

Arthropods and invertebrates allergy (with the exclusion of mites): the concept of panallergy

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It is becoming increasingly evident that exposure to arthropods in our immediate environment (at home, outdoors, at work) results in sensitization to arthropods, which can lead to inhalant allergies, namely asthma, allergic rhinitis (cf the works by Figley (1), Parlato (2,3), Feinberg (4) and principally Perlman in the USA (5,6); Shipvuri et al. (7), Gupta et al. (8), Agarwal et al. (9) in India; Benaim Pinto (10) in Venezuela; Panzani and Layton in France (11); Bellas (12) and Baldo and Panzani (13) in Australia; Mitsui et al. (14), Kino and Oshima (15–17) in Japan; Bauer et al. (18,19) in Germany. More recently, as we shall see later, a lot of studies have been carried out on cockroach allergens. As yet, little information is available on the worldwide incidence and distribution of arthropod allergies, the types of allergy encountered, the extent of cross-reactivities between arthropod allergens, and the nature of these allergens. In addition, the relative importance of different species of arthropods as a source of inhalant allergy in different geographic locations is not well defined.

The world of arthropods, which has surrounded humankind for millions of years and which includes mostly true insects and arachnids, is so important and so numerous that entomologists have not yet been able to identify all the species (there are only 750 000 identified species). Wilson estimates that there are well over 10 million insect species. The social insect, which makes up about 80% of the insect biomass, outweighs the vertebrates by about 7 to 1, he says. Three of the 10 plagues of Egypt were caused by

arthropods, namely mosquitoes, flies, and locusts. Given the ubiquity of arthropods indoors and outdoors, it comes as something of a surprise that there are few reports in the medical literature of allergy to arthropods as inhalants compared with the many studies dedicated to pollens, moulds and, more recently, to mites and stinging insects. Furthermore, most of the authors interested in this field have become interested by one arthropod – the cockroach – even though it has been demonstrated since the beginning of our studies that all arthropods could be involved in IgE-mediated allergy in humans. The role of some invertebrates will be included, however, because there is probably a cross-reactivity between arthropods and some invertebrates.

I Arthropod and invertebrate classifications

1 Phylum arthropoda

The word “arthropod”, which means “which has articulated feet”, was coined in 1845 by Siebold and Stannius. The phylum arthropoda exceeds all other groups of animals in number of species and diversity; it accounts for approximatively 80% of all known animal species.

The arthropods are characterized by a segmented body, jointed limb and a strong external skeleton, the cuticle. The possession of this exoskeleton, which gives the animal its form and which can be hardened or armoured to provide protection, requires that the animals grow by moulting. The old exoskeleton is

discarded as an exuviae, allowing the newly emerged animal to enlarge before the new exoskeleton hardens.

Every species of living animal is characterized by two Latin names: the first one is the name of the genus and the second one is the name of the species. An example is *Musca domestica*. Furthermore, species are assembled by affinities into larger groups: family, order, class, and phylum (or division). After and more general are the subkingdom and the kingdom.

There are four principal classes (Table 1):

- 1 Myriapoda;
- 2 Crustaceans which have at least five pairs of legs and two pairs of antennae; among them we found Decapoda (lobster, crab) and Branchiopoda (Daphnia). Crustaceans are mostly aquatic, have gills and possess several pairs of legs;
- 3 Arachnida, which possess four pairs of walking legs, are always wingless, and all exhibit gradual metamorphosis. Spiders feed on small animals such as flies;
- 4 Hexapoda, i.e. true insects (insecta), have a body divided into a head, a thorax, and an abdomen. In the adult, the thorax possesses three pairs of legs and may have one or two pairs of wings, or may be wingless. In some groups, such as moths and beetles, the larval forms undergo a complete metamorphosis before emerging as the adult or imago. In others, such as cockroaches or silverfish, each larval stage becomes progressively more like the adult, so that there is no abrupt change in form. Insects live in terrestrial and aquatic habitats, and exploit a huge variety of foods both of plant and animal origin.

The class Hexapoda contains over 29 orders. The chief orders are as follows:

- 1 Hemiptera, two pairs of wings, sub-orders – Heteroptera and Homoptera;
- 2 Orthoptera, two pairs of wings;
- 3 Coleoptera, one pair of elytra and one pair of wings;
- 4 Lepidoptera, two pairs of wings;
- 5 Hymenoptera, two pairs of wings;
- 6 Diptera, one pair of wings; the second pair is modified – halteres;

Table 1 Arthropod classification

Phylum Arthropoda						
:						
Subphylum	Mandibulata			Chelicerata*		
:						
Superclass	Hexapoda		Crustacea Myriapoda			
:						
Class	Insecta	Noninsect hexapoda			Arachnida	
:						
Subclass				Araneida	Acarina	Scorpionida

*Chelicerata: preoral multiarticulated appendages with a grasping function.

Table 2 Invertebrate classification

I	Arthropoda	<i>Tenebrio molitor</i> (a fish bait)
II	Annelia	<i>Eisenia foetida</i> , <i>Marphysa sanguinea</i>
III	Porifera	<i>Dysidea herbacea</i>
IV	Mollusca	<i>Gasteropoda</i> : limpet snails (<i>Helix terrestra</i> , <i>Euparipha pisana</i>) <i>Cephalopoda</i> : squid, octopus <i>Lamelibranchia</i> : abalone (<i>Haliotis ruber</i>) clam, cuttlefish, oyster, mussel, scallop shellfish

Note: Among the 29 phyla of invertebrates, we shall mention only the few relevant to our study.

- 7 Orders of minor interest: Siphonaptera, Flea, Ephemeroptera, Mayfly, Trichoptera, Caddis fly, Thysanura, Silverfish, Dermaptera, Neuroptera. There are also noninsect Hexapoda.

2 Invertebrates

Since we shall deal in this section with some invertebrates besides the phylum arthropoda, we have included here a general classification of the invertebrates (Table 2).

II Source and aerobiology of allergens

A Source of the allergens within the arthropod's body

Shulman (20) thought that many components of the body and, in particular, the haemolymph (blood) besides the cuticle might be allergenic. These proteins can change during metamorphosis from eggs to larvae and adult stages. However, it has been shown in the case of *Lucinia cuprina* (21), by RAST and protein blotting, that allergic reactions can occur in the three developmental stages (adult fly, larval, and egg) but the relationship between bands of the same molecular weight in extracts from the three stages is not yet clear, although it is likely there are the same proteins.

To become an inhalant allergen, proteins from the arthropods must be airborne. This can occur in several ways. (Bellas). First, the dry dead body of an arthropod can become pulverulent and act as a dust. Helm et al. (22) have been able to identify aeroallergens from living cultures of cockroaches (*Periplaneta americana* and *Blattella germanica*). It is the first study to demonstrate aerosolized cockroach-specific allergens from cockroach colonies, indicating that the dead bodies are not the unique potential antigens.

Second, some terrestrial arthropods possess hairs, setae (fine leg hairs), and scales that are shed more or less readily. In this category we also find the exuviae (old exoskeleton) left by the insects during their metamorphosis. (Exuviae have been shown to be a major source of allergens for mayflies, cockroaches, carpet beetle larvae and wasps.)

Third, the metabolic products of the arthropods have an important role. The role of feces has been emphasized for dust mites, locusts, fleas, and mentioned for other species. If the faeces were merely the waste products after digestion of food, it would be difficult to explain any specific activities. Edge and Burge (23) employed an immunofluorescence technique to study the site of production of allergen in the locust *Schistocerca gregaria*. Their results showed that allergen was present in the cuticle but the main sites of production of the allergen were found to be the epidermal cells of the midgut and the associated gastric caeca. These cells are also the site of production for the peritrophic membrane which travels through the gut and is expelled with the faeces. These authors suggested that it is the peritrophic membrane that is the source of the specific allergens associated with the faeces. Peritrophic membranes are produced by most species of insects. Wharton and Brody (24) showed that a similar membrane is present in dust mites and raised the question of its allergenic capabilities in man. It is also worth noting here that insects are contaminated with ingested bacteria, moulds and other agents.

B Aerobiology or dissemination of the allergens

Although we know that in some cases of respiratory allergy to pollens (proximity pollinosis) all the five postulates of Thommen are not fulfilled, we shall see whether they can be applied to arthropod allergens.

These postulates are fulfilled for arthropods in the same way as they are for pollens or moulds (Perlman). Not only are most insects capable of active migration, but perhaps more important to us is the passive dispersion of these arthropods. There is scarcely an insect that, at some time in its life cycle, does not take to the air. Just as the ocean is adrift with marine plankton, so the air may be filled with insect fauna that might well be named "aerial plankton" (as Perlman called it). In 1953, Griffiths (25) showed in the Savannah area (Georgia, USA) that it was possible to grow insect larvae from wind-borne eggs. The method of collecting wind-borne insect eggs was the same as that used in collecting wind-borne spores for mould colonies. In this peculiar case, the insect identified was *Drosophila melanogaster*. While collecting insects from the level of 50 to 14 000 feet, Coad (26) found the densest population up to 1000 feet. Airborne organisms (hollow particles 0.5 µm in diameter) collected in an atmospheric aerosol at Harwell Atomic Energy Research Establishment were identified by insect physiologists as brochosomes, which originate in the Malpighian tubules of the gut of homopterous insects.

In the beginning, when examining their slides for collecting pollens, allergists looked for insect debris such as scales of Lepidoptera but, obviously, this technique was not accurate enough to identify all these particles. Fortunately, since 1980, new techniques have been developed to measure airborne allergens. Immunological methods are becoming increasingly important for quantification of allergens: RIA inhibition, RAST inhibition, ELISA.

