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ORIGINAL ARTICLES

INSECT ALLERGENS*: THEIR INTERRELATIONSHIP AND DIFFERENCES

A Comparison of Skin Reactivity and a Proposal for Routine Skin Testing
With Insect Allergens

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WITH the broader acceptance of insects as inhalant allergens^{1, 2} the problem has arisen of reducing the variety of testing material to workable and practical numbers for routine clinical studies. It must of course be solved without serious omissions or unnecessary duplications.

Arthropods are the most numerous group of animals in the world. The number of described species is approximately 80 per cent of all animals. Biologic classification is invariably complex. It is often controversial and sometimes arbitrary, but it is an attempt at orderliness. With insects it appears to be a never ending problem. Many changes have occurred through the years in the orders and families, but, at the level of genus and specie, changes and additions are continually occurring. This presentation proposes a practical path through the apparent entomological maze confronting most allergists. Conclusions and recommendations will be drawn from clinical observations correlated with basic entomological principles.

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*Common usage has caused the term "insect" to include most of the arthropods. This inexact terminology will be used throughout the discussion. It will conform with the broad title of the Insect Allergen Committee of the Research Committee of the American Academy of Allergy. When the class of true insects is singled out, the term "Hexapoda" will be used.

True insects (Class Hexapoda) are a class of the Phylum Arthropoda as indicated in Table I, and it is around this class that the major part of this discussion centers. According to Sabrosky,³ at the United States National

TABLE I. PHYLUM ARTHROPODA⁴
(FIVE MAJOR CLASSES)

Class Onychophora	(peripatus)
Class Myriapoda	(millipedes, centipedes, symphilides, etc.)
Class Crustacea	(crabs, shrimp, sowbugs, daphnia, etc.)
Class Arachnida	(spiders, mites, ticks, scorpions)
Class Insecta (Hexapoda)	(true insects)
Subclass Apterygota (wingless insects)	(silverfish, springtails)
Subclass Pterygota (winged insects)	(most common insects)

Museum, the number of species throughout the world has been variously estimated at from 625,000 to 1,500,000. In North America the last figure was 82,000. These fall, according to Essig⁴ of The Department of Entomology of The University of California, into 33 orders and are further divided into 839 families, as seen in Table II. Six of these 33 orders embrace 96 per cent of the known species which surround us and whose distribution is world-wide. It therefore becomes evident that the list of insects to arouse clinical concern may be quickly narrowed.

TABLE II. ORDERS OF TRUE INSECTS^{3, 4}
(SIX OF THESE EMBRACE 96 PER CENT OF DESCRIBED SPECIES)

ORDER	COMMON NAMES	FAMILIES (APPROX. NO.)	PER CENT OF SPECIES	NUMBER OF SPECIES	
				WORLD- WIDE	NORTH AMERICA
Coleoptera	beetles, weevils	154	40	277,000	26,076
Lepidoptera	moths, butterflies	138	17	112,000	16,300
Hymenoptera	bees, wasps, ants	151	15	103,000	14,528
Diptera	flies, mosquitoes	144	13	86,100	16,938
Hemiptera	true bugs, aphids, scales, cicadas	72	8	55,000	8,742
Heteroptera					
Homoptera					
Orthoptera	grasshoppers, cock- roaches, etc.	21	3	23,600	1,033
Minor orders		159	4	28,200	3,865
		839	100	684,900	87,482

These insects vary in numbers on a geographical and seasonal basis. It is not difficult to gain knowledge of the appearance of certain insects in large numbers in an area at a specific period. Regional entomologists can often anticipate such occurrences. If clinical symptoms were to occur at such times (as we witness with pollens or even with such insects as caddis fly and May fly^{5, 6, 7}), then current entomological information would be most valuable. There is as yet no well-documented evidence of the occurrence of hay fever and asthma in relation to the appearance of large numbers of insects other than the caddis fly and the May fly. However, in this discussion it is presumed

that those remaining insects not immediately implicated may become, over a period of time, inhalant allergens in the house and outdoor dust as part of the debris contaminating the air we breathe. Without a reliable technique for detecting such insect debris by air sampling (as can be done for pollens) it becomes necessary to accept the ubiquitous nature of certain insects together with positive skin tests and the clinical picture to correlate insect allergens to symptoms. Such presumptions have led to the establishment of a routine in our clinical studies.

