IMMUNOLOGICAL STUDIES OF HARVESTER ANTS IN TUCSON, ARIZONA

by

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This Thesis

is

Dedicated with Love

to

My Parents

Dr. & Mrs. Chung K'uei Wang

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BIOGRAPHICAL SKETCH

Tien Min Wang was born in Kaoshung, Taiwan, Republic of China, on May 9, 1950. She attended public schools in Taichung, Taiwan, and graduated from First Girl's Public High School in 1968. She then attended National Chung-Hsing University, Taichung, Taiwan, where in 1972 she received a Bachelor of Science degree in agriculture with a major in entomology. Subsequently she worked there as a research assistant in the Entomology Department for the remainder of the year.

In January 1973, she entered The University of Arizona as a graduate student in the Entomology Department, and began working toward her master's degree with an interest in medical entomology.

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ABSTRACT

An immunological study of Harvester ants in Tucson, Arizona was undertaken to determine the reasons for a case of anaphylaxis secondary to an ant sting, which represents an instance of ant hyposensitization failure, and to select a proper extract for hyposensitization treatment against native harvester ants.

According to the accepted classification, members Pogonomyrmex rugosus Emery, maricopa Wheeler, and desertorum Wheeler were collected within the city limits of Tucson, Arizona, for immunological study. After homogenization in saline, prepared extracts of the whole body P. rugosus, maricopa, desertorum, the gaster P. rugosus, and the head and thorax P. rugosus were sterilized by micropore filtration and analyzed for protein content. Using these extracts, ant-sensitive patients were skin tested and rabbits were immunized.

The Ouchterlony double immunodiffusion test demonstrated that the antigens from several Harvester worker ant species cross react immunologically with one another but not with commercial extracts from other stinging Hymenoptera such as honey bee, wasp, hornet, yellowjacket, fire ant, and carpenter ant. A partial key for classification of harvester ant species is presented. The importance of diagnosis of the offending insect is emphasized in order to select the proper extracts for use in hyposensitization treatment of Hymenoptera sensitive patients.

INTRODUCTION

In 2641 B.C., King Menes of Egypt died following a sting of a wasp or hornet as recorded on his tomb. (Waddell, 1930). However, these reactions were not recognized as allergic until the early 1900's, (Waterhouse, 1914; Benson and Semenov, 1930). The number of severe and sometimes fatal stinging insect reactions are now reported yearly, and according to a recent report of the Insect Sting Submittee of the American Academy of Allergy, more than 400 fatal reactions to one or more Hymenoptera stings have been recorded during the past 10 years. (Barnard, 1973). Nevertheless, the total number of deaths each year is not known, since many reactions probably go unreported. The general public has a growing awareness of the life-threatening reactions which can occur in insect sting sensitive individuals.

In the past two decades, most of the studies involving insect stings of the order Hymenoptera have dealt with the honey bee (Apidae), vespid wasp (Vespidae), hornet (Vespidae), yellowjacket (Vespidae), (Barr, 1971), and imported fire ant (Formicidae: Solenopsis spp.). (Lockey, 1974). Allergic reactions to harvester ants are less well known and studied. In Tucson, Arizona, harvester ant stings may be more common and potentially as life-threatening as the other stinging insects.

As Lockey (1974) pointed out, most studies of Hymenoptera stinging insect allergy have suffered from inadequate documentation of

the responsible insect, i.e., it is based only on clinical history, clinical findings, and not on proper identification of the offending insect. For the medical entomologist, it is emphasized that identification of the offending insect is as vital as the patient's clinical history or skin test reactivity (Schwartz, 1965) in choosing proper extracts for hyposensitization therapy.

In the summer 1973, six severe allergic reactions in children stung by the harvester worker ants were recognized by allergists at the College of Medicine at The University of Arizona, in Tucson, Arizona. Among those cases, one patient experienced an anaphylactic reaction following a sting by a red harvester ant, subsequently identified as Pogonomyrmex maricopa. After one year, on maintenance hyposensitization therapy with a commercial ant extract (A) and a stinging insect mixture (S), which contained extracts of bee, wasp, hornet, and yellowjacket, the patient was stung a second time and required hospitalization. The purpose of this study was to determine the reasons for this hyposensitization treatment failure and to help select a proper extract for hyposensitization treatment against these native harvester ants. A partial classification key to the harvester worker ants in Tucson, Arizona is presented to aid in identification of the offending species.

BIOLOGY

The genus POGONOMYRMEX is one of the largest genera within the subfamily Myrmicinae, family Formicidae of the order Hymenoptera, and it is the major group of harvesting ants in North America, especially in the semi-arid and arid regions of Mexico and the southwestern United States, such as Arizona, New Mexico, and Texas. The workers of this genus collect and store seeds for food, "harvesting" the plants around their nesting areas by removing seeds with their mandibles.

A member of the worker caste is the individual to which we commonly apply the name "ant". Its functions are primarily fighting, foraging, nursing, and caring for the nest. The worker ant differs from the female (queen) in several respects. It is usually smaller, lacks wings, and seldom bears ocelli (simple eyes) except in certain restricted groups of ants. The worker ant has a thorax superficially composed of three divisions, but in reality composed of four: prothorax, mesothorax, metathorax, and the epinotum which is formed by the first abdominal segment of the embryo which has fused with the metathorax. Additional features of Pogonomyrmex workers are: 12-segmented antennae; 4-segmented maxillary palpi; 3-segmented labial palpi; and finely pectinate tibial spurs of the mid and hind legs. A distinct mesoepinotal impression is absent; a psammophore is present.

These ants are monomorphic, except for Pogonomyrmex badius Lat. which is polymorphic, and free-living. (Smith, 1947).

The nests themselves are constructed in the soil, generally in areas fully exposed to the sun (Figs. 1 and 2). Some are beneath stones, whereas others are surmounted by soil craters or by mounds with or without coverings of gravel. The conformation of the nest is one of the important ecological characteristics for species determination. Moreover, Cole (1968) described the characteristics of western harvester ant nest as follows: "The workers of some species alter the area peripheral to the nests by clearing away the plants---- felling them, bit by bit, with their powerful mandibles. Such mounds with their surrounding denuded area, characteristic of the western harvester, are familar sights to travelers in the arid West."

The significance of this genus is not only its economic aspect, but its medical importance. The economic impact is due to "harvesting" activities, and damage to range lands when nests are numerous, since the ants clean circular areas around each nest. The workers of certain species of Pogonomyrmex, such as Pogonomyrmex rugosus Emery (Fig. 3), and Pogonomyrmex maricopa Wheeler (Fig. 4), can be hostile and may violently attack and sting an invader. Other species in contrast are non-violent and retiring. When they clip and rasp the skin with their mandibles, a stinger (modified ovipositor) protrudes from the abdomen, pierces the skin, and injects ant venom almost simultaneously (Fig. 5). The site of this sting can be quite painful with localized redness, swelling and inflammation, expanding rapidly. People with

Figure 1. Nest hole of Pogonomyrmex desertorum Wheeler.

Figure 2. Nest of Pogonomyrmex rugosus Emery underneath stones.

Figure 3. Black Pogonomyrmex rugosus Emery undergoing electric stimulation for venom extraction.

Figure 4. Red Pogonomyrmex maricopa Wheeler walking on the ground with their gasters up in the air.

Figure 5. Red Pogonomyrmex maricopa Wheeler undergoing electric stimulation with stinger protruding.

hypersensitivity to ant stings may demonstrate signs of immediate hypersensitivity, such as anaphylaxis, generalized hives, shock or death. There may also be delayed or contact type hypersensitivity, but this has not been well studied. Prompt medical attention is essential.

MATERIALS AND METHODS

Classification

This part of the study is an examination of the external morphology of live specimens of several species of harvester worker ants in Tucson, Arizona, and a review of several references, especially Pogonomyrmex Harvester Ants by Cole (1968).

Sampling

Previously identified samples were selected from the Arizona Insect Collection of the Entomology Department, University of Arizona. Live ant samples were collected at different locations within the city limits of Tucson, Arizona, and preserved in 70% alcohol (Fig. 6). From each sampling area, at least 15 individuals of each species were collected for study.