In 1985, Tee et al. (27) used an immunochemical method to identify the atmospheric allergen of *Musca domestica* in cases of occupational allergy to this insect. Similarly, Swanson et al. (28) used a new volumetric air sampler for studying, among other indoor allergens, cockroach antigen. More recently, Kimura et al. (29) developed an ELISA inhibition method for the detection of mites and chironomid antigen in environmental samples of dust soil and air. Also in Japan, Kino et al. (30) developed an immunochemical quantification of insect-related airborne particles smaller than 10 µm in diameter. Helm was the first to identify major cockroach aeroallergens from living cultures of german and american cockroaches (22). Pollart et al. (31) used quantitative two-site monoclonal antibody-based enzyme immuno-assays for two cockroaches allergens, Bla g 1 and Bla g 2, to measure allergen levels in house dust samples (dust from kitchen floors and cabinets contained 50-fold more allergen than dust from bedrooms and upholstered furniture). Finally, Schou et al. (32) developed a sandwich ELISA environmental assay for cockroach allergens based on a monospecific rabbit antibody preparation reactive with determinants shared by the important allergens Per a I and Bla g 1 from american and german cockroaches. During the past 10 years, new techniques of air sampling and of measurement of submicrometre particles from pollens or other aeroallergens such as soybean have been developed. Reuström et al. (33) compared different methods for a better standardization. In many cases, monoclonal antibodies to purified allergens were used. The sampling methods have also been improved. Kitto (34) has developed a new rapid biochemical technique (sandwich ELISA) for quantifying insect infection in stored cereals using antibodies directed against a protein present in relatively large quantities in the major insect life stages of a wide variety of insect species: this ubiquitous protein is the muscle myosin, which is easy to extract reproducibly and differs very little between insects.

Tovey et al. (35) have recently measured and characterized airborne particles containing Bla g 1 in homes with relatively low reservoir levels of allergen using a new method for simultaneous immuno-detection and identification of the particles (36).

III Arthropod airborne allergy as an occupational disease

The first report of an occupational disease attributed to an arthropod was that of Ramazzini who in 1713 noted that the “terrible cough and serious difficulty of respiration” among silk carders was caused “by the small fragments of dead silkworms that are scattered all through the silk rolls”. Since then, several publications described occupation insects allergies. Five categories of subjects are commonly highlighted:

- 1 bakers and persons handling cereals and seeds contaminated by *Ephestia K* and *Sitophilus granarius*;
- 2 fishermen, professional or hobbyist, and persons whose workplaces provide breeding sites for arthropods such as farmers: *Sitophilus granarius* and the botfly *gasterophilus intestinalis* (37);
- 3 entomologists and people working on insects to study biology;
- 4 people exposed to insect derived materials silk and carmine;
- 5 respiratory allergy to invertebrates.

The inhalation of a marine sponge, as an occupational allergy, has been reported by Baldo et al. Sea squirt asthma (Hoya asthma) is an allergic asthma induced by inhalation of sea squirt (*Styela plicata*) substance first and mostly described by Mitsui et al. (14). *Styela plicata* is an ascidia (Tunicate) which firmly adheres to the outside of oyster shells and which is scattered by the hammer of the labourers during the seasonal period of shucking oysters. Three antigens have been isolated from sea squirt named Gi-rep, E1M and DIIIa of average molecular weight 106 000, 22 800, and 9980 respectively.

The following cases are not occupational but occur among hobbyists. Respiratory allergy to fish bait is not rare among fishermen. Flies are used by anglers in fresh waters: *Calliphora erythrocephala* (mosca carnaria), *Echinodorus plamosus* larvae, *Tenibrio molitor* (beetle) larvae bee; *Eisenia foetida*, which is an earthworm, is also used. In sea fishing, marine worms are mostly used: *Marphysa sanguinea* in the Mediterranean region. In all these cases, we have reports of allergy. In Provence, the worms *Perenereis cultrifera*, *Nereis diversicolor*, *Sipunculus Nudus*, and some crustaceans are also used. Many of these invertebrate nonfish seafood or parasites have been sequenced and cloned:

(a) Crustaceans:

- *Metapenaeus ensis* (greasy back shrimp), Met e 1 tropomyosin;
- *Penaeus aztecus* (brown shrimp), Pen a 1 tropomyosin;

- *Penaeus indicus* (indian shrimp), Pen e 1 tropomyosin;
- *Parapenaeus fissurus* (Taiwan shrimp), Par f 1 tropomyosin;
- *Panulirus stimpsoni* (spiny lobster), Pan s 1 tropomyosin;
- *Homarus americanus* (american lobster), Hom a 1 tropomyosin;
- *Charybdis feriatus* (crab), Cha f 1 tropomyosin.

(b) Molluscs:

- *Haliotis midae* (abalone), Hal m 1;
- *Todarodes pacificus* (pacific squid), Tod p 1.

(c) Others

- *Ascaris suum* (worm), Asc s 1;
- *Anisakis simplex*, Ani s 1 and Ani s 2 Paramyosin.

We have included here the case of baker’s asthma, which is a complex allergic entity for, besides wheat and other cereals proteins, many allergens have been implicated: baking additives, baker’s yeast enolase, storage mites and, often, arthropods *Ephestia Kuchniella* being the commonest.

Almost all the cases described in this section concern patients who are exclusively or predominantly allergy to arthropods or invertebrates.

IV Insect emanations allergens acting as disseminated aeroallergens: clinical data

There are only few publications on generalized allergies to arthropods. Only Perlman and ourselves (Panzani and Layton; Baldo and Panzani) had thought that the emanations of arthropods could be as important as mites, pollens or moulds. However, recent works from Japan (3) and from India (9) support this notion.

Parlato in Buffalo, NY, was the first in 1930 to demonstrate that the emanations of caddis fly Trichoptera could act as airborne allergens (3). At about the same time, Figley (1) on the other side of Lake Erie described cases of respiratory allergy to mayfly, ephemera. Both insects develop as larvae at the bottom of lakes and then emerge as adults at the surface from June until the end of August. Almost 20 years later, Osgood (38) reported other cases of allergy to caddis fly. Two other papers are of interest: one in 1959 by Wiseman et al. (39), who concluded that insect inhalant allergy may be a significant causative factor in respiratory allergy, and the other in 1970 by Hosen (40), who also used several insect antigens and showed evidence of inhalant allergy by skin test and provocative nasal test on half of a group of 174 patients. Thirty years passed until Kagen (41) in Wisconsin reported cases of Lake fly

allergy, caddis fly, in a general population and as an occupational respiratory allergy. He showed the incidence of chironomid sensitivity in an atopic population and tried a desensitization. A recent short report showed the importance of different household insects among asthmatics in Egypt (42).

1 Chironomids respiratory allergy

Chironomid midges and larvae are known to contain potent allergens. In Sudan, it has been reported by Kay and Gad el Rab (43,44) that a small midge, *Cladotanytarsus lewisi* (green nemitti), occurred in enormous numbers in the Nile basin and that it caused asthma in people living close to the River Nile. In Germany, it has also been reported by Bauer et al. that chironomid larvae, which are widely used as fish bait either in the form of whole lyophilized insects or in mixed products, cause IgE-mediated respiratory allergic reactions in a significant number of people exposed to these larvae through their occupation or their hobby.

Bauer et al. (18,19,45) have shown that the major larval allergens of *Chironomus thummi* and *Campptochironomus tentans* for humans are cell-free haemoglobins.

They have also demonstrated that the immunological cross-reactivities between the different chironomid species are predominant haemoglobin components with common antigenic determinants. Haemoglobins of the larvae of *chironomus thummi thummi* have been characterized and labelled Chi t 1; it comprises nine homologous components. Furthermore they have demonstrated the persistence of haemoglobin allergenicity and antigenicity during metamorphosis of chironomidae.

In Japan, many species of chironomids are widely distributed in urban areas. Thus, it is probable that chironomids allergy occurs throughout the world.

2 Cockroaches allergy

Cockroach allergy was first described by Bernton (46), and later by Mendoza (47) and by Choovivathanavanich et al. (48) in Thailand. Until now, it has been the most studied of the inhalant arthropod allergens (49,50).

Three principal species of cockroaches are known to induce respiratory allergy in man: *Blattella germanica* (german cockroach), *Periplaneta americana* (american cockroach), and *Blatta orientalis*. During the early 1980s, a new cockroach species, the Asian cockroach, was introduced into Florida and is now spreading across the USA. Helm et al. (51) have shown that this species share

allergenic activity with american and oriental cockroaches. Cockroach hypersensitivity has recently be found to be of increasing importance in allergic diseases, especially asthma. Lehrer et al. (52,53) compared cockroach allergenic activity in the whole body and faecal extracts of american and german cockroaches by skin test and inhibition studies: they suggested the presence of allergens common to both species and of common allergens between whole body and faecal extracts.

Cockroach allergens are among the arthropods the only ones if we except mosquitoes (*Aedes aegyptii*: Aed a 1, apyrase, Aed a 2), which until now have been identified, sequenced, and cloned.

1 *Blattella germanica* (german cockroach):

- Bla g 1, protein of unknown function;
- Bla g 2, aspartic protease;
- Bla g 4, calycin, a calcium binding protein;
- Bla g 5, a glutathione transferase;
- Bla g 6, troponin.

2 *Periplaneta americana* (american cockroach):

- Per a 1, homology with Bla g 1;
- Per a 3, homology to insect haemolymph protein (54).

The cockroach haemolymph proteins have a sequence identical to insect storage proteins, insect juvenile hormone-suppressible proteins and arthropod Hemocyanins.