The search for common excitants to embrace a larger group of potential allergens must begin at some level; it may broaden or narrow on the basis of clinical experiences. The use of biologic classification for establishing common allergenicity among insects may be fortuitous. The laborious task to find common allergenicity through such immunologic methods as gel diffusion studies would still fail to provide acceptable clinical inferences. The specific skin reaction is a more sound basis for presuming their potential or actual clinical importance as inhalant allergens. The additional search for relationship through neutralization or exhaustion studies with related insects add interest but interpretations of such studies have their shortcomings.⁸

METHODS

Our studies and conclusions were concerned with insects as inhalant allergens. We presumed that their allergenic quality could be detected by positive skin reactions and the presence of skin sensitizing antibodies in the patient. The phenomena associated with the bite or sting of certain insects are not included in this presentation since there are no consistently demonstrable antibodies of the reagin type in that form of insect allergy. The various insect material was used as it became available. Uniformity was sought by making all extracts in the same method from the live or frozen state. The final dried, powdered material after lyophilization and defatting was stored under desiccation until used. Aqueous extracts for testing were made from the stable powder using 50 per cent glycerinated buffered solution in a 1:10 dilution by weight volume. After passing through a sterilizing Seitz filter and with storage under ordinary refrigeration these extracts were presumed to remain stable and actively allergenic throughout our studies.^{9, 10} There was no significant nonspecific irritative reaction to any of these extracts.

Most of the various insects used in these studies are listed in Table III. Division was reduced to the level of family. To avoid making the tables cumbersome, only a few of the families have been listed. For example, in the order Coleoptera (beetles) and in the order Lepidoptera (moths) more than a dozen families were tried in each and showed similar but varying degrees of skin reactions. Sometimes a number of representatives of such a family available in small amounts were combined in order to supply an adequate quantity of material for extracting. In most instances a single representative of the family was used and is so indicated.

TABLE III. INSECT MATERIAL USED IN THESE STUDIES

ORDERS	FAMILIES	COMMON NAMES
Class Hexapoda (Insecta)		
1. Ephemeroptera	—	May flies
2. Orthoptera	[Acrididae	grasshoppers
3. Isoptera	Blattidae	cockroaches
4. Dermaptera	—	termites
5. Hemiptera	—	earwigs
6. Homoptera	Coreidae	box-elder bugs, also 6 minor families
7. Coleoptera	[Cicadellidae	leafhoppers, aphids
8. Neuroptera	Aphididae	
9. Trichoptera	[Dermestidae	carpet beetles, June
10. Lepidoptera	Scarabaeidae	beetles, ground beetles,
11. Diptera	Carabidae	also 12 minor families
12. Siphonaptera	Sialidae	lacewings
13. Hymenoptera	—	caddis flies
14. Class Arachnida	[Tineidae	clothes moths
15. 1. Scorpionida	Noctuidae	noctuid moths
16. 2. Aranea	Bombycidae	silkworm moths,
17. 3. Acarina		also 10 minor families
18. Class Crustacea	[Muscidae	houseflies
19. 1. Decapoda	Culicidae	mosquitoes
20. 2. Copepoda	Cecidomyiidae	midges
21. 3. Branchiopoda	Tabanidae	deer flies
22. 4. Isopoda	Simuliidae	black flies,
		also 4 minor families
	Dolichopsyllidae	fleas (rat)
	[Apidae	honeybees
	Bombidae	bumblebees
	Vespidae	wasps, hornets, yellow jackets
	[Formicidae	ants (black)
		scorpions
		spiders
		tick of Rocky Mt.
		spotted fever
		lobster, crab, shrimp
		plankton shrimp
		daphnia, fairy shrimp
		sowbugs

