L. perenne* and 4 cases with *P. pratense*.

Results with extracts from a further 11 species from the order Diptera and with an extract of the moth *A. infusa* reinforced the results obtained in the initial RAST screen (fig. 2). Reactions with discs prepared from extracts of a range of flies, a bloodworm, three species of mosquitos and the Bogong moth were uniformly high. In fact, reactions with the moth discs were some of the highest recorded in this study. Radioactive uptakes with *A. communis* discs were in every case less than the uptakes recorded with the other two mosquito species. High positive reactions were also recorded with discs coupled to extracts from the grain borer *R. dominica* and locust *C. terminifera* although reactions with the locust faecal extract were sometimes in the negative to equivocal range (see, for example, sera C and J, fig. 2).

In contrast to the strong reactions observed with the insect extracts, the mixed panel of other allergens tested, namely *Parietaria* pollen, three fungi, cat and dog epithelia and the foods codfish and peanut, either failed to react at all with any of the sera or reacted with only one or two of the 13 sera examined (see results with sera I, 4 and 21, fig. 2).

Detection of Insect Allergens by Electrophoretic Blotting. SDS-PAGE followed by protein staining of the gels identified multiple components over the MW range <14,000 to $\geq 200,000$ daltons in extracts of the

flies *M. domestica* and *C. stygia* and in the non-Diptera species *B. germanica*, *T. bisselliella*, *E. cautella*, *R. dominica* and *C. longicaudata* (fig. 3A). The complex pattern of bands was successfully transferred to NC membranes by electroblotting (fig. 3B) producing patterns that were almost identical to the stained pattern of bands in the separating gels.

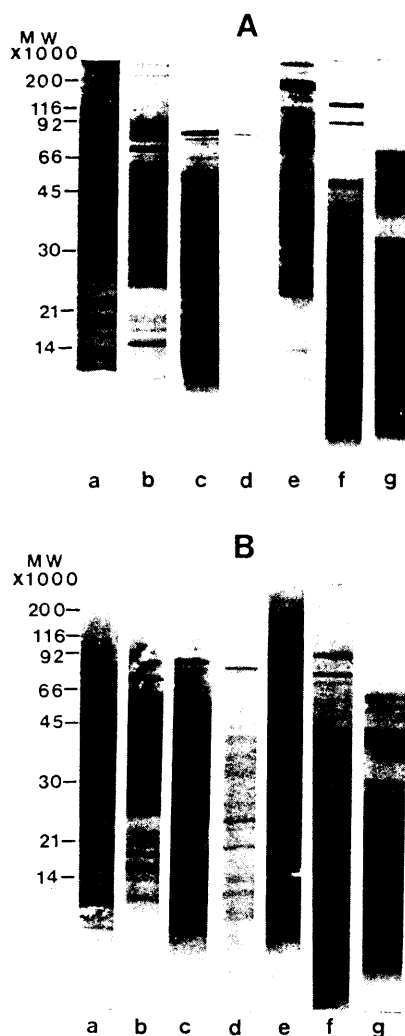


Fig. 3. A Protein-stained separation patterns following SDS-PAGE of insect extracts prepared from: (a) *Musca domestica*; (b) *Calliphora stygia*; (c) *Blattella germanica*; (d) *Tineola bisselliella*; (e) *Ephestia cautella*; (f) *Rhyzopertha dominica*; (g) *Ctenolepisma longicaudata*. Each extract was added to an 8-mm slot for separation. Gradient gels (9–27% acrylamide) 13 × 13 cm, were run for 1,700 Vh and stained with CBB. B NC transfer patterns of electrophoretically separated proteins shown in A. The transfer from gel to NC was carried out for 1.5 h at 100 V. Transferred proteins were stained with amido black.

Fig. 2. RAST results expressed as radioactive uptakes of ^{125}I -anti-human IgE with discs prepared from extracts of insects and some other common allergen sources including fungi, foods, animal epithelia and pollen. Uptakes of anti-IgE >3.5 times the uptakes obtained with cord and normal sera were considered positive. Sera examined were selected on the basis of strong positive reactions to the insect species listed in figure 1. The allergen sources examined were: (1) *Musca vetustissima*; (2) *Lucilia cuprina*; (3) *Calliphora augur*; (4) *Chrysomya rufifacies*; (5) *Chrysomya bezziana*; (6) *Chironomus duplex*; (7) *Parasarcophaga* spp.; (8) *Dacus tryoni*; (9) *Agrotis infusa*; (10) *Rhyzopertha dominica*; (11) *Chortoicetes terminifera*; (12) *C. terminifera* faeces; (13) *Aedes aegypti*; (14) *Aedes communis*; (15) *Culex fatigans*; (16) *Parietaria judaica* pollen; (17) *Cladosporium herbarum*; (18) *Aspergillus fumigatus*; (19) *Alternaria alternata*; (20) cat epithelium; (21) dog epithelium; (22) codfish; (23) peanut.

