

Occupational allergy in an entomological research centre.

II. Identification of IgE-binding proteins from developmental stages of the blowfly *Lucilia cuprina* and other species of adult flies

B. A. BALDO,* T. E. BELLAS,† E.R. TOVEY* and G. L. KAUFMAN‡

*Kolling Institute of Medical Research, and ‡Allergy Clinic, Royal North Shore Hospital of Sydney, NSW, and

†CSIRO Division of Entomology, Acton, ACT, Australia

Summary

Sera from 30 workers in an entomological research institute and from five Sydney asthmatics, all with serum IgE antibodies to sheep blowfly (*Lucilia cuprina*) antigens, were studied with the aim of identifying individual IgE-binding proteins in extracts of *L. cuprina* adult flies, larvae and eggs, and in extracts of related species of flies from the order Diptera. Using protein blotting, 21, 18 and nine different IgE-binding components were identified in extracts of *L. cuprina* adult flies, larvae and eggs, respectively. A component(s) of MW 67 kD found in all three developmental stages, showed the highest frequency of IgE-binding; reacting, for example, with 70% of the sera tested with the adult fly extract. Some components were detected in only one of the three developmental stages. Investigations of possible allergenic cross-reactivity between *L. cuprina* and extracts from six other related species of flies revealed IgE-binding bands in each of the extracts as well as in an extract of *Agrotis infusa*, a cutworm not belonging to the order Diptera. One strongly reacting component of MW 20 kD was detected in extracts of five different fly species as well as in the *A. infusa* extract. The results suggested that allergenic cross-reactivity between some fly species exists, and may extend to taxonomically unrelated insect species.

Clinical and Experimental Allergy, Vol. 19, pp. 411–417. Submitted 8 August 1988; revised 4 January 1989; accepted 11 January 1989.

Introduction

Inhalant allergies induced by flies of the order Diptera have been reported in workers following occupational exposure to insects. Allergic respiratory symptoms occurred in a scientific worker engaged in the rearing of the common house fly *Musca domestica* [1], in laboratory workers exposed to the fruit fly *Drosophila melanogaster* [2], and in individuals involved in the aerial dissemination of the sterilized screw-worm fly *Cochliomyia hominivorax* [3]. Following reports of adverse respiratory, skin and eye reactions in workers rearing the blowfly *Lucilia cuprina* in an entomological research laboratory, and demonstration of an IgE-mediated allergic reaction to *L. cuprina*

antigens [4,5], we set out to identify the IgE-reactive fly antigens in sera from the allergic workers. Using protein blotting methods, human IgE-binding components in *L. cuprina* eggs, larvae and adult flies, and in some related species of flies, were investigated.

Materials and methods

Insects

Sheep blowfly (*L. cuprina*) adults, larvae and eggs were obtained from the CSIRO Division of Entomology, Canberra; Eastern golden-haired blowfly (*Calliphora stygia* (Fabricius)) and lesser brown blowfly (*Calliphora augur* (Fabricius)), were caught by traps in the field and supplied by GW Crompton and Dr WG Vogt; hairy maggot blowfly (*Chrysomya rufifacies* (Macquart)), reared in the laboratory and supplied by S Runko and Dr

Correspondence: Dr B. A. Baldo, Kolling Institute of Medical Research, Royal North Shore Hospital of Sydney, St Leonards, 2065 NSW, Australia.

WG Vogt; Queensland fruit fly (*Dacus tryoni* (Froggatt)), taken from a laboratory culture and supplied by Dr PH Smith; the bloodworm (*Chironomus duplex* Walker), supplied by Dr J Martin, Department of Genetics, University of Melbourne; bogong moth or common cutworm (*Agrotis infusa* (Boisduval)), harvested at a resting site in the mountains and supplied by Dr ES Nielsen; and bush fly (*Musca vetustissima* Walker), was supplied by J. Walker.

Preparation of insect extracts, radio-allergosorbent test and total IgE estimation

The procedures used have been described previously [4]. Total IgE levels in sera were determined using the QuantiClone IgE test (Kallestad, Austin, U.S.A.).