Over a period of 2 years, 728 patients were tested with various insect extracts that were on hand. The expanding list of materials slowly gathered made statistical analysis of very limited value. It soon became apparent, however, that a recurrent pattern of relationship and differences in skin reactivity to the insects existed. This was pursued further by re-testing patients chosen because of previous strong skin reactions as well as because of the presence in their sera of skin sensitizing antibodies. Approximately the same degree of whealing reaction was repeatedly elicited among families within an order.

OBSERVATIONS

The following most consistent and significant observations are offered. Random cases with skin reactions exemplify these observations in the accompanying tables.

1. Multiple reactions occurred in a number of patients to most of the insect extracts used. Where such multiple reactions occurred, the least reactive insects were in the isolated orders or in orders not abundantly present in the patient's environment—Isoptera (termites), Dermaptera (earwigs), Ephemeroptera (May fly), Trichoptera (caddis fly).

2. Among patients with multiple reactions most of these occurred within a single class. This sometimes fell within the class Hexapoda and at other times within the class Arachnida, or the class Crustacea (Table IV).

TABLE IV. REACTIONS TO SEVERAL ORDERS WITHIN A CLASS

PATIENT	CLASS	ORDER	COMMON NAME	REACTION
W. B.	Arachnida	Aranea	spider	++++
		Scorpionida	spider web scorpion	++++ ++++
	Hexapoda	Lepidoptera	moth	—
		Diptera	mosquito	—
		Hymenoptera	honeybee	—
R. K.	Crustacea	Isopoda	sowbug	++++
		Branchiopoda	daphnia	++++
		Decapoda	crab	++++
		Copepoda	shrimp (plankton)	+++
			shrimp eggs	—

3. Periodically isolated or single reactions within a class or even within a single order occurred (Table V).

TABLE V. PERIODIC ISOLATED REACTION OF A SINGLE ORDER WITHIN A CLASS

PATIENT	CLASS	ORDER	COMMON NAME	REACTION
A. S.	Hexapoda	Orthoptera	grasshopper	±
		Homoptera	leafhopper	±
		Coleoptera	June beetle	—
		Lepidoptera	moth	±
		Diptera	mosquito	—
		Hymenoptera	honeybee	++++
C. P.	Hexapoda	Orthoptera	grasshopper	±
		Isoptera	termite	—
		Dermaptera	earwig	+++
		Coleoptera	June beetle	—
		Diptera	housefly	—
P. B.	Hexapoda	Isoptera	termite	+++
		Hymenoptera	ant	±
		Lepidoptera	moth	±

4. Those patients reacting to a single order of insects within a class reacted to some degree to all of the families represented. Variation in degree of reaction to various families may have been in part the result of variation in allergenicity of the extract used, or possibly the presence of other allergens unrelated to the insect itself, as might be the case with the honeybee (Table VI).