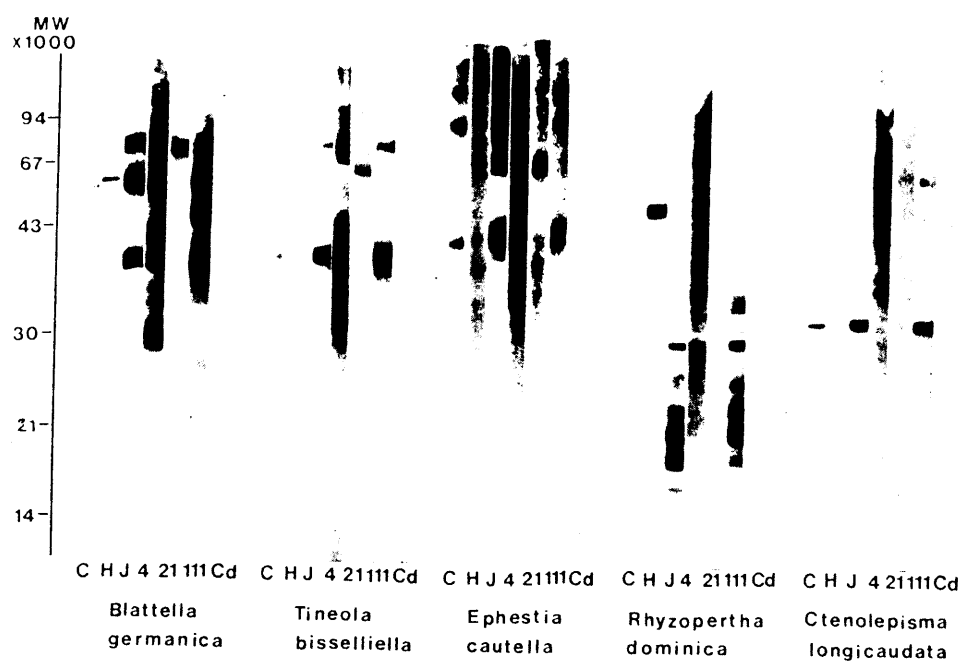


Fig. 4. Identification by autoradiography of IgE-binding components transferred to NC following SDS-PAGE of the insect extracts shown. Gels for subsequent transfer were loaded with 50 μ l/cm of the extract at a concentration of 20 mg/ml. Electrophoresis and transfer conditions were as described in the legend to figure 3. Each strip was incubated with subject's serum (C, H, J, 4, 21 or 111) or cord serum (Cd) followed by ^{125} I-anti-human IgE (Kallestad) 70,000 cpm/strip.