Sera

Sera from subjects employed in the entomological research institute that showed a positive radio-allergosorbent test (RAST) reaction to at least one of the extracts prepared from *L. cuprina* eggs, larvae or adult flies were selected for use in electroblotting studies designed to identify IgE-binding components from the fly. Thirty different sera were selected for this purpose. Sera from five Sydney adult asthmatics (serum numbers, 659, 660, 674, 681 and 684) with no occupational, or other known abnormal exposure to blowflies, but with positive serum RAST reactions to *L. cuprina* egg, larval or adult fly extracts, were obtained while the patients were attending the chest clinic at the Prince of Wales Hospital, Randwick, NSW. Cord sera were obtained from The Department of Obstetrics, Royal North Shore Hospital (RNSH), St Leonards, NSW. Sera from 28 subjects diagnosed on the basis of case history and skin-prick tests as allergic to pollens, foods, fungi or drugs (or in some cases to combinations of these), but not allergic to insects, were obtained from the Allergy Clinic, RNSH. All of these control sera, used in electroblotting studies with insect extracts, showed RAST uptakes of 2+ to 4+ on the Pharmacia classification scale. 'Normal' serum samples from non-allergic, healthy adults were obtained from 10 members of the hospital staff.

Gel electrophoresis

Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE) was carried out as previously described [6,7] using 9–27% gradient acrylamide gels. High and low MW markers (Bio-Rad, Richmond, U.S.A. or Pharmacia, Uppsala, Sweden) were included in each electrophoretic separation. Except for the MW markers, protein extracts were not treated with mercaptoethanol prior to electrophoresis.

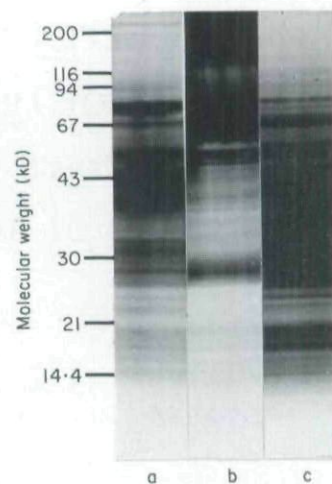


Fig. 1. Protein-stained separation patterns obtained following SDS-PAGE of blowfly (*L. cuprina*) extracts prepared from: a, adult flies; b, larvae and c, eggs. Adult and egg extracts (150 μ l, 20 mg/ml), and larval extract (150 μ l, 10 mg/ml) were separated over a gel width of 2.5 cm.

Electrophoretic transfer of proteins to nitrocellulose

This was as previously described [6,7], except for the time allowed for transfer which was extended to 120 min.

Probing of nitrocellulose membrane strips and autoradiography

Nitrocellulose (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 phosphate-buffered saline (PBS)), and then with [^{125} I]anti-human-IgE (50 000 c.p.m./strips) 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, Rochester, U.S.A.) together with photographic film (Fuji Rx-safety, Fuji Photo Film Co. Ltd, Tokyo, Japan). The cassette was then stored at -70°C for 24–72 hr. Foetal cord serum and sera from non-allergic individuals were used as controls.

Protein staining

Polyacrylamide gels were stained with Coomassie brilliant blue G250 (CBB; Sigma Chemical Co., St Louis, U.S.A.) using the enhancing method described by Neuhoff and Stamm [8]. NC transfers were stained with amido black.

Results

*Analysis of *L. cuprina* antigens and allergens*

Polyacrylamide gel electrophoresis followed by CBB staining of separated proteins identified multiple compo-