- Per a 7, tropomyosin: These allergens have been purified sequenced and cloned mostly thanks to the works of Chapman et al. (55–57), Arruda et al. (58–60), Wu et al. (61–66), Chou et al. (67), Helm et al. (68,69), and Lehrer et al. (52,53).

As a result of the wealth of publications on cockroach allergy published during the past 15 years and the scope of our paper, we want to quote only two excellent reviews: one by Chapman et al. (70) and the other by Helm et al. (71).

3 Moths and butterflies allergy: *Lepidoptera*

Already known as aeroallergens since the works of Parlato, moths and butterflies have been implicated in Japan by Kino and Oshima (15–17) as a possible cause of respiratory allergy. They found that more than half of a randomly selected asthmatic population showed not only positive skin tests and positive RAST, but also positive bronchial provocation reactions to these outdoor insects. Their results indicated that *Lepidoptera* sensitivity in Japan is as common as mite sensitivity but there appears to be no cross-reactivity between the two groups of arthropods. However, the species involved are not specified. Wynn et al. (72) measured immunochemically in the Minnesota prairie

the airborne concentration of Lepidoptera and concluded that moths may be a seasonal allergen.

Recently in Spain, Vega et al. (73) have described cases of allergy (urticaria, asthma, shock) to another Lepidoptera, the pine processionary caterpillar (*Thaumetopoea pityocampa*), which generally gives only toxic reactions.

4 Silk allergy

Silk culture started in China and spread to Japan, India, and other Asian countries. Silk allergy offers a transition between professional and nonprofessional allergy, because allergy to silk products is well known as an occupational hazard in sericulture, and silk allergy often follows (especially in China) exposure to bed quilts or clothes filled with silk waste.

In contrast, textile products made of pure silk have not, to the best of our knowledge, been incriminated as a cause of allergic reaction, because they do not contain the allergens.

5 Silverfish: *Lepisma saccharina*

The presence of this insect in houses is common in The Netherlands, as shown by Witteman et al. (74). This insect is also common in Rome, as recently reported by Barletta and ourselves (75). An extract of silverfish has been characterized and the different allergenic components have been identified. In a general population, 6.4% of subjects are positive to at least one silverfish fraction. In addition, silverfish extracts can be recognized by sera from subjects allergic to household insects. Furthermore, the results indicate that the classical aqueous extract procedure is not completely satisfactory, because several components are not soluble at the pH normally used to produce extracts; the insoluble allergenic material appears to play an important role.

6 House fly: *Musca domestica*

We found only two reports describing cases of respiratory allergy to house fly – one by Tee et al. (27) and the other by Rosemary et al. (76) – in the context of occupational disease.

7 Arthropods and fish pets: “the aquarium syndrome”

In Europe, the USA, and all over the world, people keep fish in aquaria and feed them with fish foods which vary in their composition according to their trade mark, and which contain dried Daphnae and other crustaceans, some fresh water worms, dried black larvae of culex (mosquito), and mostly dried red

chironomus larvae. All these arthropods are able to give rise to a respiratory allergy (77).

8 The underground ecological niche

In a recent study, Leveque (78) found many arthropods in the Paris Underground railway where the atmosphere is humid with a constant temperature between 28 and 35 °C all year around, realizing a kind of subterranean tropical entomological jungle. The more important species were: the cricket, *Acheta domestica*, a spider, *Tenegeria domestica*, a fly, *Drosophila melanogaster*, two species of cockroaches, *Blattella germanica* and *Blatta orientalis*, a mosquito, *Culex pipiens*, the silverfish, *Lepisma saccharina*, and also *Psychoda phalaenoides*, a dermestid, and a *Psocus* whose species were not specified.

We want to stress here the fact that, as far as we know, we found no report on the house spider *Tegenaria domestica*, which according to our observations, is one of the more important household insects.

V Arthropod allergy in Southern France and Italy: clinical and early immunological data

A Early clinical findings

Our first clinical data from the past 40 years can be summarized as follows.

- 1 A positive skin test reveals a true positive reaction and not an irritative reaction (false positive). However, it is clear that a positive skin test may be the result of a cross-reactivity with other invertebrates or a latent allergy, i.e. without any clinical significance reaction.
- 2 The clinical relevance of arthropods, which is obvious in cases of monosensitization, has been assessed in cases of polyensitization by a nasal provocation test. However, a few objections to the reality of this clinical entity can be made.
 - (i) Arthropods can be contaminated by mites but more likely other invertebrates are not. The answer is according to Bellas that “the possibility does exist for an extract derived from insects to be contaminated by mite allergens. But one would have to suspect that the amount of contamination would not be large even if it occurred” (Bellas, personal communication).
 - (ii) There is a cross-reactivity between arthropods other than mites and mites (house dust, storage, plants) but cross-reactivity between mites and invertebrates other than arthropods

seems *a priori* unlikely. However, one important aim of our research is to try to find an answer using RAST-inhibition techniques.

- 3 The commonest arthropods found to react were *Tegenaria domestica* and *Musca domestica* followed by cockroach, moth and *Ephestia* (Table 3). Furthermore, in 25% of the cases, patients reacted by skin tests to all the arthropod allergens available, monosensitization to one arthropod being found in only 26%. In general (50% of the cases), we found a reaction to several arthropods. In conclusion, and in our opinion, the cockroaches are probably not – at least in the Mediterranean region – the commonest household insects involved in respiratory allergy. Diptera (flies) and spiders are more important but this is to be demonstrated on a larger scale. Probably, cockroach allergens partly cross-react with other arthropod allergens such as house fly, spider, and moth. In 1959, Wiseman et al. (39) studied extracts of 33 insects and found that the carpet beetle *Trogoderma versicolor* is potentially one of the important insects.
- 4 The incidence of skin tests positive to this material among atopic people (allergic rhinitis, asthma, urticaria/angioedema) is rather high: 30% among patients living in the South of France, although it does not reach the high incidence of cockroach-positive skin tests described in the USA or in Thailand. In most cases, besides strong reaction to arthropods, we found reactions to mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), pollens (grasses, parietaria, cypress, olive tree), and moulds (*Alternaria alternata*). Only 10% of the cases are monosensitized.
- 5 There is quite a large difference in incidence of allergy in relation to climate. Medina and Figueroa (79) in Colombia found a high prevalence of allergy to insects and they state that, “without doubt the tropical and subtropical zones of Colombia may register a higher prevalence of allergy to insects than is commonly known”.

The possibility of a greater frequency of arthropod allergy in subtropical or tropical countries prompted us to study a population of 100 Algerian patients living in different parts of Algeria and presenting with a respiratory allergy – mostly asthma (asthma

98% of the cases, allergic rhinitis 2%) proved by clinical anamnesis, skin tests, and sometimes RASTs and respiratory function tests. All the patients were tested with the usual battery of allergens. In 50% of the cases, we found positive skin tests to one of several allergens. In 24% of the positive cases, the patient's skin reacted to all the allergens tested, realizing a panallergy. We found the same percentage of panallergy among Caucasian patients from the South of France but with only 30% of the cases having at least one positive test (80). Continuing with this study of the influence of the environment in relation to the climate (81), we compared (besides these two groups) three other groups of patients: Algerians born and living in France; Caucasians born and living now in France but who lived at least 20 years in North Africa; ‘Asiatics’ born in Asia and living now in France. All these patients presented a typical respiratory allergy (asthma or allergic rhinitis) with positive skin tests and sometimes RAST to various airborne arthropods. As is evident from the results (Table 4), the percentage of positive skin tests is the highest (70%) among Asiatics. To a considerable extent, it is the same, on the one hand, among Algerians and Caucasians born in North Africa and, on the other hand, among Algerians born and living in France and Caucasians born and living in France. We can conclude that the genetic and ethnic background has no influence, the role of the environment i.e. the climate with its temperature and humidity being determinant.

B Recent clinical findings

Since 1997, using three allergens (cockroach, *Blattella germanica*, mosquito, and horse fly) together with a large panel of aeroallergens, we found among 1432 allergic patients the following positive responses to insects: *Blattella germanica*, 206 (14.38%); mosquito (*Aedes aegypti*), 298 (20.8%); horse fly, 278 (19.41%).

We have considered as positive only the cases in which the skin test reactivity was >1, i.e. classes 2, 3, and 4. The symptomatology among the 1432 allergic patients was as follows: asthma, 288 (20.11%); allergic rhinitis, 644 (45%); conjunctivitis, 214 (15%); atopic dermatitis, 286 (19.97%).

The positive reaction to household insects is in general associated with house dust mites (HDM). However, in some cases, household insects allergy stands alone:

- with blattella, 60% positive HDM vs. 40% negative;
- with mosquito, 75% positive HDM vs. 25% negative;

Table 3 Percentage of skin tests positive in 200 patients to the five commonest antigens

Spider	70%
Housefly	80%
Moth	52%
Ephestia	48%
Cockroach	58%

Table 4 Incidence of inhalant arthropod allergy among atopics according to climate

	No. of cases	No. of skin tests + ve to arthropods	Percentage of skin tests + ve to arthropods
Caucasians born and living in the south-east of France	260	78	30%
Algerians living in Algeria	100	50	50%
Algerians born and living in France	50	13	26%
Caucasians born in North Africa but now living in France	50	24	48%
Asiatics born in Asia and living in France for less than 10 years	20	14	70%

- with horse fly, 70% positive HDM vs. 30% negative.

A more specific investigation was carried out with 25 patients allergic to *Blattella germanica* tested in parallel with a natural tropomyosin. A nasal provocation test (NPT) was carried out using the same cockroach extract; in all cases, we found a positive NPT and a positive skin test to tropomyosin. The correlation of skin test reactivity between *Blattella germanica* extract and tropomyosin was good (Table 5).