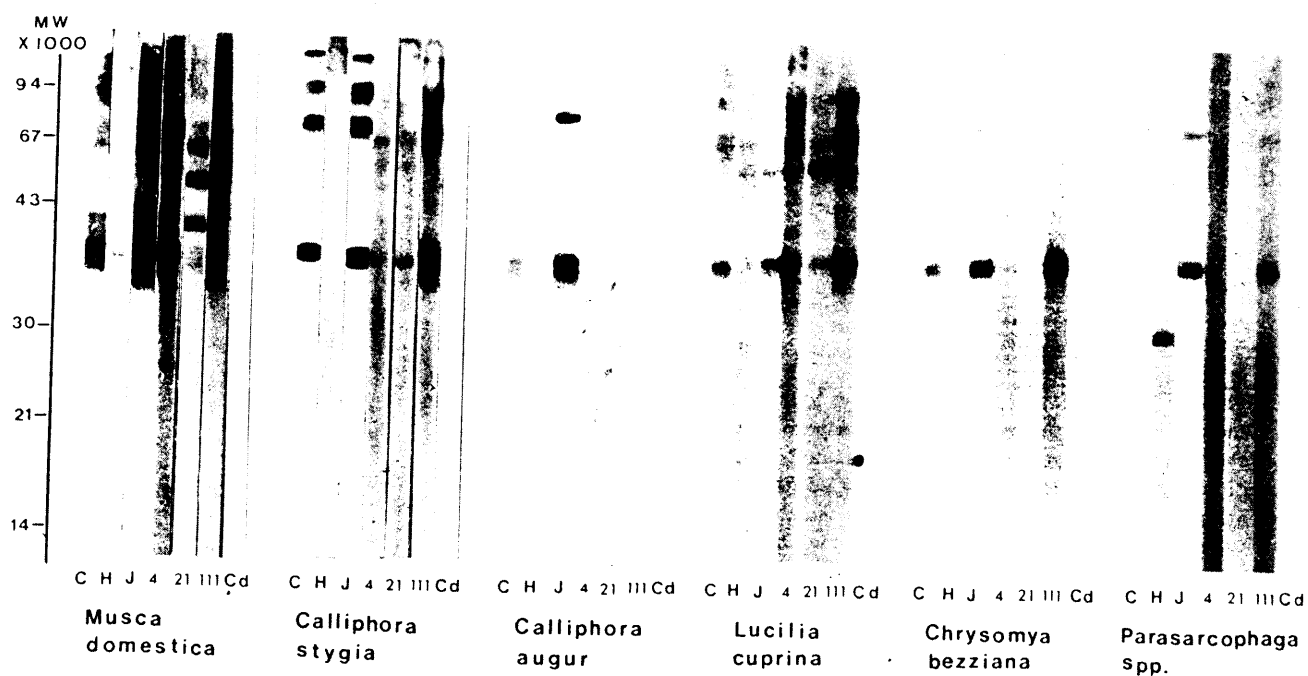


Fig. 5. Identification by autoradiography of IgE-binding components transferred to NC following SDS-PAGE of Diptera (fly) extracts shown. For other details see legend to figure 4.

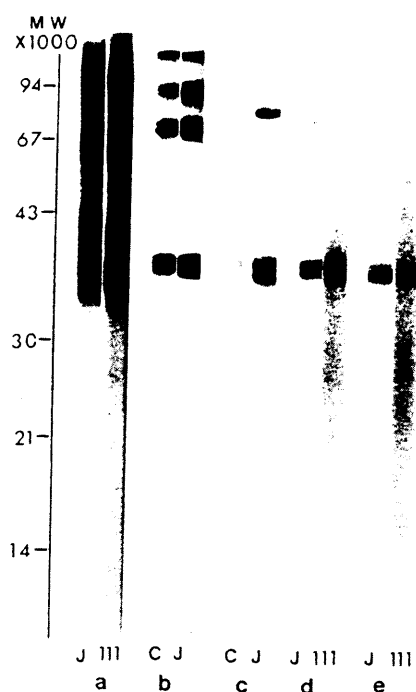


Fig. 6. Examples of identical insect allergen recognition patterns on NC transfers shown by some sera (C, J, and III): (a) *Musca domestica*; (b) *Calliphora stygia*; (c) *Calliphora augur*; (d) *Chrysomya bezziana*; (e) *Parasarcophaga* spp. quantities used and electrophoresis, transfer and detection conditions were as described in the legends to figures 3 and 4.

Using six of the sera that were highly reactive with insect antigens, distinct IgE-binding bands were located in each of the seven insect extracts (fig. 4, 5). The most striking features of the blot results were the preponderance of labelled bands from *B. germanica*, *T. bisselliella*, *E. cautella* and *C. longicaudata* with MWs in the region of approximately 30,000 daltons and above, the cluster of *R. dominica* allergenic proteins in the MW range 16,000–35,000 daltons and the dense band at MW approximately 32,000 daltons on the *C. longicaudata* transfer (fig. 4). From results with the six sera used for probing, *B. germanica* and *T. bisselliella* extracts were each shown to contain at least 14 allergens, *E. cautella* and *C. longicaudata* extracts at least 12 and *R. dominica* extract at least 15 allergens. The allergenic patterns of bands obtained with the two moth extracts *T. bisselliella* and *E. cautella*, were very similar while the *R. dominica* pattern was clearly different to the other four patterns (fig. 4).

When six different species of flies were compared side-by-side in blotting studies with the allergic sera and ^{125}I -anti-IgE (fig. 5) two main features emerged. Firstly, almost all of the IgE-binding proteins were found to have MWs greater than 35,000 daltons, and, secondly, a dense band of MW approximately 37,000 daltons was found to occur on the NC blots of each of the six fly species.