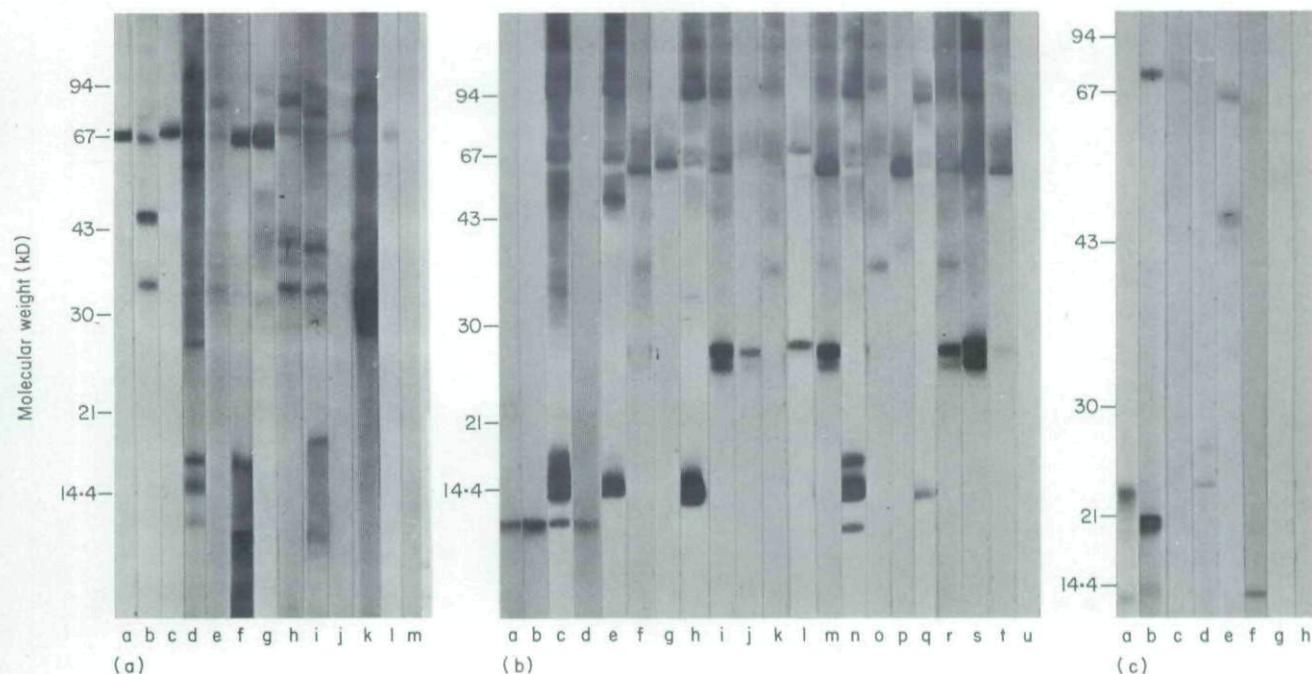


Fig. 2. Identification by autoradiography of IgE-binding components transferred to nitrocellulose following SDS-PAGE of blowfly (*L. cuprina*) (a) adult fly, (b) larvae and (c) egg extracts. Strips were incubated with the following sera from subjects: (a) a, 028; b, 041; c, 045; d, 066; e, 075; f, 086; g, 090; h, 093; i, 131; j, 141; k, 160; l, 226; m, cord serum. (b) a, 028; b, 045; c, 066; d, 086; e, 090; f, 118; g, 127; h, 131; i, 133; j, 137; k, 157; l, 160; m, 169; n, 193; o, 196; p, 212; q, 226; r, 660; s, 674; t, 684; u, cord serum. (c) a, 041; b, 066; c, 075; d, 076; e, 090; f, 093; g, 226; h, cord serum. Prior to transfer, adult (10 mg in 400 μ l), larval (5 mg in 400 μ l) and egg (15 mg in 400 μ l) extracts were electrophoretically separated over a gel width of 11 cm.

nents in extracts of *L. cuprina* adult flies, larvae and eggs (Fig. 1).