C Early immunological data

The insects studied and their taxonomic relationship are shown in Table 6. Unfortunately, most of the extracts used in our first immunological studies (Table 7) were only prepared for research purposes and not for clinical testing. These first results have been extended later as we shall see in the following section dedicated to the concept of 'panallergy'.

Results of RASTs test

In order to assess the spectra of arthropod reactive IgE antibody responses in subjects with diagnosed inhalant allergies to arthropods, with Baldo (12), we tested the sera of 41 subjects presenting with an arthropod inhalant allergy documented by case history and skin testing using water-soluble extracts prepared from a wide range of insect species. Unfortunately, we were unable to obtain an extract of spider. All insect extracts but one – a locust faecal extract – were whole body extracts.

An initial screening of sera against seven different insect species was carried out: house fly (*Musca domestica*), blowfly (*Calliphora stygia*), common clothes moth (*Tineola bisselliella*), german cockroach (*Blattella germanica*), silverfish (*Ctenolepsisma longicaudata*), carpet beetle

Table 5 *Blattella germanica* versus natural tropomyosin

25 cases	Allergic rhinitis	18	72%	Monosensitized	4%
	Asthma	6	24%	Polysensitized	96%
	Spasmodic cough	1	4%		

Skin test reactivity scale from 1 to 4

(1) <i>Blattella germanica</i>	1	3	12%
	2	9	36%
	3	10	40%
	4	3	12%
(2) Tropomyosin	1	3	12%
	2	11	44%
	3	10	40%
	4	1	4%

We adopted the following scores: a 3+ reaction corresponds to the wheal and erythema of the histamine (1 mg for 1 mL prick test); a 4+ reaction corresponds to a greater reaction than that to histamine; a 2+ reaction is 75% and a 1+ reaction is half the size of the histamine reaction.

(*Anthrenus verbasci*), tropical warehouse moth (*Ephestia cautella*). The screening showed that 13 (approximately one third) of the sera examined reacted with all the seven extracts, while over half of the 41 sera reacted with four or more species; only one was negative. The number of positive-reacting sera ranged from a high of 35 with *Ephestia cautella* discs down to 15 with *Blattella germanica*. Also, the highest individual RAST uptakes were recorded with *Ephestia cautella* and the two diptera *Calliphora stygia* and *Musca domestica*.

Tests for IgE antibodies to *Lolium perenne*, *Phleum pratense* and *Dermatophagoides farinae* showed that sera from 26 subjects reacted to *Dermatophagoides farinae*, 18 reacted to *Lolium*

Table 6 List of insects used in our early immunological data

Order	Family	Genus and species	Common names
Blattodea	Blattellidae	<i>Blattella germanica</i> (Linnaeus)	German cockroach
Coleoptera	Dermestidae	<i>Anthrenus verbasci</i> (Linnaeus)	Variagated carpet beetle
	Bostrychidae	<i>Rhyzopertha dominica</i> (Fabricius)	Lesser grain borer
Diptera	Calliphoridae	<i>Calliphora augur</i> (Fabricius)	Lesser brown blow fly
		<i>Calliphora stygia</i> (Fabricius)	Eastern golden-haired blow fly
		<i>Chrysomya bezziana</i> Villeneuve	Oriental screw worm fly
		<i>Chrysomya rufifacies</i> (Macquart)	Hairy maggot blow fly
		<i>Lucilia cuprina</i> (Wiedemann)	Australian sheep blow fly
	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> (Biosduvai)	Bogong moth
	Pyrilidae	<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>Ctenolepsisma longicaudata</i> (Escherich)	Silverfish

Table 7 IgE binding bands (kDa) detected in the household arthropod extracts by SDS-PAGE immunoblotting

Arthropod extract	Detected in less than 50% sera	Detected in more than 50% sera
Fly	70/49/40/20	36/34/31/27/17
Moth	57/48/40/37/20/17	34/31/24
Spider	40/22	110/38/35/26/19

perenne and 13 reacted to *Phleum pratense* pollen.

With 13 of the 41 sera that reacted strongly with all seven insect extracts, we performed RASTs with a further 11 species of Diptera and another species from the order Lepidoptera Bogong moth (*Agrotis infusa*). Reactions were generally high; in fact, reactions with the moth discs were some of the highest recorded in this study.

Detection of insect allergens by electroblotting

Because most single allergen sources are multi-allergen systems yielding several chemically different allergenic proteins, we must expect that insect allergens will be a mosaic of proteins.

SDS-PAGE followed by protein staining of the gels identified multiple components over the molecular weight (MW) range from 14 to 200 kDa in extracts of the flies *Musca domestica* and *Calliphora stygia*, and in the non-Diptera species *Blattella germanica*, *Tineola bisselliella*, *Ephestia cautella*, *Rhyzopertha dominica*, and *Ctenolepisma longicaudata*.

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. The most striking features of the blot results were the preponderance of labelled bands from *Blattella germanica*, *Tineola bisselliella*, *Ephestia cautella*, *Rhyzopertha dominica*, and *Ctenolepisma longicaudata* with MWs in the region of approximately 30 kDa and above; the cluster of *Rhyzopertha dominica* allergenic proteins in the MW range 16–35 kDa; and the dense band at MW approximately 32 kDa on the *Ctenolepisma longicaudata* transfer (Fig. 1). From results with the six sera used for probing, *Blattella germanica* and *Tineola bisselliella* extracts were each shown to contain at least 14 allergens; *Ephestia cautella* and *Ctenolepisma longicaudata* extracts contained at least 12; and *Rhyzopertha dominica* extracts contained at least 15 allergens. The allergenic patterns of bands obtained with the two moth extracts *Tineola bisselliella* and *Ephestia cautella*, were very similar while the *Rhyzopertha dominica* pattern was clearly different to the other four patterns.

When six different species of flies (*Musca domestica*, *Calliphora stygia*, *Calliphora augur*,

Lucilia cuprina, *Chrysomya bezziana* and *Parasarcophaga* spp.) were compared side by side in blotting studies with the allergic sera and 125-I-anti-IgE two main features emerged (Fig. 2) First, almost all the IgE-binding proteins were found to have MWs greater than 35 kDa and, second, a dense band of MW approximately 37 kDa was found to occur on the NC blots of each of the six fly species.

Recently using the sera of 95 patients who gave a positive skin test result to a mixture of household insects we obtained by immunoblotting the results shown in Table 7 with 40% of positive reactions to house fly and 30% to spider and *Ephestia*.

In conclusion, we found a high proportion of sera that showed reactions to more than one insect species. Also as already mentioned, one important feature of electroblotting for allergen identification is the capacity of the method to utilize and compare large numbers of different sera and different extracts side-by-side in the same experiment. A typical example is shown in Fig. 2, where, in all the sera tested, IgE binding fractions of 37–39 kDa are visible in extracts of the six different species of flies. This common IgE binding fraction of 37–39 kDa looked like a candidate “panallergen”.

VI Recent evidence for a panallergy among arthropods and invertebrates

As already stated, arthropod allergy is often a “panallergy” or, at least, a rather extensive cross-allergy between the different families and orders of arthropods, which sometimes extends to all invertebrates. This observation leads us to hypothesize that there is among all the invertebrates a broader biological relationship than the usual taxonomy suggests.

In 1966, we were the first with Layton working at the US Department of Agriculture, Albany, CA, to describe an unusual form of cross-reactivity when studying allergy to the emanations of insects and arthropods. Allergy to household insects was already well known thanks to the works of Perlman. I also knew that many patients with asthma or allergic rhinitis in the South of France and North Africa were allergic to several household insects, house fly, spider, clothes mites, *Ephestia* and that there was, in 50% of the cases, a cross-reactivity between these species which belong to true insects (class Hexapoda) and to class Arachnida.

At this time, the RAST technique and the *in vitro* diagnosis of allergy were unknown; this is why we used (thanks to Layton (82) who developed this technique) the allergic transfer test on monkeys, which is the equivalent of the PK test in humans.

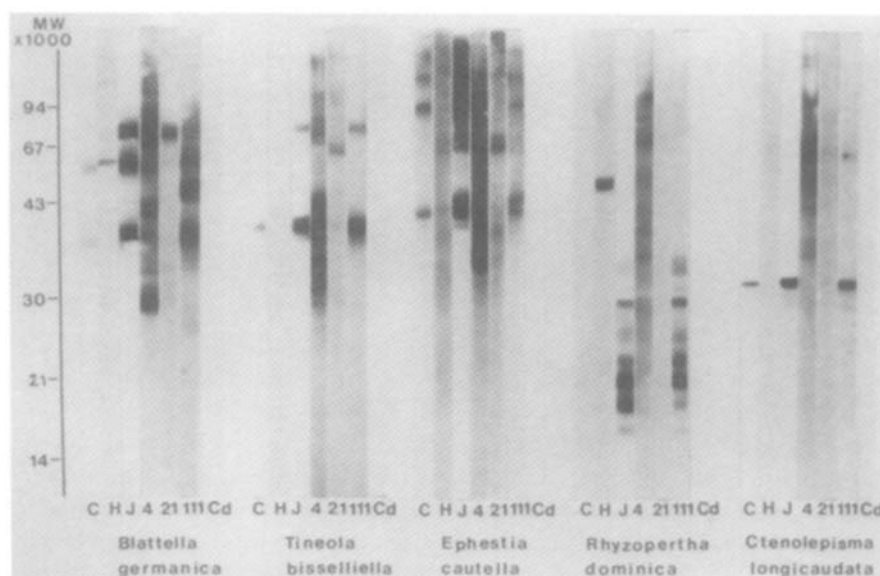


Figure 1 Identification by autoradiography of IgE binding components transferred to NC following SDS-PAGE of extracts of the insect species shown. Strips were incubated with sera from six subjects (C, H, J, 4, 21, 111) with inhalant allergy to arthropods (Cd being a control with cord serum) followed by ^{125} I-antihuman IgE. From BALDO, BA, PANZANI RC. Int Arch Allergy Appl Immunol 1988;**85**:278–287. Reproduced with the kind permission of Karger SA.