External Morphological Examination

After intensive study of external morphology of each live sample, the antennae, right mandible, pedicel, thorax and pedicel, and head were removed and cleaned in 100% KOH for 24 hours at room temperature. After transfer to 3% acetic acid for 5 min, each piece was glued to a microscope slide to prevent drying. Examination under a dissecting microscope with an ocular micrometer permitted the following drawings: contours of the head; conformation of the right

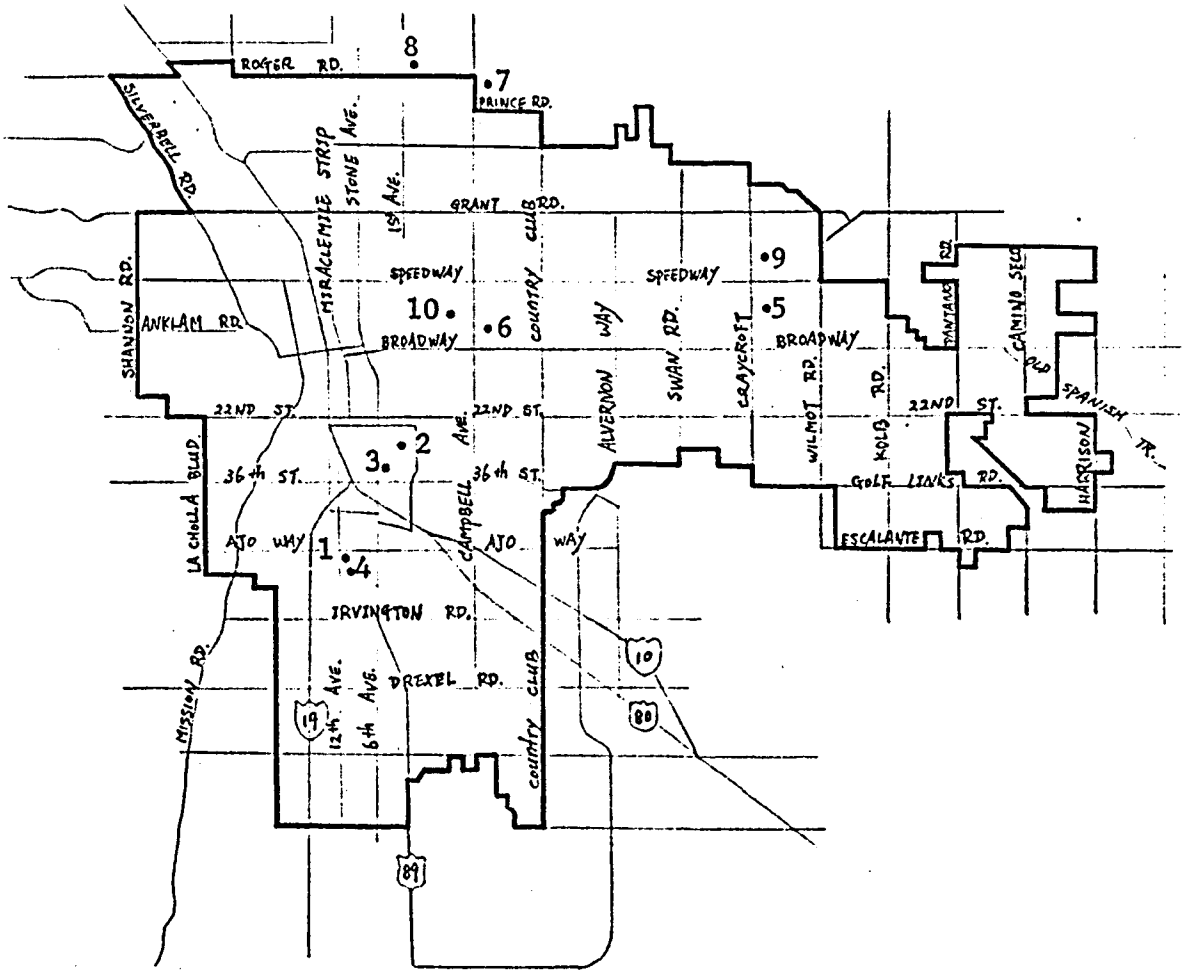

Figure 6. Localities where live specimens of the Genus Pogonomyrmex spp. were sampled in Tucson, Arizona.

Pogonomyrmex (P.) maricopa Wheeler:
1,2,3,4,5,7,8,9,10.

Pogonomyrmex (P.) rugosus Emery:
2,6,7,8,9,10.

Pogonomyrmex (P.) desertorum Wheeler:
2,5,7,8.

Pogonomyrmex (P.) californicus (Buckley)
3.

1, 2, 3, & 4 are localities of patients' houses.

mandible; base of right antennal scape; contours of thorax and petiole; and contours of petiole and postpetiole.

Preparation of the Keys

Based on the collection of the Entomology Department, at the University of Arizona, a list of ants in Tucson, Arizona was compiled. Second, following Smith (1947), and Creighton (1950), modified and simplified keys, from family to genera, were made. Then, simplified partial keys suitable for classification of the Pogonomyrmex species in Tucson, Arizona were compiled from Cole (1968).

Antigen Preparation

Sampling

Samples of three species: Pogonomyrmex (P.) rugosus, maricopa, and desertorum were collected with forceps within the city limits of Tucson, Arizona, during the summer of 1974. After collection, samples were placed in screened containers and brought to the laboratory. After washing with distilled water and allowing samples to dry, ants were killed by freezing at -20 ^{o}C, at which ants were stored until used for antigen extraction.

Using sterilized small scissors, gasters (abdomens) of P. rugosus were removed from the heads and thoraxes by sectioning behind each postpetiole. Gasters and front parts were stored in the freezer (-20 ^{o}C) until ready for use.

Extraction

The extracts of whole body P. rugosus, P. maricopa, P. desertorum (WR, WM, WD), gaster P. rugosus (GR), and front P. rugosus (FR) were homogenized by means of a POLYTRON, size 20, Brinkmann instrument, Westbury, N. Y. The procedures involved as follows:

The Polytron was cleaned by washing with soap water for 10 sec, followed by distilled water for 10 sec X 3, and a final wash for 15 sec in steriled saline (0.15 N NaCl). One gram of each sample was homogenized in a polypropylene centrifuge tube, 28.7 X 103 mm, containing 10 ml of precooled saline. In an ice bath, homogenization schedule consisted of three "10 sec" periods at "1 min" intervals to allow for cooling. Homogenates were centrifuged at 23,000G X 45 min at 4 ^{o}C, and supernatants were stored at 4 ^{o}C. Aliquots of 0.5 ml of each sample were removed for protein determination. For sterilization, supernatants obtained were passed through 25 mm micropore filters, membrane filter GMB, Porengross, pore size 0.45 μ. The filtrates were stored in sterilized sealed glass vials at 4 ^{o}C.

Sterilization of the Extracts

To test for sterilization, each filtered extract was inoculated into tubes, which contained thioglycollate broth which permits growth of bacteria and fungi. Cultures were checked at 3 and 7 days, and only sterile extracts were used in these studies.

Each sterile extract prepared at 1:10 weight to volume (w/v) as described above was used at this concentration to immunize rabbits, but diluted to 1:100 in a saline-phenol solution (sodium chloride 0.5%

phenol 0.4% buffered with sodium phosphate) for skin testing ant sensitive patients.

Commercial Antigens

Commercial extracts which were purchased for comparison of antigenity of prepared extracts in this study were as follows:

Ants, Red Ant, Carpenter Ant, Fire Ant, Stinging Insect Mixture, Yellowjacket, Wasp, Hornet, and Bee honey, from Hollister-Stier Lab. Inc. Spokane, Washington.

Red Ant, from Greer Lab. Inc. Lenoir, North Carolina.

Red Ant, from Meridian Bio-medical Inc. Denver, Colorado.

Extract Analyses

Protein Content Determination

The protein content of the extracts was determined by the method of Lowry et al. (1951). Use bovine serum albumin (fraction 5) as the reference standard. Absorbance readings were obtained spectrophotometrically at an O.D. of 550nm.

Allergenic Activity

Extracts were tested for allergenicity in patients with histories of hypersensitivity reactions following an stings by scratch testing followed by direct intradermal skin test (Schwartz, 1965; Barr, 1972). Saline was used as the negative control, and histamine as the positive control, since it produces a wheal-and-flare reaction unless a patient has taken a drug with antihistamine activity.