Using the 30 sera from the sensitized subjects working in the entomology institute, and the 5 sera from Sydney asthmatics, all of whom had serum IgE antibodies to blowfly antigens, a total of 27 bands in the electrophoretically separated fly, larval and egg extracts were found to bind IgE. Twenty-one different bands were detected in the fly extract, 18 in the larval extract and 9 in the egg extract. In general, reactions were easily detected as clear bands on the autoradiographs. Figure 2 shows the bands that reacted when NC transfers of the *L. cuprina* extracts were probed with subjects' sera followed by [^{125} I]anti-human IgE. The examples of serum reaction patterns have been selected to show the full range of the IgE-reactive bands detected in this study. Table 1 summarizes the number and MW of the components detected in the fly, larval and egg extracts, and Fig. 3 shows the incidence of IgE-binding to each band by the 35 sera studied. Although a common numbering system has been used in Table 1 and Fig. 3, to avoid unnecessarily complicating the data, it should be emphasized that the identity of individual proteins in adult, larvae and eggs cannot be assumed simply on the basis of similar MWs. Component number

21 of the adult fly extract (67 kD) was the most reactive band on NC transfers (Fig. 2) and was found to bind to 70% of the sera examined (Table 1; Fig. 3c). Other components showing a fairly high frequency of reaction with IgE antibodies in the patients' sera were components 10 (33 kD, 40%), 8 and 13 (28 kD; 40 kD, 23%, respectively), and 23 and 27 (75 kD; 94 kD, 17%) (Fig. 2). With *L. cuprina* larval extracts, 18 of the 27 bands detected in the adult fly extracts bound IgE, and again the components with MWs of 67, 40, 33 and 28 kD showed a high frequency of reactions with the patients' sera (Table 1; Fig. 3b). However, some clear differences were observed, for example, some patients' sera reacted with larval extract components of MW 60 and 10 kD, in contrast to results with the adult extract where no binding was observed with bands of these MWs. Another major difference was the binding of adult components of MWs 75, 80 and 85 kD (Table 1; Fig. 3c). A small number of patients' sera reacted with a component in one extract but not in another—e.g. bands with MWs 8, 16, 30, 38, 55, 58 kD and so on (Table 1; Fig. 3b, c).

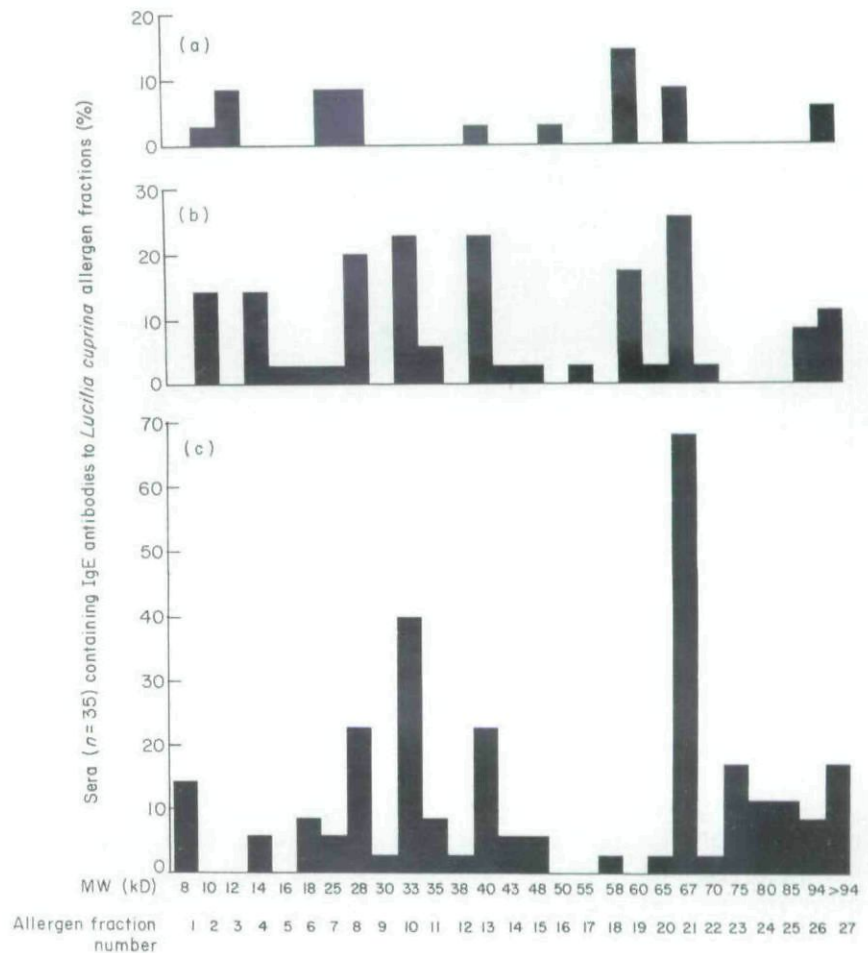
Reactions of the separated egg components with patients' sera were few, and only nine of the components that corresponded to the adult and larval extracts bound