This test enabled us to show the cross-reactivity between arthropods. Among the observed reactions some were *a priori* suspected – for instance, a cross-reactivity between *Mosca domestica* and another Diptera, such as *Ephestia* – but one was particularly interesting. We found a strong cross-reactivity using sera from French patients and the extract of a stinging insect, *Triatoma protracta*, known in the USA and Argentina, but unknown in France (Fig. 3). Obviously, the French patients who have never been to the USA, had never been

in contact with *Triatoma protracta*. This is the first time, as far as we know, that the word “panallergy” has been coined and used.

In a paper with Layton (11), we said (p. 264): “that the basis we wanted to emphasize is that arthropods allergy is a panallergy”. This concept was fully expanded in the presentation ‘Does pan-allergy to insects exist’ (83) and in a paper on arthropods allergy published in 1994 (84,85). In these works, we have shown that the concept of panallergy to arthropods extends to invertebrates.

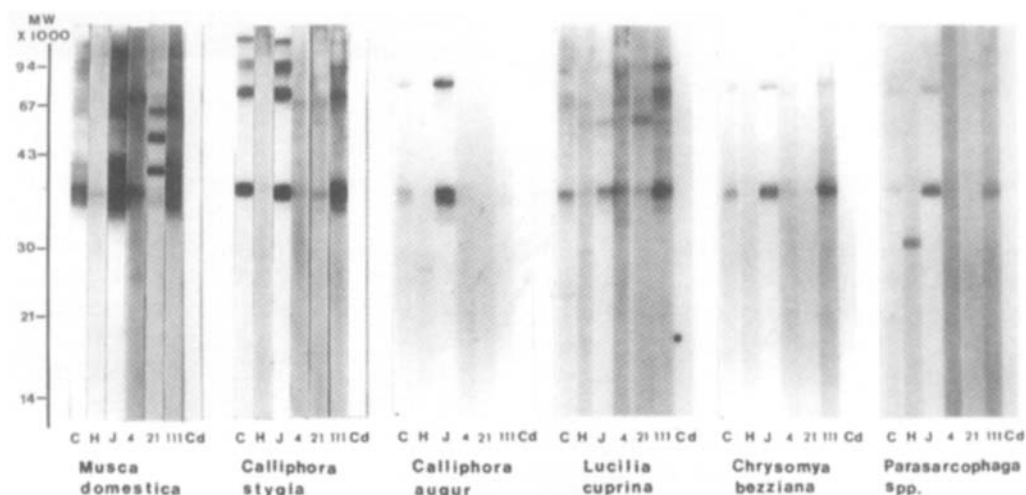


Figure 2 Identification by autoradiography of IgE binding bands transferred to NC following SDS-PAGE of the extracts of the Diptera (fly) species shown. Strips were incubated with sera from six subjects (C, H, J, 4, 21, 111) with inhalant allergy to arthropods (Cd being a control with cord serum) followed by ^{125} I-antihuman IgE. From BALDO, BA, PANZANI RC. Int Arch Allergy Appl Immunol 1988;**85**:278–287. Reproduced with the kind permission of Karger SA.

Because the world of insects and arthropods is almost limitless, with only 20% or less of this whole population having been identified, it seems to us important to isolate one or several panallergens in order to simplify the practical approach of the diagnosis and treatment of arthropod allergy.

A Cross-reactivity among arthropods and other invertebrates: the concept of panallergy

1 Relationship between arthropod antigens and storage or house dust mite (*Dermatophagoides pteronyssinus* and *Farinae*) antigens.

According to Van Hage Hamsten et al. (86), there is a lack of allergenic cross-reactivity between *Dermatophagoides pteronyssinus* and storage mite (*Lepidophyphus destructor*, *Glycyphagus domesticus*, *Tyrophagus putrescentiae*, and *Acarus siro*). However, keeping in mind the fact that mites belong to the class of Arachnids, one could expect to find a cross-reactivity between arthropods at large and mites. It seems that it is not the case as far as cockroach antigens are concerned. In a recent study, after a characterization and a partial purification of the cockroach antigens (*Periplaneta americana*, *Blattella germanica*, and *Blatta orientalis*), Kang et al. (87) showed using hyperimmune rabbit sera that crude cockroach extracts do not have an antigenic fraction that cross-reacts with house dust or with house dust mite. Kino and Oshima (88) used the RAST inhibition technique and have shown that there was no cross-reactivity between Lepidoptera (moths and butterflies) and the house mite *Dermatophagoides farinae*. A lack of cross-reactivity between different chironomid allergens and *Dermatophagoides farinae* has been reported by Ikegami et al. in Japan (89). From a clinical point of view, the majority (80%) of our patients allergic to mites are not allergic to other arthropods. Furthermore, in a group of 41 subjects allergic to arthropods, we found only 26 cases of RAST positive to *Dermatophagoides farinae*. Surprising as it is, this absence of cross-reactivity between mites and other arthropods has not yet received a clear explanation; a possible explanation lies in the different origins of the antigens, i.e. in the case of mites, faecal origins and, in the case of other arthropods, the whole body, and especially the integument. Studies with purified antigens of mites that are now available and purified antigens from the commonest arthropods using the RAST inhibition technique will provide a more precise answer. As a matter of fact, in the experiments we did, whole body extracts of different arthropods gave the stronger reactions by RAST, while a locust faecal antigen proved to be less allergenic; however, this aspect of the problem needs further research. We



Figure 3 Macarus monkey whose right check has been injected with serum of a patient allergic to different household insects. Subsequent intravenous challenge with an extract of triatoma associated with the dye Blue Evans shows a positive coloured reaction at the site of the injection of serum.

must recall here that, in 1969, Mitchell et al. (90) studied the relationship between house dust, mites, and insects, and concluded that cross-reactions between *Dermatophagoides farinae* and house dust, on the one hand, and spider and insects, on the other hand, are substantially less frequent than cross-reactions between the mites and house dust.

2 Relationship between inhalant arthropod allergy and allergy to stinging insects: hymenoptera, fleas, mosquitoes...

Numerous observations have been made of beekeepers with inhalant allergy to bee body components. Hypersensitivity to airborne bee allergen has been reported since 1932 (91) and, more recently, by Reisman et al. (92) and Ostrom et al. (93). In these publications, the patients demonstrated skin tests positive to honey-bee whole body extract and negative to honeybee venom. However, similar studies have not been carried out with wasps or other stinging insects. The possibility that the dead bodies of other stinging insects can become allergenic is discussed by Agarwal et al. (94) in India for the mosquito *Anopheles Stephensi* and by Trudeau et al. (95), in Florida for the cat flea *Ctenocephalides felis*. However, in both studies, on the one hand, no inhibition studies were undertaken to see whether there is a cross-reactivity between the mosquito, the flea, and other arthropods, such as house fly, cockroach, and spider; and, on the other hand, no skin tests to different arthropods have been performed. It is worth noting here that the major allergen of *Aedes aegypti* have been cloned and sequenced: Aed a 1, Aed a 2. In one case of respiratory allergy to lyophilized *Limulus polyphemus* (horse crab) amoebocyte lysate, Ebner et al. (96) found no cross-reactivity with

other related arthropod allergens (crab, shrimp, lobster and chironomids).

From a clinical point of view, we must add that patients who have an inhalant allergy to arthropods do not generally have a notable reaction to Hymenoptera stings. It seems that the antigens of the venoms from Hymenoptera (bees, wasps, and ants) and the antigens from the salivary secretions of Diptera (flies and mosquitoes) and Siphonaptera (fleas) are different from the antigens of the whole body.

3 Relationship between food allergy to crustaceans and airborne arthropod allergy

Food allergy to crustaceans is a well known phenomenon, the ingestion of this food causing urticaria or angioedema. In our experience, these patients very often do have skin tests positive to inhaled arthropods and, more especially, spiders. Clinically, in some cases, there is also a respiratory allergy. One of our rules is that a patient clinically allergic to crustaceans and to molluscs or gastropods and having a respiratory allergy is suspected of having an inhalant allergy to some arthropods. O'Neil et al. (97) found by the immunoelectrophoretic technique and RAST inhibition that cockroach antigens cross-react with those of several species of crustaceans.

4 Relationship between a specific inhaled or ingested allergy to molluscs and gastropods and a more generalized respiratory allergy to arthropods

People who have a food allergy to molluscs (snails, clams, and oysters) sometimes have positive skin tests and, sometimes, a respiratory allergy to arthropods. It has been shown by Clarke (98) that abalone could be an inhalant allergen causing asthma. In a study of hypersensitivity to larvae of chironomids, Eriksson et al. (99) found a cross-sensitization between chironomids, on the one hand, and crustaceans (shrimp, crab, and lobster) and molluscs (clam, oyster), on the other hand. Koshte et al. (100) found a cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. More recently, Crespo et al. (101) showed a cross-reactivity between boiled Atlantic shrimp and german cockroach. Terrestrial snails (*Helix* species) have been implicated by Vuitton et al. (102) as possible cross-allergens with house dust mites.