Rabbit Immunization

Different saline aqueous antigen extracts: WR, WM, WD, FR, and GR were diluted 1 to 3 fold to obtain final concentrations of 2 mg/ml for antigen preparation. Antigen extracts were emulsified with equal volumes of Freund's adjuvant, complete or incomplete, for immunizing rabbits. Each rabbit received 1 ml of extract containing 1 mg of antigen protein. Two rabbits were immunized with WR, one with WM, one with WD, one with FR, and one with GR, according to the schedule shown in Table 1. Rabbit antisera were stored in the freezer (-20 ^{o}C) until ready for use.

Antigen-Antibody Studies

Precipitating antibody was detected by immunodiffusion, according to the principles of Ouchterlony (1958 and 1962), Williams and Chase (1971), and Humphrey and White (1970).

The buffer stock solution and agarose used in this study were prepared following the methods described by Wieme (1959), modified by using Agarose (Matheson Coleman and Bell Company), instead of Noble Agar (Hjerten, 1961). Thimerosal 10 mg per 100 ml of agarose was used as preservative. Microscope slides were cleaned and on each slide, 2.5 ml melted agarose was poured and allowed to gel. Slides were stored at 4 ^{o}C. After 24 hours, wells were punched and filled with antigen extract or rabbit antiserum. After 24 and 48 hours at room temperature, slides were examined for precipitins. Slides were then photographed and stained with Coomassie Brilliant Blue R-250, Bio Rad Lab. Richmond, Cal. (Axelsen, Kroll and Weeke, 1973).

Table 1. Immunization Schedule of Rabbits

	Date	Freund's Adjuvant	Methods
1st Immunization	Sep. 23, 1974	complete	0.2 ml in 2 front footpads 0.3 ml in 2 sites on back subcutaneously (SQ).
2nd Immunization	Sep. 30, 1974	incomplete	4 sites on back. SQ. 0.25 ml for each one
Bleeding	Oct. 14, 1974	___	from ear vein, by using 20 G needle

RESULTS

Classification

Previously identified specimens of harvester ant worker in the collection room of the Entomology Department include Pogonomyrmex (P.) barbatus (F. Smith), bicolor Cole, desertorum Wheeler, rugosus Emery, occidentalis (Cresson), californicus (Buckley), maricopa Wheeler, and P. (Ephebomyrmex) pima Wheeler. According to habitat, identification with prepared keys, and a comparison with of previously identified specimens, the live samples were composed of four species: P. (P.) maricopa, rugosus, desertorum, and californicus. In addition, periodic field sampling from the middle of September to early November 1973 revealed that the black P. rugosus and red P. maricopa were the most widely distributed species in the Tucson area.

A key to the subfamilies of the family Formicidae, a key to the genera of the subfamily Myrmicinae, and a list of the ants in Tucson, Arizona are given in the Appendix A, B, and C. The terminologies of both the generalized mandible and the antennal scape base, which are main characteristics used in the identification of the species of Pogonomyrmex workers are taken from Cole (1968), and illustrated in figures 7 and 8.

Drawings of contours of the head, conformation of the right mandible, base of right antennal scape, contours of thorax and pedicel, and contours of petiole and postpetiole are shown in the figures 9, 10, 11, 12, and 13.

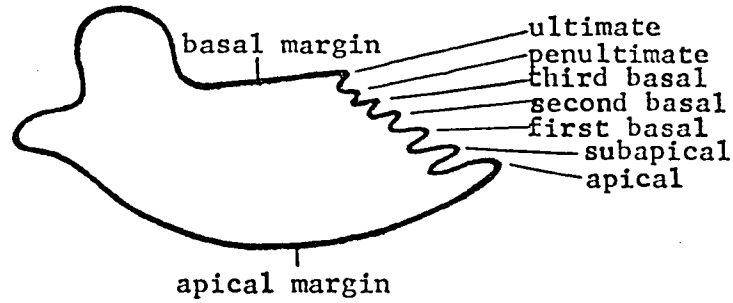

Figure 7. Terminology of the generalized mandible of the worker (after Cole, 1968).

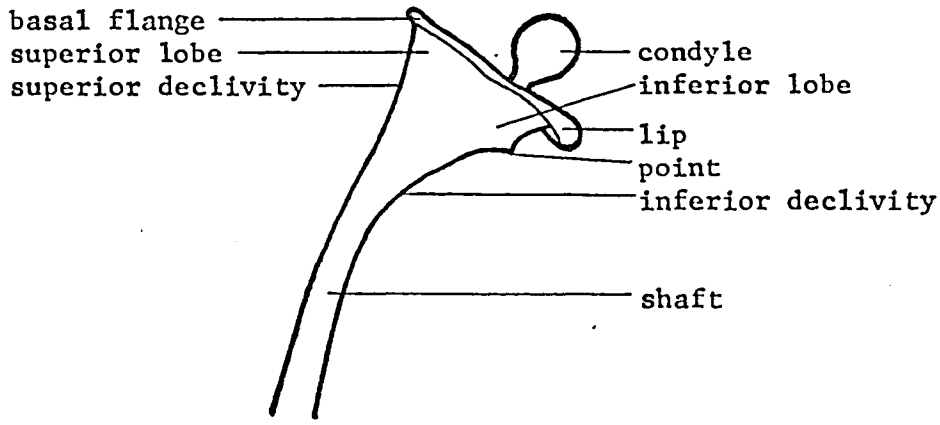

Figure 8. Terminology of the scape base of the worker (after Cole, 1968).

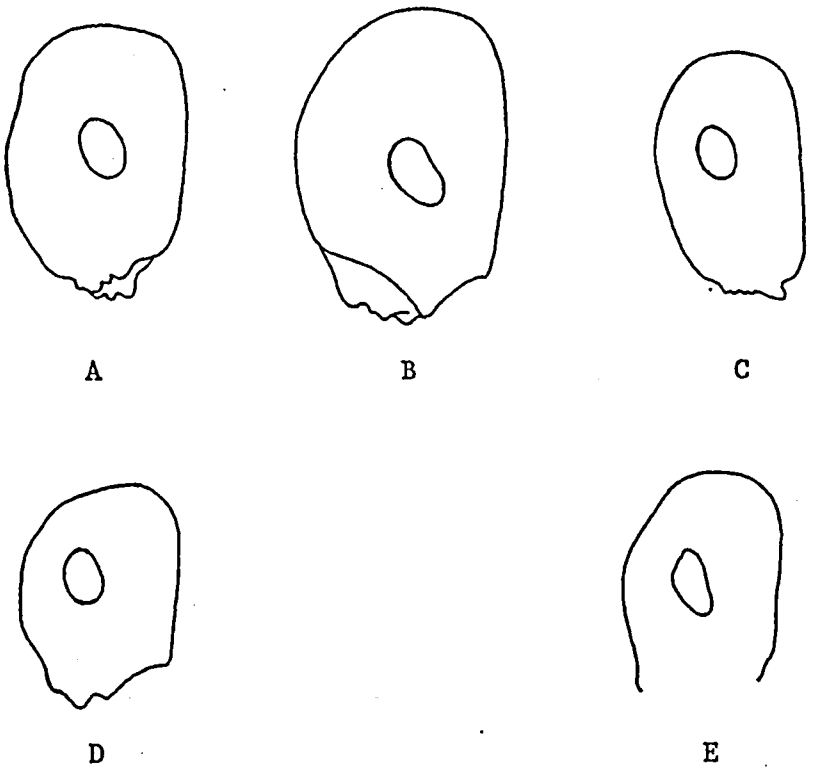

Figure 9. Contours of head.

A. P. (P.) maricopa Wheeler; lateral view, Tucson, Arizona. (18 X).

B. P. (P.) rugosus Emery; lateral view, Tucson, Arizona. (18 X).

C. P. (P.) californicus (Buckley); lateral view, Tucson, Arizona. (18 X).

D. P. (P.) desertorum Wheeler; lateral view, Tucson, Arizona. (18 X).

E. P. (P.) occidentalis (Cresson); lateral view, Minden, Nev.*

* After Cole, 1968. The magnification was not mentioned.

