CONTENTS

Guidelines for Contributors ........................................................................................................... ii

Proceedings

23rd SOVE Annual Conference, Sparks (Reno), Nevada
Symposium: Delusions of Parasitosis
18 November 1991

Delusions of Parasitosis: A Symposium; Coordination Among Entomologists, Dermatologists, and Psychiatrists ................................................................. J. P. Webb 1
Cryptic Arthropod Infestations: Separating Fact from Fiction .................................................... J. H. Poorbaugh 3
The Clinical Presentation. Diagnosis and Treatment of Delusions of Parasitosis—A Dermatologic Perspective .................................................................................................................. C. S. Koblenzer 6
Case Histories of Individuals With Delusions of Parasitosis in Southern California and a Proposed Protocol for Initiating Effective Medical Assistance ..................................................... J. P. Webb 16

7th SOVE Annual European Branch Meeting, Bologna, ITALY
25 August 1992

The Use of an Insect Activity Monitor in Behavioral Studies of the Flea, Xenopsylla cheopis (Rothschild) ................................................................................................................................. F. Clark, M. T. Greenwood, and J. S. Smith 26

24th SOVE Annual Conference, San Francisco, California
16 November 1992

Critical Issues in Vector-Borne Disease ......................................................................................... A. R. Barr 33

Scientific Note

Plexiglas™ Box System for Mosquito Rearing ................................................................................... J. Olejnicek 38

Submitted Papers

Prevention of Mosquito Production at an Aquaculture Wastewater Reclamation Plant in San Diego Using an Innovative Sprinkler System ................................................... R. Ebipane, E. Heidig, and D. W. Gibson 40
New Jersey's Approach to Encephalitis Prevention ........................................................................... W. J. Crans and L. J. McCuiston 45
A Comparison of Abdominal Scale Patterns in the Mosquito Aedes aegypti ........................................ R. E. Duhrkopp, W. K. Hartberg, and R. Novak 49
The Impact of Environmental Factors on the Efficacy of Bacillus sphaericus Against Culex pipiens ......................................................................................................................... N. Becker, M. Ludwig, M. Beck, and M. Zgomba 61
An Ixodes scapularis (Deer Tick) Distribution Study in the Minneapolis-St. Paul, Minnesota Area ....................................................................................................................... D. F. Neitzel, J. L. Jarnefeld, and R. D. Sjogren 67
Guidelines for Contributors

The Bulletin of the Society for Vector Ecology is an international journal concerned with all aspects of the biology, ecology, and control of arthropod vectors and the interrelationships between the vectors and the disease agents they transmit. The journal publishes original research articles and research notes, as well as comprehensive reviews of vector biology based on presentations at Society meetings. All papers will be reviewed by at least two referees who are qualified scientists and who recommend their suitability for publication. Acceptance of manuscripts is based on scientific merit and is the final decision of the editor, but these decisions may be appealed to the Editorial Board.

Scientific contributions should be sent to Dr. Marc J. Klowden, Editor, Division of Entomology, University of Idaho, Moscow, Idaho 83843, U.S.A. Manuscripts must be double spaced on a single side of bond paper with 25 mm margins. An original and two clear copies are required. Draft mode dot matrix type should not be used. Submission of text on a 3-1/2" computer diskette formatted in MS-DOS is encouraged. Microsoft Word, Word Perfect, or Wordstar formats are acceptable, as well as unformatted text files. Please indicate the type of format on the diskette label. Papers must be organized under the following headings, each on a separate page, in order: Title page, abstract, text, acknowledgments (if appropriate), references cited, tables, figure legends, and figures. The title page should contain the names of all authors and their affiliations and the identification and address of the corresponding author. Pages should be numbered consecutively starting with the title page. References should conform to the style in recent volumes. Illustrations that are submitted must be clearly labeled and legible after reduction.

Page charges, which partially defray the cost of publication, are $35 per printed page. Reprint charges are shown in the table below.