5 Relationship between worms used as fish bait and other arthropods

Finally, many subjects who have a respiratory allergy to sea invertebrates used as fish bait have positive skin tests to arthropods and, in some cases, a concomitant

respiratory allergy to arthropods. In fact, several of these sera showed the stronger reactions to other arthropods in our immunoblotting studies. To study the possible cross-reactivity between inhaled arthropods and crustaceans and seafood invertebrates, some of the sera that were highly reactive with insect extracts were employed in protein blotting studies with extracts of an arachnid, a crustacean, a mollusc, and a gastropod. With prawn, sea snail, and squid extracts up to 15 IgE-binding bands were detected by some of the sera, indicating the probable existence of cross-reacting allergenic components in the different arthropods and invertebrates (83).

6 Cross-reactivity between arthropods: the concept of Pan-allergy

In 1958, Pruzansky et al. (103) reported that there was one antigen common to a fly (*Phormia regina*), cockroaches, and crickets, another to the same fly and cockroaches, and a third to crickets and cockroaches as studied by tanned cell haemagglutination, haemagglutination inhibition, and agar gel diffusion. A few years later (1963), Langlois et al. (104) used the same techniques and showed a common antigenicity between a cricket (*Acheta assimilis*), a grasshopper (*Melanoplus bivittatus*), a moth (*Phlegathontius quinquemaculata*), a butterfly (*Danaus plexippus*), and the caddis fly. It is worth noting here that Pollart et al. (105) stated in one of their papers on cockroach allergy (Bla g 2): "Additionally, sampling of homes without visual evidence of cockroach infestation found that significant levels of cockroach allergen could be found in 20% of homes in which there was no visual evidence of cockroaches observed by the inhabitants and the investigators". In our opinion, a simple explanation is that Bla g 2 cross-reacted with other household insects present in these homes.

In a significant fraction (30%) of patients with respiratory allergy to household insects, we found positive skin tests to all the arthropod extracts available and even with extracts of insects from another continent, as we have demonstrated with *Triatoma protacta* (USA) and with *Agrostis infusa* (Australia). Similarly, in our RAST experiments, the IgE reacted with a wide range of both closely related and poorly related arthropod species in one-third of the cases. In a recent work, Kaufman et al. (106) have found positive skin tests to sheep blowfly (*Lucilia cuprina*) in 83% of the Sydney patients. This high rate of sensitization might reflect cross-reactivity between the insect species more often encountered in the urban environment. This lack of species specificity is of interest in the diagnosis and specific treatment of arthropod allergy.

Because an individual may be exposed to a large number of different insects in different places, i.e. at home, in workplace, or outdoors, it is difficult to determine whether a patient has multiple sensitivities, because of multiple insect exposures or because of widespread insect cross-reactivity. Of course, both these mechanisms may sometimes be operative; cross-reactions and separate sensitization may occur in the same subject. However, the first of the two explanations seems more likely. There is probably a "primary allergen" that has a special and particular molecular structure, and which opens the way to minor or secondary allergens by epitope spreading. This hypothesis will partly explain the concept of panallergy.

Taken together, our clinical and immunological findings show that allergy to one species of arthropods is rather uncommon; that cross-reactivity is the rule; and that, in one third of clinical cases, this cross-reactivity extends to all the arthropods and even to some invertebrates, leading us to the concept of panallergy.

Recently, Lehrer et al. (107) described a cross-reactivity between cockroach, arthropods, nematodes, and mammalian allergens, but we found no indication of these mammalian allergens. Obviously, a cross-reactivity between invertebrate and mammalian allergens seems to us unlikely.

Furthermore, in a recent paper, Aguinado et al. (108) pointed in our direction. As the authors say in their conclusion:

"These studies provide evidence that the nematodes are not primitive metazoans but are protostomes related to arthropods. They also support a monophyletic protostome clade. Considering the greatly differing morphologies, embryological features and life histories of the moulting animals, it was initially surprising that the ribosomal RNA tree should group them together. However, given that all moulting taxa sampled are in this clade, and given the significant anatomical modifications associated with moulting, such as the lack of locomotory cilia, ecdysis appears to be a defining synapomorphy for this group, although additional molecular data from other molecules are necessary to test further or confirm the monophyly of the moulting animals."

In summary, in a poster entitled "Does panallergy to insects exist?", a high proportion of sera showed serum IgE antibody responses to more than one insect species. Reactions, especially to flies, mosquitoes, and moths, were strong and the IgE antibodies reacted with a wide range of both closely related and poorly related insect species in the phylum of arthropods and

of nonfish seafoods (crustaceans, mollusks, gastropods) (83).

B Tropomyosin as a panallergen in invertebrate allergy

In a paper published in 1988 (12), we found that a 36–38 kDa protein was the prominent panallergen within arthropods. IgE reacting bands of 36–40 kDa identified later as tropomyosin have been found in extracts from organisms such as crustaceans, insects, and molluscs, but not in vertebrates (chicken or mouse).

Recently, the actine binding protein tropomyosin has been identified as a relevant allergen in extracts from arthropods and invertebrates. Shanti et al. (109) isolated from shrimp *Penaeus indicus* a major allergen of 34 kDa, which is a tropomyosin and shows a high degree of cross-reactivity with tropomyosin from related crustaceans and the fruit fly *Drosophila melanogaster*.

At the same time, Daul et al. (110) detected the presence of a 36 kDa allergen in 82% of sera from patients allergic to shrimp *Penaeus aztecus*. This allergen named Pen a 1 was identified as a tropomyosin which also has great homology with different arthropods and, in particular, with the fruit fly. In the same manner, Witteman et al. applied RAST inhibition assays using sera from shrimp-allergic patients and a monoclonal antibody against *Dermatophagoides pteronyssinus* tropomyosin and found that there is a cross-reactive allergen, presumably tropomyosin, in shrimp, mites, and insects. They also found that IgE antibody reactive with silverfish, cockroach, and chironomid is frequently found in mite-positive allergic patients (111).

As reported more recently by Leung et al. (112), another shrimp tropomyosin termed Met e 1 (34 kDa), from the shrimp *Metapenaeus ensis* has been cloned, expressed, and sequenced. Aki et al. (113) described the presence of tropomyosin (37 kDa) as a major allergen in the house dust mite *Dermatophagoides farinae*, which has a frequency comparable with that of Der f 1 and Der f 2. Finally, the cross-reactivity to tropomyosin allergens has been extended to invertebrates such as crustacea and mollusca by Leung et al. (114), and to squid (*Todarodes pacificus*) by Miyazawa et al. in 1996 (115). In 1993, Lin et al. (116) identified and characterized a 30 kDa major allergen from the shrimp *Parapenaeus fissurus*, i.e. Par f 1, which cross-reacts with a crab 39 kDa allergen and is probably a tropomyosin, as are also Hom a 1 (*Homarus americanus*) and Pan s 1 (*Panilurus stimpsoni*) (117). More recently, Leung et al. isolated from the

common crab, *Charybdis feriatus*, a 34 kDa protein Cha f 1 which is a tropomyosin (118).

In our studies with Martinez (119), examining 100 sera from patients exhibiting positive skin tests with one or several household insects, and using the three extracts house fly (*Mosca domestica*), moth (*Ephestia* spp.), and spider (*Tegenaria domestica*), we selected the 29 most reactive to perform immunoblotting (Table 8). As can be seen from Fig. 4, the higher frequency and intensity of IgE binding was observed within the 34–38 kDa molecular weight interval in the three extracts; results which correspond to tropomyosin. In this study, we used the following extracts:

- 1 Insects: *Dactylopius coccus* (cochineal); *Periplaneta americana* (american cockroach); *Blatta orientalis*, *Blattella germanica* (german cockroach); *Apis mellifera* (bee); *Vespula* spp. (wasp); *Culex pipiens* (mosquito); *Crematogaster* spp. (black ant);
- 2 Mites: *Dermatophagoides pteronissinus*; *Dermatophagoides farinae*; *Euroglyphus maynei*; *Lepidoglyphus destructor*; *Blomia kulagini*; *Tetranychus urticae*;
- 3 Seafood:
 - (a) crustaceans – *Parapenaeus longirostris* (prawn), *Hommarus gammarus* (bobster), *Cancepagurus* (cancer crab), *Maia squinado* (spider crab), *Macropipus puber* (swimming crab), *Nephrops norvegicus* (norway lobster);
 - (b) molluscs/gastropods – *Helix aspersa* (snail), *Euparipha pisana* (sea snail), *Mytilus edulis* (mussel), *Ruditapes* spp. (clam), *Ostrea edulis* (oyster), *Octopus vulgaris* (octopus), *Tadarodes pacificus* (squid), *Sepia officinalis* (cuttle fish);
 - (c) parasites – *Ascaris suum*, *Anisakis simplex*.

Cross-reactivity within other arthropod and invertebrate extracts was evaluated by immunoblotting incubating the different extracts in parallel with the serum pool of household insect allergic patients and with the commercial polyclonal antiserum antichicken tropomyosin.

Although several cross-reactive allergens could be detected in the immunoblotting of mite extracts revealed with the serum pool of household arthropod allergic patients (Fig. 5), the presence of tropomyosin as one of the allergens involved could only be demonstrated through its immunoglobulin binding capacity, in the extract of *Tetranychus urticae*, despite the documented presence of this important allergen at least in mites of *Dermatophagoides* genus.

Crustacean extracts showed a large number of cross-reactive allergens revealed with the human

serum pool, with well-represented tropomyosin molecules, as judged by the intense IgE and even IgG binding of this molecules (Fig. 6). Although exhibiting some molecular weight differences (37–41 kDa), depending on the crustacean species analysed, in all cases tropomyosin appeared as the most important cross-reactive allergen, except in the crab (*Cancepagurus*), where tropomyosin could not be detected at all.