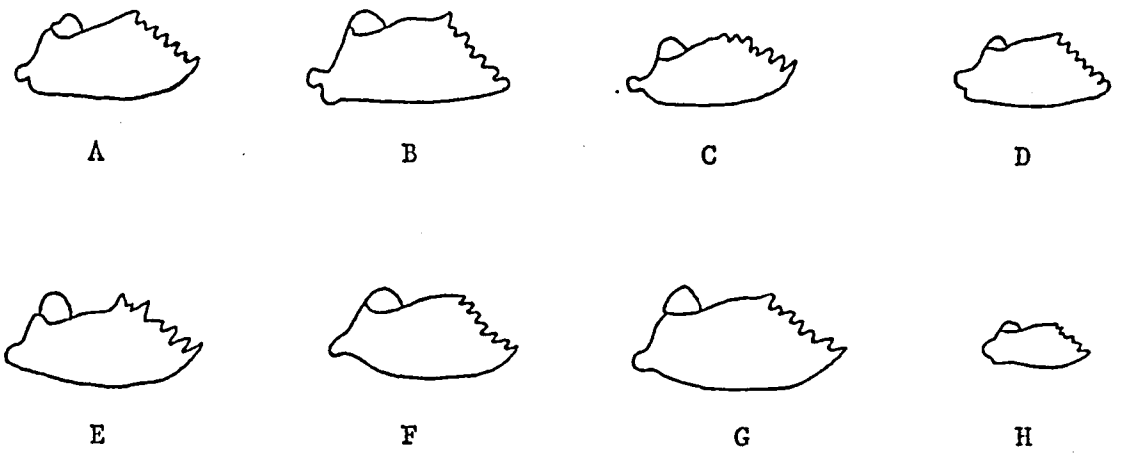

Figure 10. Conformation of the right mandible.

A. P. (P.) maricopa Wheeler; Tucson, Arizona. (18 X).
B. P. (P.) rugosus Emery; Tucson, Arizona. (18 X).
C. P. (P.) californicus (Buckley); Tucson, Arizona. (18 X).
D. P. (P.) desertorum Wheeler; Tucson, Arizona. (18 X).
E. P. (P.) occidentalis (Cresson); Portal, Arizona.*
F. P. (P.) bicolor Cole; Continental, Arizona.*
G. P. (P.) barbatus (F. Smith); Dallas, Texas.*
H. P. (E.) pima Wheeler; Tucson, Arizona.*

* After Cole, 1968. The magnification was not mentioned.

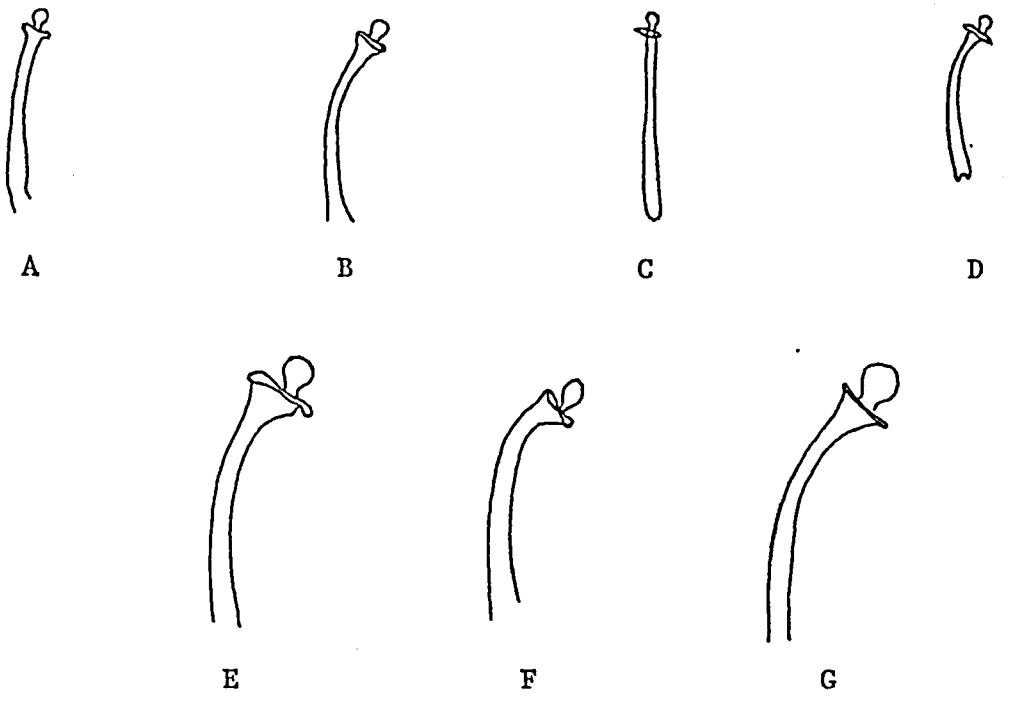

Figure 11. Base of right antennal scape in outer lateral view.

A. P. (P.) maricopa Wheeler; Tucson, Arizona. (18 X).
B. P. (P.) rugosus Emery; Tucson, Arizona. (18 X).
C. P. (P.) californicus (Buckley); Tucson, Arizona. (18 X).
D. P. (P.) desertorum Wheeler; Tucson, Arizona. (18 X).
E. P. (P.) occidentalis (Cresson); Ludlow, Colorado.*
F. P. (E.) pima Wheeler; Tucson, Arizona.*
G. P. (P.) bicolor Cole; Continental, Arizona.*

* After Cole, 1968. The magnification was not mentioned.

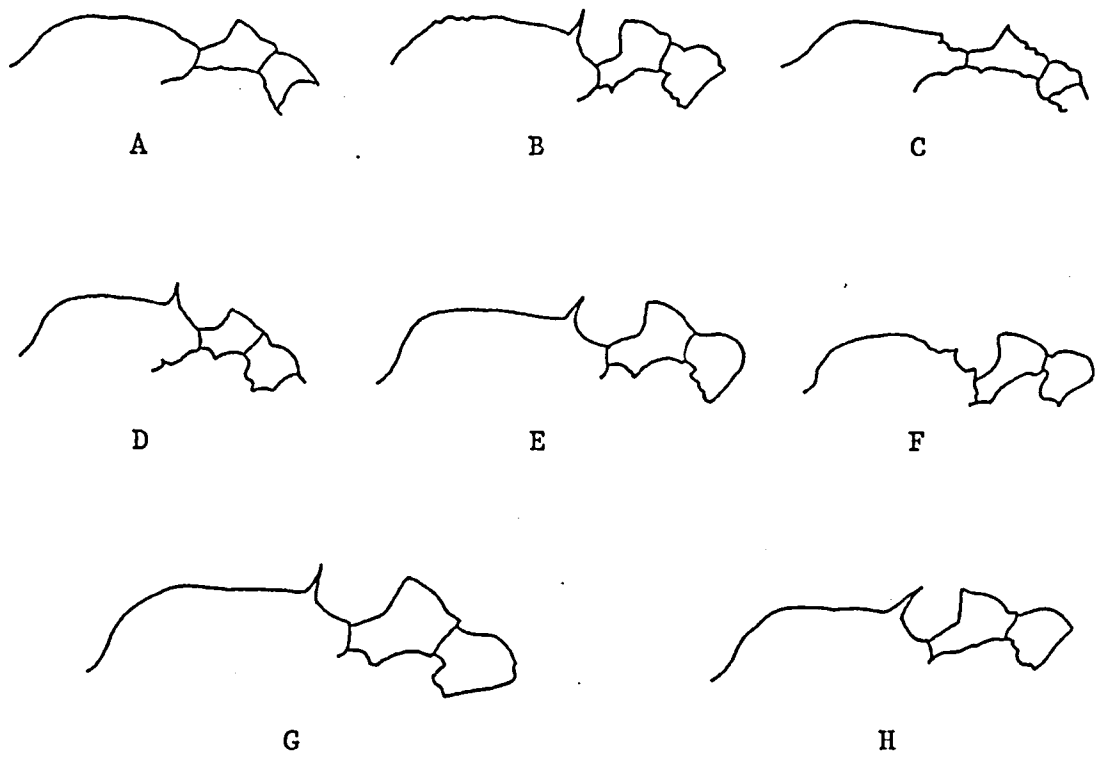

Figure 12. Contours of thorax, petiole, and postpetiole, in lateral view.