TABLE VI. REACTIONS OF REPRESENTATIVE FAMILIES WITHIN EACH ORDER

PATIENT	CLASS	FAMILY	COMMON NAME	REACTION
G. D. M.	Hymenoptera	Apidae	honeybee	++++
		Vespidae	hornet	+++
		Vespidae	yellow jacket	++++
		Noctuidae	moth	—
T. L.	Lepidoptera	Cicadelladae	leafhopper	+++
		Aphididae	aphid	+++
	Dermoptera		earwig	—
	Coleoptera		June beetle	—
	Trichoptera		caddis fly	—
L. E.	Homoptera	Aphididae	aphid	++++
		Cicadelladae	leafhopper	++++
	Orthoptera	Blattidae	cockroach	±
		Acerididae	grasshopper	±
	Diptera	Muscidae	housefly	+++
		Culicidae	mosquito	++++
		Cecidomyiidae	midge	++++
		Tabanidae	deer fly	+++
	Diptera	Muscidae	housefly	++
		Culicidae	mosquito	+++
		Tabanidae	deer fly	+++
		Simuliidae	black fly	++
			moth	±
			May fly	—
G. L.	Diptera		caddis fly	—
			leafhopper	—
	Lepidoptera			
	Ephemeroptera			
P. R.	Trichoptera			
	Homoptera			
	Orthoptera	Acerididae	grasshopper	++++
		Blattidae	cockroach	++++
			moth	++
	Lepidoptera		mosquito	++
			earwig	—
	Diptera		wasps	—

5. Reaction to one or two families and with less or no reactions to others within the same order occurred only in a few cases (Table VII).

6. Comparable anatomic structures and secretory products (silk, venom, etc.) showed no consistent allergenic interrelationship between classes.

TABLE VII. OCCASIONAL REACTIONS TO ONE OR TWO FAMILIES AND NOT TO ALL WITHIN AN ORDER

PATIENT	ORDER	FAMILY	COMMON NAME	REACTION
D. K.	Diptera	Muscidae	housefly	++++
		Culicidae	mosquito	±
		Cecidomyiidae	midge	++
		Tabanidae	deer fly	+++
A. P.	Diptera	Muscidae	housefly	+++
		Culicidae	mosquito	+++
		Cecidomyiidae	midge	—
		Tabanidae	deer fly	+
W. W.	Hymenoptera	Apidae	honeybee (worker)	++++
		Vespidae	hornet	—
			yellow jacket	±
		Formicidae	ant	—
		Apidae	honeybee (drone)	++++
		Bombidae	bumblebee	+++

7. Unusual observation continually occurred as exemplified by (a) reaction to silk and not to moth and vice versa,² (b) reaction to shrimp egg and not to the hatched plankton (brine) shrimp from the same lot of eggs, and vice versa, and (c) reaction to spider and not to spider web (Table VIII).

TABLE VIII. REACTIONS TO ANATOMIC STRUCTURES AND SECRETORY PRODUCTS

PATIENT	CLASS	ORDER	COMMON NAME	REACTION
W. B.	Hexapoda	Lepidoptera	silkworm silk	-
	Arachnida	Aranea	spider web	++++
	Hexapoda	Lepidoptera	caterpillar web	±
D. T.	Arachnida	[Aranea	[spider	++++
			spider web	+++
		Scorpionida	scorpion	++
	Hexapoda	Diptera	housefly	-
		Lepidoptera	moth	-
		Coleoptera	beetle	+
D. K.	Arachnida	Aranea	[spider	++++
			spider web	±
		Scorpionida	scorpion	++++
	Hexapoda	Lepidoptera	[moth	+++
			caterpillar	+++
			caterpillar web	-
M. B.	Hexapoda	Lepidoptera	[silk	++++
			silk moth	-
G. R.	Crustacea	Isopoda	sowbug	-
		Branchiopoda	daphnia	-
		Copepoda	[shrimp (plankton)	-
			shrimp eggs	+++

8. Exhaustion tests with serum from several extremely sensitive patients suggested differences in allergenicity between orders. These demonstrations of exhaustion were fraught with contradiction.⁸ Some observations with exhaustion tests, however, such as that shown in Table IX, suggest that each order of insects may possess reagin-stimulating allergens both common to and differing from those of other orders.