The specific patterns of bands in a fly extract recognised by individual sera were sometimes identical. This was observed with five different species and is illustrated in figure 6.

Discussion

The most interesting features of the results outlined here are the high proportion of sera that showed reactions to more than one insect species, the strength of many of the reactions detected, particularly towards flies, mosquitos and moths, and the wide range of both closely related and poorly related insect species that reacted with IgE antibodies in the subjects' sera.

Of the 41 subjects examined, only one and possibly two others, lacked serum IgE antibodies that reacted with at least one of the seven insect species selected for testing in the initial screen. The insects selected for examination covered a broad range of species from seven different families and 5 different orders. The finding that a large proportion of the sera reacted with a number of different species suggested that the sera contained cross-reacting IgE antibodies that recognised insect allergens. An alternative explanation is that the subjects may have encountered and become separately sensitized to a number of different insect species. Of course, both of these mechanisms may sometimes be operative and cross-reactions and separate sensitization may occur in the same subjects. However, the former of the two explanations seems more likely especially since strong positive reactions were found to all 13 species of Diptera tested and to both fly and other insect species which are unlikely to have ever been encountered by the subjects studied. A good example of this is illustrated by results with the Bogong moth (*A. infusa*), a species indigenous to Australia. When *A. infusa* and other insect extracts were compared side-by-side in tests with the 13 strongly reactive sera, the moth extract produced the highest RAST uptakes with eight of the sera. From these results and the findings with the Diptera extracts the in-

terpretation one is left with is that the subjects were already sensitized to antigens from another moth (or fly, or mosquito, or some other insect), and the IgE antibodies produced were highly cross-reactive with antigens from the Australian species.

The finding of components that react with IgE antibodies and which have the same MWs appears to demonstrate a possible basis for allergenic cross-reactivity between closely-related species of flies. Overall though, when one considers the RAST results with a large number of sera, the range of insect extracts tested and the protein blotting results, it is difficult to escape the conclusion that at least some IgE antibodies may cross-react widely with antigens from taxonomically distantly related, or even unrelated, insect species. Wide-ranging cross-reactivity of this type has been observed in studies of insect allergies currently underway in our laboratory [Baldo, Tovey, Kaufman and Bellas, in preparation] and has been reported briefly by others [Edge and Sherwood Burge, 1980]. It seems likely that 'pan allergy' to insects may occur in subjects who have been sensitized to a few, or perhaps only one, insect species [Panzani, 1986]. In fact, such wide-ranging allergy may extend beyond insects to arthropods in general and embrace arachnids and even crustaceans. In the present study, subjects showed a high incidence of positive reactions to the arachnid *D. farinae* and high RAST uptakes to insects were frequently accompanied by high uptakes to the mite extract. Edge and Sherwood Burge [1980] also suspected an association between mite and insect allergic sensitivities and found that a number of components were common to some insects and the mite *Dermatophagoides pteronyssinus*. Detection of cross-reactions between antigens from cockroaches and several species of crustacea were claimed by O'Neil et al. [1985] who stated that their findings suggested a relationship between sensitization via the inhalant and ingestion routes. In the present study subject J sought medical attention not because he was aware of any allergic sensitivity to insects but because he was highly allergic to some non-fish seafoods, crustaceans and molluscs. On examination, the subject proved highly skin test positive to some insect extracts and his serum contained IgE antibodies that reacted strongly with extracts from a variety of insect species.

It seems, therefore, that allergenic similarities may exist between both closely-related and apparently unrelated species within the class Insecta and cross-allergenicity may extend to some non-insect species in

the phylum Arthropoda. To identify allergenically-related proteins from the many diverse species, extensive protein blotting studies of the type outlined in this study should be undertaken.

A lack of species specificity shown by IgE antibodies in the sera of subjects allergic to insects has important implications for our understanding of underlying mechanisms and for the specific diagnosis of insect hypersensitivities. Perhaps potentially related to this is the fact that 36 of the 41 subjects studied here were asthmatic and, in a recent survey in Sydney [Kaufman, Baldo, Tovey and Gandevia, unpubl.], a high incidence of IgE antibodies to a number of insect species were found in 50 asthmatics in whom insect hypersensitivity was not suspected. It seems likely that allergic reactions to inhaled insect 'emanations' may be more common than is currently believed.

Acknowledgements

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