A. P. (P.) maricopa Wheeler; Tucson, Arizona. (10 X).
B. P. (P.) rugosus Emery; Tucson, Arizona. (10 X).
C. P. (P.) californicus (Buckley); Tucson, Arizona. (10 X).
D. P. (P.) desertorum Wheeler; Tucson, Arizona.*
E. P. (P.) bicolor Cole, Continental, Arizona.*
F. P. (E.) pima Wheeler; Tucson, Arizona.*
G. P. (P.) barbatus (F. Smith); Dallas, Texas.*
H. P. (P.) occidentalis (Cresson); Murray, Utah.*

* After Cole, 1968. The magnification was not mentioned.

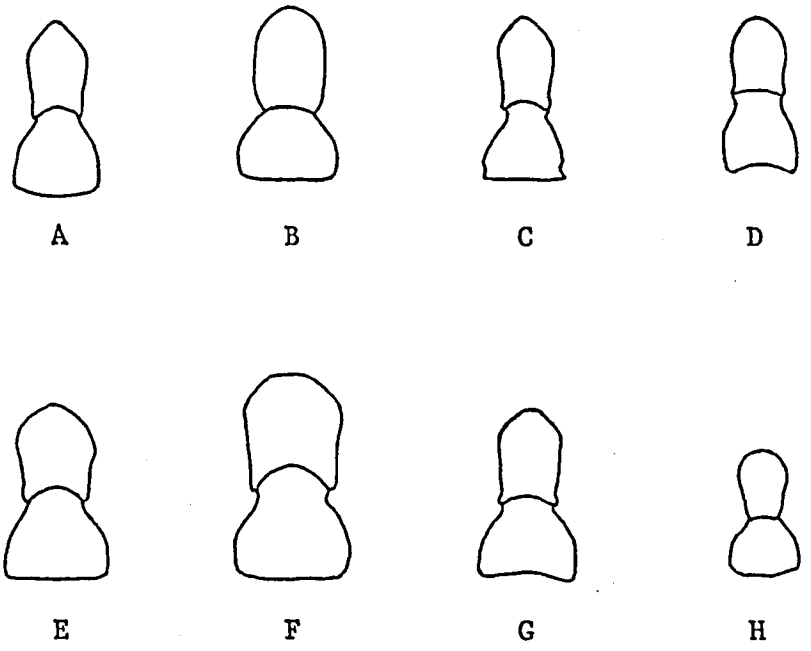

Figure 13. Contours of petiole and postpetiole, in dorsal view.

A. P. (P.) maricopa Wheeler; Tucson, Arizona. (18 X).
B. P. (P.) rugosus Emery; Tucson, Arizona. (18 X).
C. P. (P.) californicus (Buckley); Tucson, Arizona. (18 X).
D. P. (P.) desertorum Wheeler; Tucson, Arizona. (18 X).
E. P. (P.) occidentalis (Cresson); Murray, Utah.*
F. P. (P.) barbatus (F. Smith), Dallas, Texas.*
G. P. (P.) bicolor Cole; Imuris, Sonora, Mexico.*
H. P. (E.) pima Wheeler; Tucson, Arizona.*

* After Cole, 1968. The magnification was not mentioned.

Key to the Subgenera of the Genus
Pogonomyrmex (after Cole, 1968)

1. Basic (maximum) number of mandibular teeth 6, the ultimate basal tooth much reduced (Fig. 10-H); eye placed decidedly below approximate center of side of head; scape strongly bent in proximal one-quarter of its length; outer margin of frontal lobes nearly straight, subparallel; psammophore weakly developed; head and thorax extensively and coarsely rugo-reticulose; femora, especially those of forelegs, strongly incrassate; epinotal spines connected basally by a prominent and usually straight keel; postpetiole, viewed from the side, massive, the ventral process very large and bulbous, the height much greater than the length (Fig. 12-F). . Subgenus EPHEBOMYRMEX Wheeler. . . . Sp. P. (E.) pima Whlr.

1'. Basic (maximum) number of mandibular teeth 7, the ultimate basal tooth usually not at all reduced (Fig. 10-A); eye placed at approximately center of side of head; scape not strongly bent, the bend involving a notably greater extent of the scape length; outer margin of frontal lobe distinctly convex; psammophore strongly developed; head and thorax not extensively and coarsely rugo-reticulose; femora not strongly incrassate; epinotal spines (when present) not connected basally by a prominent keel; postpetiole, viewed from the side, smaller, the ventral process less well developed and not especially bulbous, the height not notably greater than the length (Fig. 12-A). . . Subgenus POGONOMYRMEX Mayr

Partial Key to the Complexes of the Subgenus
POGONOMYRMEX (after Cole, 1968)

1. Lateral lobe of clypeus produced in front of antennal insertion forming a peripheral portion which extends anteriorly beyond level of median clypeal lobe as a broad blunt process of variable strength; medio-anterior portion of frontal lobe ascending very steeply from adjoining peripheral part of median clypeal lobe, the two forming a sharp angle and producing a deep impression for the median clypeal lobe and the adjoining frontal triangle; head (excluding mandibles) notably broader than long; eye small, weakly convex, not extending beyond side of head with head in full-face view, the head length between occipital corner and mandibular insertion more than three times the greatest eye length; outer surface of extreme base of antennal scape strongly, broadly, and longitudinally compressed, the area involved flattened or concave; with head in full-face view, the longitudinal cephalic rugae nearly straight and parallel, diverging slightly into posterior corners of head; in lateral view, the longitudinal rugae nearly straight and parallel and diverging slightly into posterior corners where they may meet the extremities of the frontal longitudinal rugae, not forming whorls behind the eye; venter of petiolar peduncle with a few, long, erect hairs extending downward from the peduncular process and vicinity or from that region when a process is absent; first gastric segment broader than long. . . . . BARBATUS COMPLEX

1'. Not as mentioned above 2

2. Base of antennal scape strongly enlarged, broad, robust, the basal flange (when present) thick, the lip strong and prominent (Fig. 11-E); frontal lobes strongly developed, broad, moderately to very strongly convex medially; cephalic rugae usually not forming concentric whorls above the eye; thoracic dorsum, in lateral view, not strongly arched, gradient of epinotal base at most very slight (Fig. 12-H); epinotal armature (angles, denticles, or spines) present; postpetiole, viewed from above, very robust, generally no longer than broad (Fig. 13-E). . . . . OCCIDENTALIS COMPLEX

. . . . . Sp. P. (P.) occidentalis (Cresson)

2'. Base of antennal scape weakly enlarged, the basal flange thin, the lip rather weak (Fig. 11-A); frontal lobes less strongly developed, narrower, weakly convex medially; cephalic rugae generally forming crescentric whorls above the eye; thoracic dorsum, in lateral view, rather strongly arched, gradient of epinotal base moderate to strong (Fig. 12-A); epinotal armature present or absent, denticles or spines, when present, appearing to be directed strongly upward; postpetiole, viewed from above, less robust, generally longer than broad (Fig. 13-A). . . . . MARICOPA COMPLEX

Partial Key to the Species of the Barbatus Complex (after Cole, 1968)

1. Cephalic rugae extremely fine, very closely set, producing a silky luster. 2

1'. Cephalic rugae notably less fine, not so closely set, not producing a silky luster. 3

2. Posterior corners of head without rugae, smooth and strongly shining . . . . . .Sp. P. (P.) desertorum Wheeler

2'. Posterior corners of head with rugae, not smooth and strongly shining . . . . . .Sp. P. (P.) bicolor Cole

3. Cephalic rugae very coarse, widely spaced, usually wavy; pronotal rugae very coarse, irregular, widely spaced, wavy, tending to form prominent reticulations; dorsum of pronotum generally somewhat flattened; dorsum of petiolar node with coarse, irregular rugae, often reticulose; color generally black or deep reddish black, gaster often contrastingly lighter. . . .Sp. P. (P.) rugosus Emery*

3'. Cephalic rugae notably finer, not widely spaced, not especially wavy; pronotal rugae not particularly coarse or wavy, not forming prominent reticulations; dorsum of pronotum generally evenly and broadly convex; dorsum of petiolar node without coarse, irregular rugae, not reticulose; body generally concolorous, light to deep ferrugineous red. . . . . Sp. P. (P.) barbatus (F. Smith)

*They are all dark in the Tucson population.