TABLE IX. EXHAUSTION TEST
(SERUM OF P. R.* TO H. W.* SITE PREPARED DEC. 7, 1957)

SITE	PREPARED WITH	DAYS AFTER INITIAL SITE PREPARATION					
		2	3	4	5	6	7
A	P. R. serum	12-9-57	12-10-57	12-11-57	12-12-57	12-13-57	12-14-57
		G. H.	G. H.	G. H.	No testing		C. R.
B	P. R. serum	++++	++	±	A and B still indurated		-
		G. H.	G. H.	G. H.			M.
C	P. R. serum	++++	++	±			++
D	P. R. serum						C. R.
E	Unprepared						+++
F	Unprepared						M.
							+++
							-
							±

Antigen used: G. H. (grasshopper); M. (moth); C. R. (cockroach).
Serum of P. R.

*P. R., patient; H. W., recipient.

DISCUSSION

A comparison of interrelated antigenicity among the insects to that among plants or vertebrates offers interesting though vulnerable analogies. Among hayfever producing plants the families *Amaranthaceae* and *Chenopodaceae* have pollens which are structurally and antigenically closely related (Russian thistle and pigweed). The families within the large heterogeneous group of composite plants all frequently react to some degree on sensitive individuals, and yet among grasses (the family *Gramineae*) the skin reactions may show wide variation on a clinically sensitive patient with one tribe of grasses producing much larger whealing reaction than another. This is probably more a matter of degree rather than differences in allergens.

In the animal kingdom within the class *Mammalia* the dander of cow, deer, and camel, all within the suborder *Ruminantia*, usually react on patients sensitive to cow hair. Within the order *Carnivora* and its suborder *Tissipedia*, the cat and dog families both often react to varying degrees in a patient sensitive to one or to the other.

TABLE X. PROPOSED LIST OF INSECTS FOR ROUTINE TESTING

ORDER*	COMMON NAMES OF INSECTS†
Class Hexapoda	
1. Coleoptera	beetles, weevils
2. Lepidoptera	moths, butterflies
3. Hymenoptera	honeybees, hornets, yellow jackets
4. Diptera	flies, mosquitoes
5. Orthoptera	grasshoppers, locusts, cockroaches
6. Hemiptera	box-elder bugs, squash bugs
7. Homoptera	aphids, leafhoppers, cicadas, scales
8. Minor orders	mixture of common representative
Class Arachnida	
9. Aranea	spiders
10. Acarina	mites
Class Crustacea	
11. Isopoda	sowbugs
12. Branchiopoda	daphnia, fairy shrimp

*The first four orders will encompass 80 per cent of the common species found in North America.

†Families and species not indicated. A mixture of those most abundant and most readily available may be used.

TABLE XI. REGIONAL AND LESS COMMON INSECTS FOR SPECIFIC PROBLEMS

Class Hexapoda	
1. Order Ephemeroptera	May flies
2. Order Trichoptera	caddis flies
3. Order Neuroptera	lacewings
4. Order Thysanura	silverfish
Class Myriapoda	
5. Order Chilopoda	centipedes
6. Order Diplopoda	millipedes
Class Crustacea	
7. Order Copepoda	plankton (brine) shrimp

Classification of plant and animal life is based on many factors including anatomy, physiology, biology, ecology, and even cytology. The allergist who must accept allergenic relationship only on the basis of immunologic similarity must, however, be impressed by a close relationship between the taxonomist's arrangement and his own observation, particularly with skin testing. There is thus a strong temptation to use entomological classification in grouping our skin testing with insects.

The arbitrary but apparently valid acceptance of the classification level of order for grouping of tests, together with the reduction of the orders to those embracing 96 per cent of the known species, has brought the list of important insects to a less formidable number. It has reduced the number of recommended tests to a mere dozen (Tables X and XI). This should make the task of routine studies with insect allergens easier.

SUMMARY AND CONCLUSIONS

1. Allergenic interrelationship of insects at the level of orders and families is suggested as a result of extensive skin testing.
2. Routine "screening" for probable sensitivity to insects would require about 12 of the most common classes and orders of Arthropods.
3. A proposed list of extracts for routine testing is offered.
4. More detailed testing with various families, genres or even species within a reacting order may follow and may reveal occasional variation in allergenicity.

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