Table 1. Sera from *Lucilia cuprina*-allergic subjects, showing reactions of each with the identified allergenic components obtained by transfer of SDS-PAGE-separated *L. cuprina* proteins

Serum number (n=35)	<i>L. cuprina</i> allergen fraction number (1-27) and estimated MW (kD)																											Total IgE (IU/ml)				
	RAST (% uptake)																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27					
017	●											●									●							5.1	5.9	3.7	420	
028		■																			●							4.1	10.7	2.5	135	
041							▲			●				●				▲										▲	11.5	14.7	12.3	140
045		■																			●							6.2	13.1	4.8	120	
061																		▲										1.3	12.4	3.2	115	
065																		▲										4.1	3.3	3.3	210	
066	●	■	▲	■	■	●	▲	●	■	■	■	■	■	●				■	■	●	●							29.7	31.5	31.7	>1000	
067							●																					8.1	21.7	4.2	420	
068												●	■															12.1	24.4	7.4	180	
075									●									▲			●							19.5	19.9	12.2	>1000	
076							▲																					6.3	8.0	5.4	>1000	
086		■																			●							5.4	9.5	3.7	660	
090				■					■	■	■	■	▲	■							●	▲						34.1	28.5	30.5	450	
093	●		▲	■		●	■	■	●	●				●				■			●							16.6	8.7	7.5	>1000	
118									●												●							3.7	7.1	2.0	150	
127																					●							4.6	6.7	4.1	270	
130												●	■															14.5	14.9	5.1	120	
131	●				●		▲		●					●							●	▲						23.1	19.6	28.1	>1000	
133							■	■	■	■	■	■									●							5.7	15.8	2.4	280	
137							■	■	■												●	■						1.3	7.5	1.5	66	
141	●							■													●							13.7	15.6	3.9	720	
157												●	■															5.9	18.6	4.5	88	
160	●						■	●	●	●	●							■			●							29.3	23.1	18.7	>1000	
169							■	■	■	■											●							2.5	16.2	2.1	45	
193	■	▲		■	■	■	■	●													●	■						19.7	28.2	19.9	215	
196																												7.8	16.2	3.6	135	
197																					●							6.5	7.8	3.3	64	
212							▲			●											●							13.4	19.2	5.1	84	
223									●	●	●	●	●								●	■						8.1	3.9	2.2	130	
226				■																								8.5	9.3	8.6	130	
659									●									▲			●							14.2	16.5	4.6	>1000	
660							■	■	■	■	■	■	■								●							10.4	18.9	1.4	100	
674							■	■	■	■	■	■	■								●	■						13.9	19.7	2.3	98	
681												■									●							5.0	8.0	1.7	94	
684							●														●							7.4	8.5	4.2	300	
Cord																												1.5	1.1	1.3		

● Adult, ■ larvae, ▲ egg.

Fig. 3. Frequency histograms of allergic subjects' sera containing specific IgE antibodies to each of the *L. cuprina* (a) egg, (b) larvae and (c) adult allergenic components separated by SDS-PAGE.



human IgE antibodies (Fig. 2). As with the larval extract, where a high incidence of reactivity was seen, but in contrast to the adult extract, the 60 kD component was the most reactive in the egg extract. Components 21 (67 kD), 3 (12 kD), 7 (25 kD) and 8 (28 kD) were the next most reactive components present (Table 1; Fig. 3a).

Investigation of possible allergenic cross-reactivity between the blowfly L. cuprina and other species of flies

The species studied for cross-reactivity, together with their common names, are listed in Table 2. All were members of the Diptera family except the bogong moth (*A. infusa*).