Partial Key to the Species of the Maricopa Complex (after Cole, 1968)

1. Cephalic interrugal punctulation rather strong; interrugal punctulation of epipleura moderate to strong; interrugal spaces subopaque . . . . . .Sp. P. (P.) maricopa Wheeler

1'. Cephalic interrugal punctulation absent to moderate; interrugal spaces strongly shining . . . . .Sp. P. (P.) californicus (Buckley)

Extract Analyses

Protein Content Determination

Using the method described by Lowry et al. (1951), the results of protein determination are summarized in Table 2.

Table 2. Protein Concentration of Our Prepared P. rugosus, maricopa, and desertorum extracts

Extracts*	O. D. (550 nm)	Protein (mg/ml)	Dil. Factor	Prot. Conc. (mg/ml)
WR	0.196	0.041	100	4.1
WM	0.310	0.071	100	7.1
WD	0.110	0.025	100	2.5
GR	0.217	0.045	100	4.5
FR	0.145	0.030	100	3.0

*WR= whole body P. rugosus; WM= whole body P. maricopa; WD= whole body P. desertorum; GR= gaster P. rugosus; FR= head and thorax P. rugosus.

Allergenic Activity

Extracts of WR and WM elicited positive skin test within 15 min in ant sensitive human as shown in the Table 3. This indicates that antigens have been extracted and retain their antigenicity. In addition, results showed that the antigenicity of prepared extracts were equal to or greater than that of commercial ones for patients in the Tucson area.

Antigen-Antibody Studies

By analyzing precipitins formed on double-immunodiffusion slides, several important results were obtained. When antigen extracts prepared from different parts of the ant body (whole body, gaster, and front) were allowed to diffuse against a rabbit antiserum of a whole body extract from the same species, the precipitin lines formed demonstrating that antigens are distributed throughout the entire body of the ant. Some antigens, however, are common to every part, while other antigens are most concentrated in certain parts (Fig. 14). When extracts from members of different families in the order Hymenoptera diffused against a rabbit antiserum to whole P. rugosus or whole P. maricopa, no cross-antigenicity was observed, i.e., bee, hornet, wasp, yellowjacket, and stinging insect mix do not appear to share antigens with P. rugosus or P. maricopa (Fig. 15). Rabbit anti whole body P. rugosus and anti whole body P. maricopa do not cross react with either extract of fire ant (Solenopsis spp.) or extract of carpenter ant (Camponotus spp.) (Fig. 16). In addition, figure 17 shows that the different species of genus Pogonomyrmex cross-react with one another,

Table 3. Intradermal Skin Test with Our Prepared P. rugosus P. maricopa, and Commercial Extracts

Patient$	Size of wheal**	Size of neg. control	Size of pos. control	Titers* WR	WM	Com.
S. D.	12 (13)	0	11	ND	10^5	$10^{5\neq}$
C. M.	15 (11)	0	11	10^5	10^6	$10^{3/}$
O. S.	11 (10)	0	11	ND	10^5	$10^{4/}$
E. L.	14 (12)	0	12	10^6	10^6	$10^{6/}$
W. A.	19 (13)	0	13	ND	10^6	$10^{5/}$
B. B.	10	0	10	ND	10^4	ND
W. T.	12	0	10	10^5	10^5	ND
Control						
S. A.	12	0	10	10^3	10^3	ND
S. R.	11	0	10	10^2	10^3	ND

* Each value represents the reciprocal of the greatest dilution of the extracts which produced a wheal-and-flare reaction.

**Size of wheal is measured by length plus width in mm, and values in parenthesis are that of commercial extracts.

/ Extracts purchased from Hollister-Stier Lab. Inc.

≠ Extract purchased from Meridian Bio-medical Inc.

$ Initials of patient's name.

ND Not done.

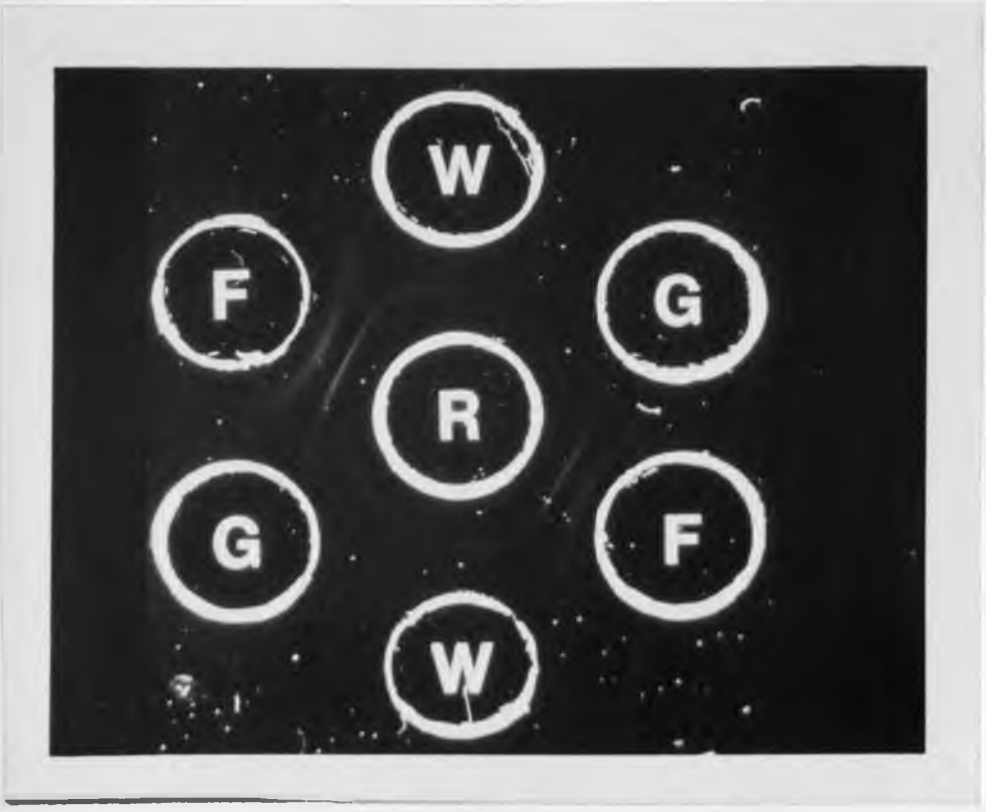

Figure 14. Ouchterlony Immuno-gel-diffusion between different portions of the species Pogonomyrmex maricopa.

R= rabbit anti-whole body P. maricopa serum; W= whole body extract; G= gaster extract; F= front extract.

Figure 15. Ouchterlony Immuno-gel-diffusion between different families of Hymenoptera.

R= rabbit anti-whole body P. rugosus serum; P= whole body P. rugosus extract; B= bee extract; H= hornet extract; W= wasp extract; Y= yellowjacket extract; S= stinging insect mixture extract.

Figure 16. Ouchterlony Immuno-gel-diffusion between different genera of family Formicidae (a).

R= rabbit anti-whole body P. maricopa serum; Pm= whole body P. maricopa extract; Pr= whole body P. rugosus extract; F= fire ant extract; C= carpenter ant extract.