Then, molluscs from the classes Gasteropoda (*Helix aspersa*), Bivalvia (*Mytilus edulis*, *Ruditapes* spp., *Ostrea edulis*), and Cephalopoda (*Octopus vulgaris*, *Tadarodes pacificus*, *Sepia officinalis*) were assayed. Important cross-reactive allergens (IgE binding bands) were found in the range 39–42 kDa, but their identification as tropomyosins through IgG binding only could be demonstrated for snail (*Helix aspersa*) and octopus (*Octopus vulgaris*) extracts (Fig. 7).

Two parasites, the nematodes *Anisakis simplex* and *Ascaris suis*, that could be involved in cases of food allergy by fish and meat ingestion were also analysed. Both extracts showed several cross-reactive allergens (Fig. 8) and, among them, the presence of tropomyosin was also evidenced, exhibiting a molecular weight of 42 kDa in the *Anisakis* extract and two variants of 42 and 38 kDa in the *Ascaris* extract. It is worth noting here that, as far as invertebrates are concerned, two allergens have been purified and sequenced: Abalone (120) and the major of allergen of *Ascaris Ani s 1* (121,122). *Ani s 1* showed no homology to previous described proteins, although *Anisakis simplex* is known to produce allergens similar to the ABA 1 of *Ascaris*, which belongs to the fatty acid and vitamin A-binding proteins (123). In addition, there is an antigenic relationship between *Anisakis simplex* and *Ascaris suum*, *Ascaris lumbricoides* and the dog parasite *Taxocara canis* (124).

It can be concluded that tropomyosin is a relevant panallergen in household arthropods, being also present in many other invertebrates: mites, crustaceans, molluscs, and nematodes (125).

Finally, in 1999, Asturias et al. (126), using part of the same sera we used in the previous experiments, characterized the american cockroach tropomyosin (*Periplaneta americana* allergen 7) as a cross-reactive allergen. Cross-reactivity between

Table 8 RAST on 95 patients using extracts of house fly, spider and *Ephestia*

	House fly class			Spider class			<i>Ephestia</i> class		
	1	2	3	1	2	3	1	2	3
	16	15	6	7	15	5	12	15	0
Total	37 (40%)			27 (30%)			27 (30%)		
<2	21			20			15		

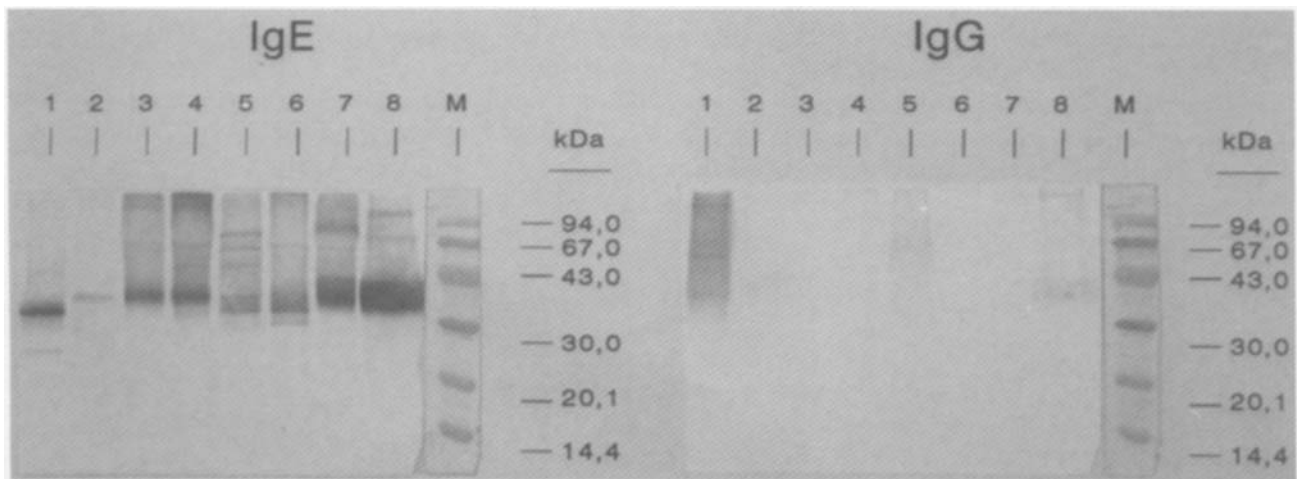


Figure 4 SDS-PAGE IgE and IgG immunoblottings of insect extracts: (1) *Dactylopius coccus*, (2) *Periplaneta americana*, (3) *Blatta orientalis*, (4) *Blattella germanica*, (5) *Apis mellifera*, (6) *Vespula* spp., (7) *Culex pipiens* and (8) *Crematogaster* spp. IgE immunoblotting was revealed with the human serum pool from 15 patients allergic to household arthropods. IgG immunoblotting was revealed with the polyclonal antiserum antichicken tropomyosin. From MARTINEZ A, MARTINEZ J, PALACIOS R, PANZANI RC. Allergol Immunopathol 1997;25(3):118–126. Reproduced with the kind permission of Editorial Garsi SA (Spain).

cockroaches has been reported, but analyses using cloned or purified cockroaches allergens have shown that only Per a 1 and Bla g 1 share significant homology; *Blattella germanica* allergens Bla g 2, Bla g 4, and Bla g 5 are not present in *Periplaneta americana* extracts, and Per a 3 from *Periplaneta americana* is not detected in *Blattella germanica*. The Western blotting experiments in this study showed that Per a 7 is the second cross-reacting allergen described in cockroaches, because it is present in the

two cockroaches species tested, *Blattella germanica* and *Blatta orientalis*.

From the experiments reported here, it appears that tropomyosin is an important panallergen among arthropods and invertebrates. However, because the homology between the different extracts used is not perfect and even, in some cases, is nil, it may be assumed that other panallergens are to be found. The first panallergen seems to us to be paramyosin. Similarly to tropomyosin, paramyosin is a protein

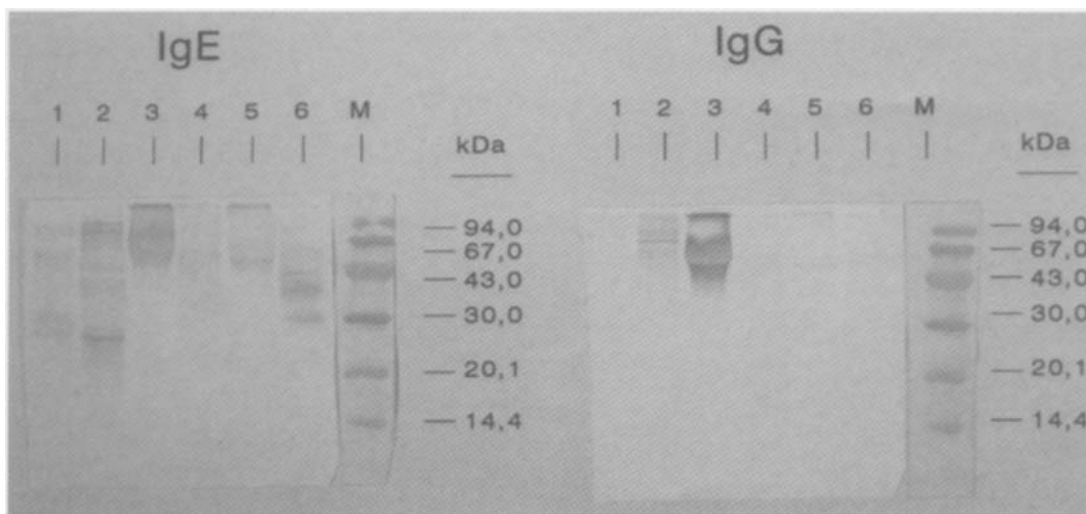


Figure 5 SDS-PAGE IgE and IgG immunoblottings of mite extracts: (1) *Dermatophagoides pteronyssinus*, (2) *Dermatophagoides farinae*, (3) *Euroglyphus maynei*, (4) *Lepidoglyphus destructor*, (5) *Blomia kulagini*, and (6) *Tetranychus urticae*. From MARTINEZ A, MARTINEZ J, PALACIOS R, PANZANI RC. Allergol Immunopathol 1997;25(3):118–126. Reproduced with the kind permission of Editorial Garsi SA (Spain).

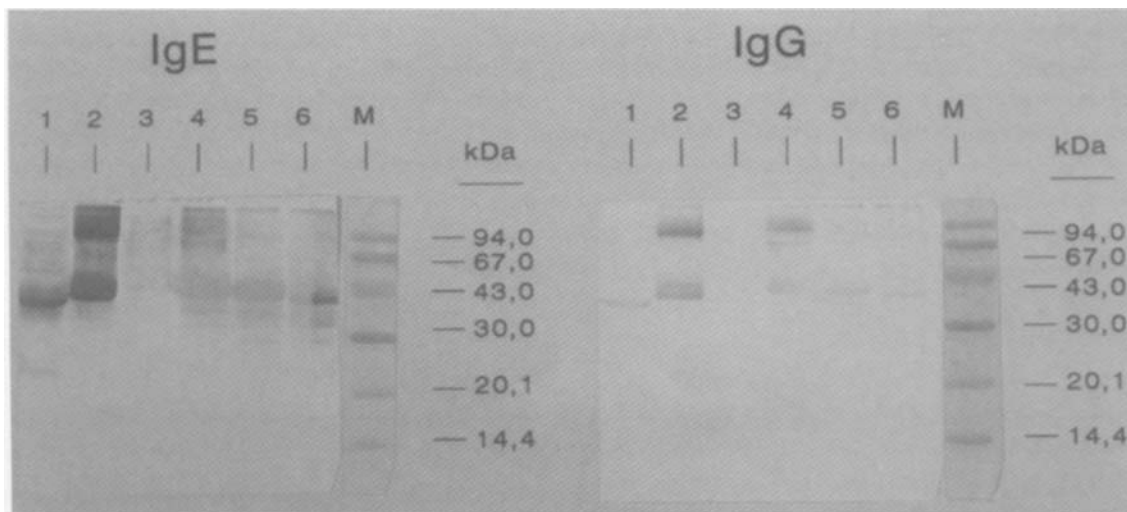


Figure 6 SDS-PAGE IgE and IgG immunoblottings of crustacean extracts: (1) prawn, (2) lobster, (3) cancer crab, (4) spider crab, (5) swimming crab, and (6) norway lobster. From MARTINEZ A, MARTINEZ J, PALACIOS R, PANZANI RC. Allergol Immunopathol 1997;25(3):118–126. Reproduced with the kind permission of Editorial Garsi SA (Spain).

associated with muscles but, unlike tropomyosin, paramyosin is found only in invertebrates. Up to now, paramyosin has mostly been found in mites (127) and parasites: filariasis, taenia and schistosomiasis. In mites, paramyosin is very similar to other known paramyosins. Paramyosin being an invertebrate panallergy, it was not surprising that Fernandez-Caldas et al. described the primary structure of a paramyosin of *Anisakis simplex*. The frequency of specific IgE binding to the native form of paramyosin

using the sera of 26 *Anisakis simplex* sensitive individuals was 88%. They proposed the term Anis s 2 for this new allergen (128).