Protein staining of SDS-PAGE patterns of extracts from the seven species are shown in Fig. 4. Extracts from each of the species were separated into at least 40 electrophoretically distinct bands distributed throughout the MW range of approximately 5–8 to 100 kD. Marked similarities were observed between the *C. stygia* and *C. augur* patterns (Fig. 4a, b) while the *M. vetustissima* protein band pattern resembled the pattern obtained with

Table 2. Insect species examined for allergenic cross-reactivity with *Lucilia cuprina*

Genus and species	Common name
<i>Calliphora stygia</i> (Fabricius)	Eastern golden-haired blowfly
<i>Calliphora augur</i> (Fabricius)	Lesser brown blowfly
<i>Chrysomya rufifacies</i> (Macquart)	Hairy maggot blowfly
<i>Musca vetustissima</i> Walker	Bush fly
<i>Dacus tryoni</i> (Froggatt)	Queensland fruit fly
<i>Chironomus duplex</i> Walker*	
<i>Agrotis infusa</i> (Boisduval)†	Bogong moth; common cutworm

* A bloodworm; no common name.

† Unlike all other species in this study, not a member of the order Diptera.

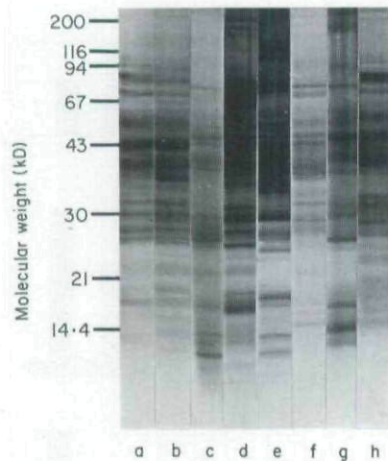


Fig. 4. Protein-stained separation patterns obtained following SDS-PAGE of extracts (20 mg/ml) prepared from: a, Eastern golden-haired blowfly (*C. stygia*) (50 μ l); b, lesser brown blowfly (*C. augur*) (50 μ l); c, bush fly (*M. vetustissima*) (10 μ l); d, hairy maggot blowfly (*C. rufifacies*) (25 μ l); e, Bogong moth (*A. infusa*) (25 μ l); f, Queensland fruit fly (*D. tryoni*) (25 μ l); g, *C. duplex* (50 μ l); and h, sheep blowfly adult (*L. cuprina*) (50 μ l). All extracts were added to an 8 mm slot for separation.

C. duplex (Fig. 4c, g). Heavy protein staining of some electrophoretically separated bands clearly distinguished the *C. rufifacies* and *A. infusa* extracts from extracts of the other five species (Fig. 4d, e).

When probed with six sera from *L. cuprina*-allergic subjects, distinct IgE-binding bands were located in each

of the seven extracts (Fig. 5). The most striking feature of these results was the sharp, heavily stained band of approximate MW 20 kD in the *C. stygia*, *C. augur*, *M. vetustissima*, *C. rufifacies*, *A. infusa*, and *D. tryoni* extracts. Using only six allergic sera, *C. stygia* was shown to contain at least eight, *C. augur* at least seven, *M. vetustissima* at least nine, *C. rufifacies* at least 11, *D. tryoni* at least 10 and *C. duplex* at least 15 allergens (Fig. 5).

Probing of insect protein blots with control sera from non-allergic and allergic subjects

As well as human cord serum, which was used as an IgE-'free' control serum to probe blots of *L. cuprina* adult, larval and egg extracts (Fig. 2(a) m, (b) u and (c) h), and the various fly extracts (Fig. 5(a) g, (b) g, (c) g, (d) g, (e) g, (f) g and (g) g), sera from 'normal' non-allergic adults and from subjects allergic to sources other than insects, were also examined for reactivity with the blotted insect proteins. No specific IgE antibody binding to the insect proteins was observed with any of the control sera.