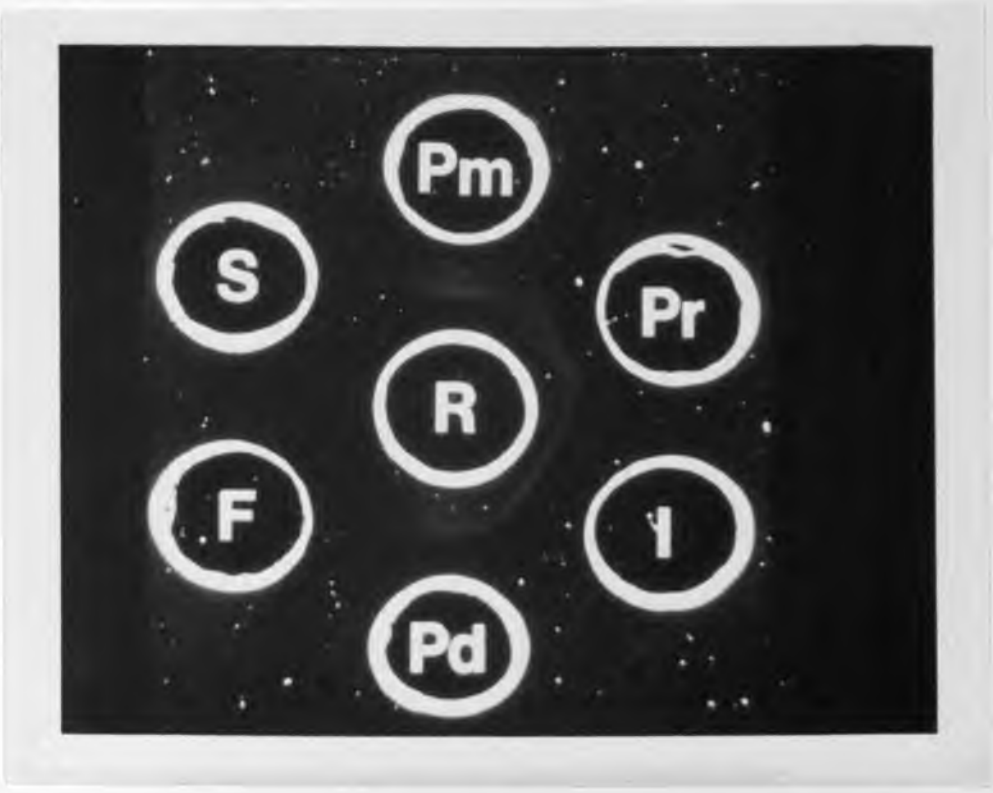

Figure 17. Ouchterlony Immuno-gel-diffusion between different species of Pogonomyrmex.

R= rabbit anti-whole body P. maricopa serum; Pr= whole body P. rugosus extract; Pm= whole body P. maricopa extract; Pd= whole body P. desertorum extract; 1= Hollister-Stier red ant extract; S= stinging insect mixture extract; F= flea extract.

i.e., they share same antigenicity, but not with stinging insect mix and flea (order: Siphonaptera). Compared with commercial P. rugosus extracts, the precipitin lines formed between the extracts produced in this study and rabbit anti-sera appeared stronger. No precipitin line was developed between rabbit anti-serum and extract A (derived from genus Formica), sold as "ants" (Fig. 18). Moreover, in figure 19, at the highest concentration of antigen extract more than one precipitin line is produced against the rabbit antiserum indicating that more than one antigen-antibody system is present.

Figure 18. Ouchterlony Immuno-gel-diffusion between commercial extracts and our prepared Pogonomyrmex maricopa extracts.

R= rabbit anti-whole body P. maricopa serum; A= Hollister-Stier ants extracts; P= whole body P. maricopa extract; 1= Meridian red ant extract; 2= Hollister-Stier red ant; 3= Greer red ant.

Figure 19. Ouchterlony Immuno-gel-diffusion between different dilutions of Pogonomyrmex rugosus gaster extracts.

RG= rabbit anti-gaster P. rugosus serum; 2= 1:2 dilution; 4= 1:4 dilution; 8= 1:8 dilution; 16= 1:16 dilution; 32= 1:32 dilution.

DISCUSSION

In this study, partial keys to the classification of harvester worker ants in Tucson, Arizona were prepared. They include only species of Pogonomyrmex found in Tucson, Arizona. They are simplified, comprehensive, and up to date.

Methods used in antigen extraction produced an extract with antigenecity equal to or greater than commercial extracts as compared by direct intradermal skin test and the Ouchterlony double-immuno-diffusion test. The method of antigen preparation used in this study is a rapid and efficient process compare with another methods, e.g. methods described by Shulman, Langlois and Arbesman (1964).

In the last two decades, several immunological studies of stinging insects investigated cross-antigenicity. Honey bee, wasp, hornet, and yellowjacket were shown to certain common antigens, some fractions common to each one, some fractions specific to one or two of them, i.e., they cross-react antigenically with one another. (Foubert and Stier, 1958; Arbesman, Langlois and Shulman, 1965). Lockey (1974) reported cross-reactivity between wasps and the imported fire ant, because patients might react to both extracts in the skin testing.

In the present study, double-immuno-diffusion tests did not demonstrate that there were cross-reactivity between harvester ant and stinging insect mix or its individual members, bee, hornet, wasp, and yellowjacket. Moreover, in the family Formicidae, there was no

cross-reactivity between harvester ant, fire ant, and carpenter ant. Therefore, antigenicity of ants appears to be genus specific. One of the double-immuno-diffusion studies (Fig. 20) showed a faint precipitin line between rabbit anti-whole body Pogonomyrmex serum and carpenter ant extract suggesting that either some cross-antigenicity exists between these two genera or the precipitin reaction is due to a non-immunological reaction between Hymenoptera extracts and rabbit anti-serum. (Dirks and Sternburg, 1972; Franklin and Baer, 1974). As Foubert and Stier (1958, p. 13) had emphasized: "The high degree of specificity of the antigen-antibody reaction necessitates accurate identification of the sensitizing allergen. If the offending insect is known, the antigen to be used for desensitization is obvious". Based on a hyposensitization treatment failure in a patient receiving injection of an extract prepared from the wrong genus, and rabbit immunization studies, which confirmed the lack of cross-reactivity with that genus, it remains of critical importance to properly identify offending insect for proper choice of antigen extract in the treatment of insect allergic patients.

The findings in the present study demonstrated that the harvester ant extracts prepared are capable of inducing antibody production in rabbits and positive skin test in humans. Further sudies of this type which provide data concerning cross-reacting and non-cross-reacting antigens uncover important relationship among insects, thereby supplementing morphological studies and possibly helping to save human lives.

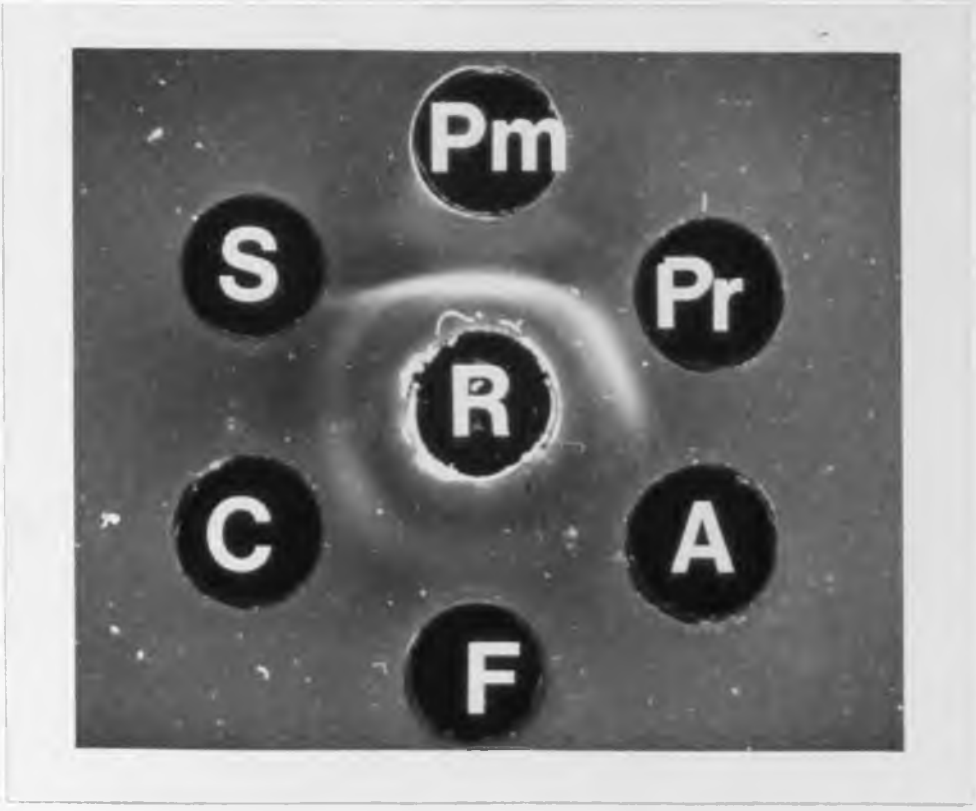

Figure 20. Ouchterlony Immuno-gel-diffusion between different genera of family Formicidae (b).