C Possible role of carbohydrate cross-reactive determinants as panallergens

Evidence for the role of cross-reactive determinants (CCD), which are common among plants, is very

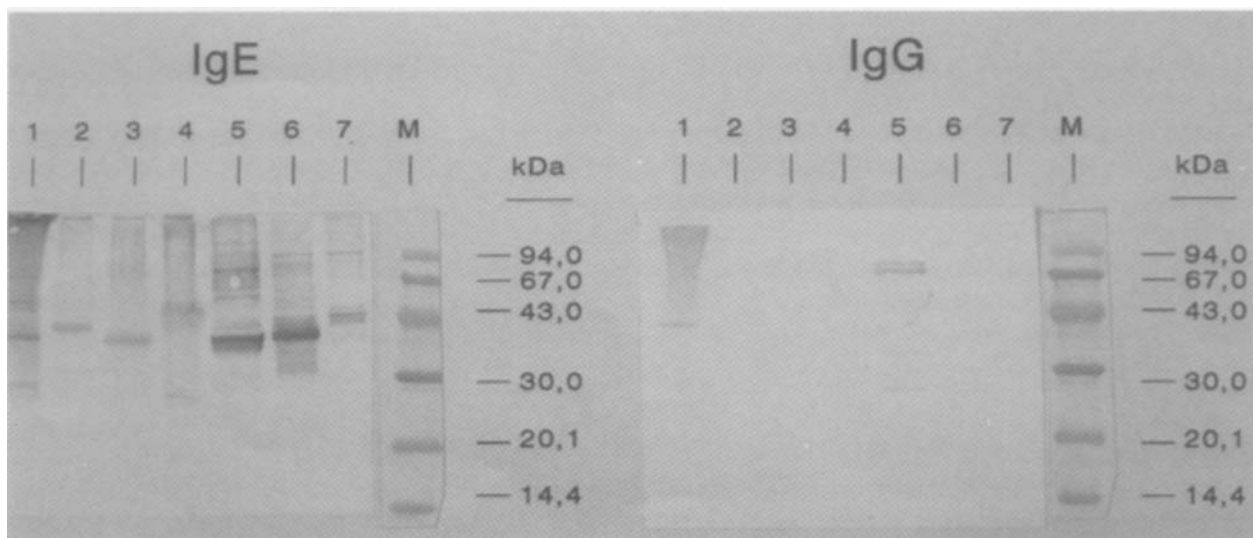


Figure 7 SDS-PAGE IgE and IgG immunoblotting of mollusc extracts: (1) snail, (2) mussel, (3) clam, (4) oyster, (5) octopus, (6) squid, and (7) cuttlefish. From MARTINEZ A, MARTINEZ J, PALACIOS R, PANZANI RC. Allergol Immunopathol 1997;25(3):118–126. Reproduced with the kind permission of Editorial Garsi SA (Spain).

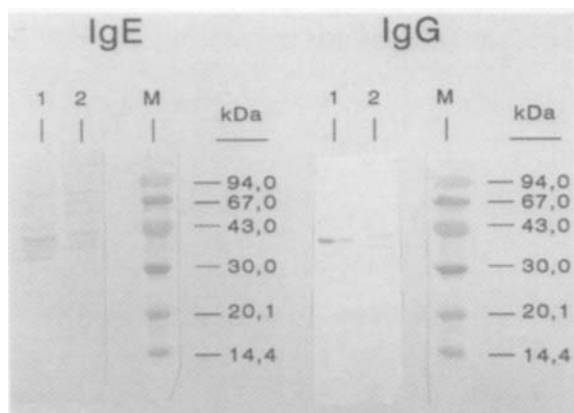


Figure 8 SDS-PAGE IgE and IgG immunoblotting of parasite extracts: (1) *Anisakis simplex* and (2) *Ascaris suis*. From MARTINEZ A, MARTINEZ J, PALACIOS R, PANZANI RC. Allergol Immunopathol 1997;25(3):118-126. Reproduced with the kind permission of Editorial Garsi SA (Spain).

scarce in invertebrate allergy if we except honey bee phospholipase A2. This study is beyond the scope of our study. However, Koshte et al. (100) studied caddis fly allergy and raised this possibility; furthermore, in sea squirt allergy (14), Oka et al. have shown that, among the three allergens identified, one of the epitopes termed Gi-rep resides in carbohydrate chains (129). Then Shigeta et al., working with the same group, suggested that an allergenic pentasaccharitol Gp-1B-b6 isolated from Gi-rep is a minimum structural unit responsible for its allergenicity (130).

Faye and Chrispeels think that O-linked as well as N-linked glycans, which modify the physicochemical properties of polypeptides, are common antigenic determinants in the glycoproteins of plants, molluscs and insects (131).

It seems to us that, by analogy with plants, the possible role of CCD, besides other already known panallergens, is worth studying in a larger scale.

Conclusion and prospects for the future

Arthropod inhalant allergy seems to us an important and underestimated cause of respiratory IgE mediated allergy (mostly asthma and sometimes allergic rhinitis) across the world. Spiders, cockroaches, house flies, and moths are the commonest insects found. This allergy is more frequent in North Africa than in the south of France or in Italy, and is much more common in subtropical and tropical areas. Very often, the sensitization extends to all arthropods (insects, arachnids, and crustaceans) and sometimes also to some invertebrates, producing a "panallergy".

In order to clarify this topic the following points must be solved.

Arthropods and invertebrates allergy

- 1 First is identification around the world – as has already been done with pollens, moulds and mites – of the most important outdoor and indoor arthropods, with the indoor arthropods probably being much more important than the outdoor arthropods.
- 2 Second, assess the reality of this clinical entity by careful clinical history, skin test, RAST, and nasal or bronchial provocation tests.
- 3 Next, we have characterization and purification of the antigens of the commonest arthropods: spider, house fly, silverfish, ephestia, moth (in the case of cockroaches, it has already been done). However, we must keep in mind the concept of panallergy, which could probably simplify specific immunotherapy (SIT) if there is a primary common antigen, such as tropomyosin, paramyosin or even CCD. SIT with the three sea squirt purified allergens has already been carried out in 1998 by Jyo et al. (132). They showed that one of the allergens named DIII a had a lower efficacy of only 36% compared with 72% and 90% for the two others. (This is only an example of SIT with purified allergens in arthropod allergy, because SIT is beyond the scope of this study.)
- 4 Fourth, specify the relationship between these antigens and the antigens already purified of mites (house dust and storage mites) by RAST inhibition studies.
- 5 Finally, human IgE mediated household insects allergy could probably throw light on the taxonomy of arthropods and of all invertebrates.

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Addendum

After the acceptance of our review we found four recent papers related to our topic. The title of the first was promising "Allergen cross-reactivity between house dust mites and other invertebrates" (133). However, this paper is a review and does not show further evidence of cross-reactivity between house dust mites and other arthropods or invertebrates.

More interesting is a paper from Japan by Onizuca et al. (134), in which they described another professional respiratory allergy among fishermen during the fishing season of spiny lobster, which was caused by a marine coelenterate, red soft coral (RSC), *Dendronephthya nipponica*, which is caught together with the spiny lobsters. They have achieved the

purification of the major allergen of RSC: Den n 1. Another red soft coral, *Alcyonium gracillimum*, has also been described and purified by the same authors (135).

The third paper is concerned with the "cDNA cloning and molecular identification of the major allergen from the Pacific oyster *Crassostrea gigas*"; this allergen is designated as Cra g 1.03 by Leung and Chu (136), and is the oyster tropomyosin. However, Ishikawa et al. (137), who have purified the two allergens Crag g 1 and Crag g 2 from the shucked

oyster *Crassostrea gigas* have previously identified Crag g 1 as a tropomyosin (138). After having isolated the major allergen of the gastropod *Turbo cornutus*, Tur c 1, as being a tropomyosin, the same authors suggested that this IgE binding epitope is different from Cra g 1 and Pen I 1, the shrimp *Penaeus indicus* (139).

Finally, we must quote an important publication from India in which *Psocoptera* spp. (book louse) is described by Niphadkar et al. as a major household allergen in Mumbai (140).

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¹Note: People interested in having a complete bibliography of household insect allergy should contact: Dr T.E. Bellas, entomologist, Camberra (Australia) on the following web site: <http://www.ento.csiro.au/inhalant/allergy-biblio.html>

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