Discussion

From the RAST and protein blotting results set out here, and from results outlined elsewhere [4,5; Kaufman *et al.*, in preparation], it can be concluded that sensitization and subsequent allergic reactions can occur to all three developmental stages of the blowfly *L. cuprina*. Some IgE-binding *L. cuprina* components in the adult, larval and egg extracts were found to have the same, or similar,

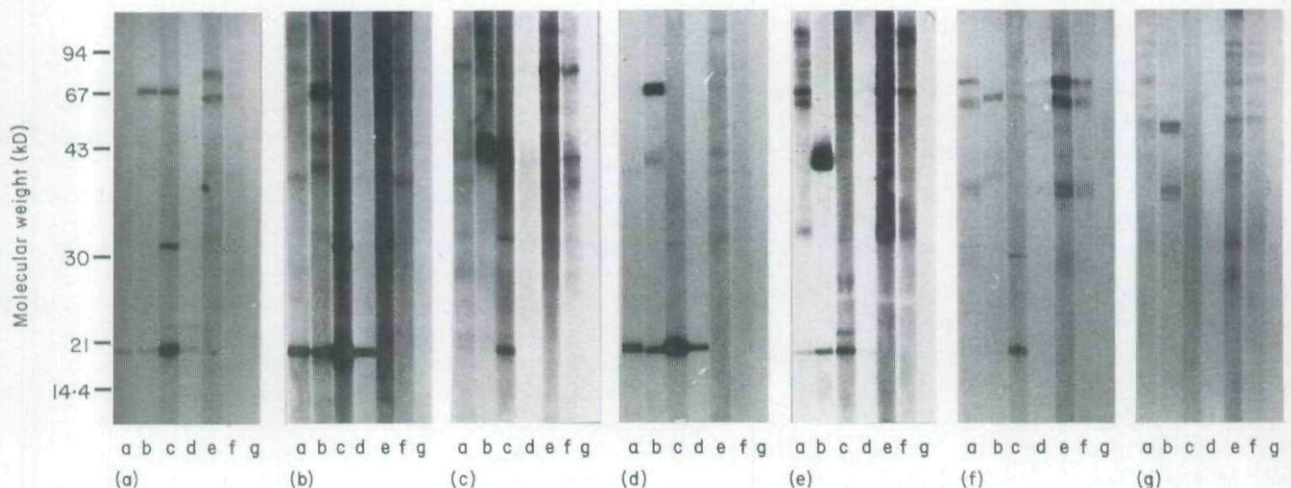


Fig. 5. Identification by autoradiography of IgE-binding components transferred to nitrocellulose following SDS-PAGE of extracts (20 mg/ml) of (a) Eastern golden-haired blowfly (*C. stygia*) (700 μ l), (b) lesser brown blowfly (*C. augur*) (560 μ l), (c) bush fly (*M. vetustissima*) (140 μ l), (d) hairy maggot blowfly (*C. rufifacies*) (140 μ l), (e) Bogong moth (*A. infusa*) (280 μ l), (f) Queensland fruit fly (*D. tryoni*) (420 μ l), and (g) *C. duplex* (700 μ l). Strips were incubated with the following sera: a, 131; b, 090; c, 066; d, 193; e, 160; f, 093; and g, cord serum. Each extract was separated over a gel width of 11 cm.

MWs but the relationship between bands of the same MW in extracts from the three developmental stages is not yet clear. Although it is likely that, in some cases at least, these are the same proteins, direct experimental evidence will be needed to establish this one way or the other, and it must be remembered that the common numbering system used in Table 1 and Fig. 3 does not imply identity of proteins of similar MWs.

Subjects' serum IgE antibody responses were generally heterogeneous both in terms of the allergens recognized within any one extract (adult, larval or egg) and between extracts. Subjects with the highest RAST uptakes generally reacted to multiple bands on the transfers while low RAST uptakes were often associated with only a small number of bands.