R= rabbit anti-whole body P. maricopa serum; Pm= whole body P. maricopa extract; Pr= whole body P. rugosus extract; A= commercial extract (Formica); F= fire ant extract (Solenopsis); C= carpenter ant extract (Camponotus); S= stinging insect mixture (bee, hornet, wasp, & yellowjacket).

SUMMARY AND CONCLUSION

Harvester worker ants belong to the order Hymenoptera, family Formicidae, subfamily Myrmicinae, and genus Pogonomyrmex Mayr. According to the Arizona Insect Collection of the Entomology Department, University of Arizona, Tucson, Arizona, there are eight species in the two subgenera Pogonomyrmex and Ephebomyrmex as follows: P. (P.) barbatus, bicolor, desertorum, rugosus, occidentalis, californicus, maricopa and P. (E.) pima. To human beings, their economic impact is due to their "harvesting" activities, and their medical importance is due to their "stinging" characteristics. When they attack an invader, they bite the victim with their powerful mandibles, pierce the skin with their stingers, and elicit venom almost simultaneously.

Based on the classification of harvester worker ants in Tucson, Arizona, several P. rugosus, maricopa and desertorum were collected and studied immunologically. Whole body P. rugosus, maricopa, desertorum, gaster P. rugosus, and head and thorax P. rugosus were homogenized to prepare extracts for immunizing rabbits. Ant-sensitive patients and normal individuals were skin tested with sterile prepared extracts of whole body P. rugosus and P. maricopa.

Rabbits immunized with extracts of harvester ants produced precipitating antibody to several species within this genus, but these rabbit antisera did not react by immunodiffusion against commercial extracts of bee, wasp, hornet, or yellowjacket. Furthermore, antisera

to harvester ants did not cross react with commercial fire ant and carpenter ant extracts. But within the genus Pogonomyrmex, the different species of harvester ants do appear to contain certain antigens in common with one another. In conclusion, antigenic cross-reactivity could be demonstrated only between species within the genus Pogonomyrmex, and not between other genera. The entomologist has an important role in the proper identification of the offending insect, so the physician can choose the proper extract for skin testing and hyposensitization treatment of ant-sensitive patients.

APPENDIX A

A KEY TO THE SUBFAMILIES OF THE FAMILY FORMICIDAE

1. A distinct constriction between the first and second gastric segments. If this is faint, the mandibles are linear or (and) the petiole is formed into a conical dorsal spine. . . Ponerinae

1'. Without constriction between the first and second gastric segments 2

2(1'). Abdominal pedicel composed of two segments 3

2'. Abdominal pedicel composed of one segment. 5

3(2). Frontal carinae located very close to each other, and the antennal insertions fully exposed when the head is viewed from above. 4

3'. Frontal carinae not located close to each other, and each often bearing a lobe which partially or wholy covers the antennal insertion when the head is viewed from above. . . . . .Myrmicinae

4(3). Eyes very large, reniform or suboval, ocelli usually present . . . . . .Pseudomyrminae

4'. Eyes either absent or else vestigial; if present, ocellus-like. No ocelli. . . . . Dorylinae

5(2'). Cloacal orifice distinctly circular and usually surrounded by a fringe of hairs. . . . . .Formicinae

5'. Cloacal orifice slit-like, the hairs, when present, not forming an encircling fringe . . . . . .Dolichoderinae

APPENDIX B

A KEY TO THE GENERA OF THE SUBFAMILY MYRMICINAE*

1. Antennae with 10 segments . . . . . .*Solenopsis*

1'. Antennae with more than 10 segments 2

2(1'). Antennae with 11 segments . . . . . .*Acromyrmex*

2'. Antennae with 12 segments 3

3(2'). Spurs of each middle and hind tibia very distinctly pectinate . . . . . .*Pogonomyrmex*

3'. Spurs of each middle and hind tibia simple or absent, very rarely with a few barbules but never pectinate. 4

4(3'). Antenna with a distinct 3-segmented club, worker caste dimorphic (rarely polymorphic) with the head of the major disproportionally large . . . . . .*Pheidole*

4'. Antenna without a 3-segmented club, worker caste monomorphic, or if polymorphic, the head of the major is not disproportionally large. 5

5(4'). Thoracic dorsum with the mesoepinotal suture absent or very faintly indicated . . . . . .*Novomessor*

5'. Thoracic dorsum with the mesoepinotal suture well-marked. . . . . .*Veromessor*

*Based on the collection of the Entomology Department, University of Arizona.

APPENDIX C

LIST OF ANTS IN TUCSON, ARIZONA*

Subfamily DORYLINAE

Genus NEIVAMYRMEX Borgmeier

1 Species Neivamyrmex harrisii (Haldeman)
2 Neivamyrmex mojave (M. R. Smith)
3 Neivamyrmex nigrescens (Cresson)

Subfamily PONERINAE

Genus ODONTOMACHUS Latreille

4 Species Odontomachus haematoda desertorum Wheeler

Subfamily PSEUDOMYRMINAE Emery

Genus PSEUDOMYRMEX Gue'rin

5 Species Pseudomyrmex apache Creighton
6 Pseudomyrmex pallidus (F. Smith)

Subfamily MYRMICINAE

Genus POGONOMYRMEX Mayr

Subgenus POGONOMYRMEX Mayr

7 Species Pogonomyrmex (P.) barbatus (F. Smith)
barbatus-complex
8 Pogonomyrmex (P.) bicolor Cole
barbatus-complex
9 Pogonomyrmex (P.) desertorum Wheeler
barbatus-complex
10 Pogonomyrmex (P.) rugosus Emery
barbatus-complex
11 Pogonomyrmex (P.) occidentalis (Cresson)
occidentalis-complex
12 Pogonomyrmex (P.) californicus (Buckley)
maricopa-complex

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*Based on the collection of the Entomology Department, Univ. od Arizona.

13 Pogonomyrmex (P.) maricopa Wheeler
maricopa-complex

Subgenus EPHEBOMYRMEX W.M. Wheeler

14 Species Pogonomyrmex (E.) pima Wheeler

Genus NOVOMESSOR Emery

15 Species Novomessor cockerelli (Andre)

Genus VEROMESSOR Forel

16 Species Veromessor pergandei (Mayr)

Genus PHEIDOLE Westwood

Subgenus Pheidole Westwood

17 Species Pheidole (P.) rhea Wheeler
macropheidole-group
18 Pheidole (P.) subdentata Pergande
fallax-group
19 Pheidole (P.) xerophila tucsonica Wheeler
subarmata-group

Genus SOLENOPSIS Westwood

Subgenus SOLENOPSIS Westwood

20 Species Solenopsis (S.) xyloni Mc Cook

Genus ACROMYRMEX Mayr

Subgenus MOELLERIUS Forel

21 Species Acromyrmex (M.) versicolor versicolor (Pergande)

Subfamily DOLICHODERINAE

Genus IRIDOMYRMEX Mayr

22 Species Iridomyrmex pruinosus analis (Andre)
23 Iridomyrmex humilis (Mayr)

Genus FORELIUS Emery

24 Species Forelius foetidus andrei Forel

Genus DORYMYRMEX Mayr

Subgenus CONOMYRMA Forel

25 Species *Dorymyrmex (C.) bicolor* Wheeler
26 *Dorymyrmex (C.) pyramicus pyramicus* (Roger)

Subfamily FORMICINAE

Genus CAMPONOTUS Mayr

Subgenus TANAEMYRMEX Ashmead

27 Species *Camponotus (T.) acutirostris* Wheeler
28 *Camponotus (T.) sansabeanus sansabeanus* (Buckley)

Subgenus MYRMENTOMA Forel

29 Species *Camponotus (M.) sayi* Emery

Genus PARATRECHINA Motschoulsky

Subgenus PARATRECHINA Motschoulsky

30 Species *Paratrechina (P.) longicornis* (Latreille)

Subgenus NYLANDERIA Emery

31 Species *Paratrechina (N.)* sp.

Genus MYRMECOCYSTUS Wesmael

32 Species *Myrmecocystus mexicanus mexicanus* Wesmael
33 *Myrmecocystus melliger melliger* Forel
34 *Myrmecocystus mimicus* Wheeler

Genus FORMICA Linnaeus

Subgenus PROFORMICA Ruzsky

35 Species *Formica (P.) perpilosa* Wheeler

Subgenus FORMICA Linnaeus

36 Species *Formica (F.)* sp.
fusca-group

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