Some of the sera studied appeared to contain IgE antibodies that cross-reacted with antigens in extracts from other species of flies. The IgE antibodies that reacted with the various fly extracts may have been formed purely as a response of the allergic subjects to *L. cuprina*. If this is so, the observed reactions with other fly species may be true cross-reactions with antigenically similar components present in the species. An alternative explanation is that the subjects may have become sensitized separately, and reacted to *L. cuprina* and at least some, and perhaps all, of the other six fly species examined here. The former of these two explanations seems more likely.

Clear reactions observed after probing the separated proteins from the bogong moth *A. infusa*, a species not belonging to the order Diptera and unrelated to the flies studied here, suggested that at least some IgE antibodies may cross-react widely with antigens from taxonomically unrelated insect species. Wide-ranging cross-reactivity of this type has been observed in studies of insect allergies [9] and has been reported briefly by others [10]. 'Pan allergy' to insects [9] may occur in many subjects who have been sensitized to one insect species. The lack of species specificity shown by IgE antibodies in the sera of patients allergic to insects has important implications for our understanding of underlying mechanisms and for the specific diagnosis of insect hypersensitivities.

For both academic and practical purposes it would be of interest to purify and characterize at least one of the important *L. cuprina* allergens, for example, the component of MW 67 kD. Comparison of the properties of a major insect inhalant allergen with major allergens from pollens, mites, etc. would be of great interest. In addition, the allergen would be valuable as a skin test and RAST diagnostic agent for detecting allergic sensitivity to blowflies. As with allergy to *L. cuprina*, more information is needed on related species of flies, both at the clinical and laboratory levels. Evidence is accumulating that insects may be an important source of allergens which provoke

allergic reactions domestically, as well as in the work place [11]. If that is so, our demonstration of the allergenic potential of fly proteins may be relevant to the study and understanding of inhalant allergy and asthma in the general population.

Acknowledgments

We thank Gail Knowland for technical assistance. This work was funded by the National Health and Medical Research Council of Australia.

References

- 1 Tee RD, Gordon DJ, Lacey J, Nunn AJ, Brown M, Newman Taylor AJ. Occupational allergy to the common house fly (*Musca domestica*): use of immunologic response to identify atmospheric allergen. *J Allergy Clin Immunol* 1985; 76:826-31.
- 2 Spieksma FThM, Vooren PH, Kramps TA, Dijkman JH. Respiratory allergy to laboratory fruit flies (*Drosophila melanogaster*). *J Allergy Clin Immunol* 1986; 77:108-13.
- 3 Gibbons HL, Dille JR, Cowley RG. Inhalant allergy to the screwworm fly. *Arch Environ Health* 1965; 10:424-30.
- 4 Kaufman GL, Baldo BA, Tovey ER, Bellas TE, Gandevia BH. Inhalant allergy following occupational exposure to blowflies. *Clin Allergy* 1986; 16:65-71.
- 5 Kaufman GL, Gandevia BH, Bellas TE, Tovey ER, Baldo BA. Occupational allergy in an entomological research centre. I. Clinical aspects of reactions to the sheep blowfly *Lucilia cuprina*. *Br J Indust Med* 1989, in press.
- 6 Tovey ER, Baldo BA. Standardization of allergens. Qualitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. *Int Arch Allergy Appl Immunol* 1984; 75:322-9.
- 7 Ford SA, Baldo BA, Geraci D, Bass D. Identification of *Parietaria judaica* pollen allergens. *Int Arch Allergy Appl Immunol* 1986; 79:120-6.
- 8 Neuhoff V, Stamm R. Quantitative aspects of protein staining by Coomassie blue dyes. In: Neuhoff V, ed. *Electrophoresis '84*. Weinheim: Verlag Chemie, 1984; 317-20.
- 9 Baldo BA, Panzani RC. Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalant allergies to insects. *Int Arch Allergy Appl Immunol* 1988; 85:278-87.
- 10 Edge G, Sherwood Burge P. Immunological aspects of allergy to locusts and other insects. *Clin Allergy* 1980; 10:347(abstr).
- 11 Bellas TE. Insects as a cause of inhalant allergies. A Bibliography. 2nd Edn. Canberra: CSIRO Division of Entomology. Report no. 25, 1982.

Copyright of Clinical & Experimental Allergy is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.