<table>
<thead>
<tr>
<th>Pages</th>
<th>1-4</th>
<th>5-8</th>
<th>9-12</th>
<th>13-16</th>
<th>17-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 copies</td>
<td>$50.00</td>
<td>$95.00</td>
<td>$140.00</td>
<td>$185.00</td>
<td>$230.00</td>
</tr>
<tr>
<td>or less</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Each add’l 50</td>
<td>$5.75</td>
<td>$11.50</td>
<td>$17.25</td>
<td>$23.00</td>
<td>$28.75</td>
</tr>
<tr>
<td>copies Same order</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DELUSIONS OF PARASITOSIS: A SYMPOSIUM; COORDINATION AMONG ENTOMOLOGISTS, DERMATOLOGISTS, AND PSYCHIATRISTS

J. P. Webb, Jr.

It may generally be stated that if one remains in the vector/pest control aspect of public health for any reasonable length of time, one will doubtless meet and possibly have the opportunity to assist an individual who suffers from the condition referred to medically as Delusions of Parasitosis (DOP). The DOP syndrome is defined by a set of descriptive characteristics (see Kushon, Koblenzer, and Webb, this volume) undertaken by an unshakable belief or conviction that living organisms (e.g., mites, "bugs," or worms) are living upon and/or within the body. This mental state persists even when consistent attempts to confirm the presence of parasitic specimens fail to demonstrate the existence of contributory organisms. Originally described by the French dermatologist Thibierge (1894) in the late 1800's, DOP was prominently characterized by the Swedish researcher Ekborn (1938) giving the syndrome international recognition. In the United States Wilson and Miller (1946) coined the term "Delusions of Parasitosis" and dispelled the use of the terms "acarophobia," "entomophobia," and "parasitophobia," which are mental conditions characterized by a fear of mites, insects, and parasites, respectively, a symptom that is usually not a component of DOP. Schrut and Waldron (1963) and Waldron (1963) further emphasized the fact that DOP does not involve a fear of insects or mites but suggested that an encounter with a real arthropod infestation may "trigger" the DOP syndrome. Keh (1983) more recently has evaluated the DOP phenomenon and has provided an excellent account of interactions between DOP victims and medical entomologists. Included in his discussion is the important suggestion to have the complainant contact his/her physician through whom "bug" specimens may be further evaluated in cooperation with the entomologist.

Until recently (Kushon, this volume), the medical community considered DOP to be a rare condition, basing this conclusion upon case studies statistics assembled from psychiatric sources. Kushon (op. cit.) surveyed a broad group of entomologists in the United States with questionnaires and obtained results that indicate that DOP is indeed more common than previously thought.

The contact at the Orange County Vector Control District Laboratory with numerous DOP diagnosed individuals (10-20 cases per year) during the past decade led to the establishment of a protocol of assistance for these people. This common endeavor brought together Dr. Don Kushon, Dr. Caroline Koblenzer, Dr. John Poorbaugh, and Dr. James Webb for a symposium focusing on various aspects of DOP. Dr. Poorbaugh, a medical entomologist formerly with the California State Department of Health Services, presented a review (this volume) of an "infested" DOP case considered for a long time to be a real situation of mite infestation. This case dramatically illustrates the extent to which an affected individual will go to prove that their infestation is real (Traver 1951) even to the point that reputable researchers (Baker et al. 1956, Baker 1958, Evans et al. 1961) will unwittingly provide false support for the DOP victim. Dr. Koblenzer (this volume), a dermatologist in private practice in Philadelphia, made an excellent presentation regarding the diagnosis and treatment (from a dermatologist's viewpoint) of DOP. Dr. Kushon, who is a psychiatrist in residence at Hahnemann University (Philadelphia), presented (this volume) the centerpiece of the symposium by effectively illustrating the extent of DOP incidence, the involvement by the entomologist, the referral for medical assistance, the need for an interdisciplinary approach including the entomologist and the physician, and the suggestion that DOP clinics be established. Dr. Webb (Orange County Vector Control District) followed with a presentation (this volume) directed toward the development of a protocol of entomologist/patient interaction to assure relevant assistance leading to consistent and competent medical/psychiatric treatment, if needed.

The future for physicians, public health biologists, entomologists, parasitologists, and others in related disciplines appears to be full of opportunities for contributions in this new approach to assisting individuals afflicted with DOP.


2Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92643, U.S.A.
REFERENCES CITED


CRYPTIC ARTHROPOD INFESTATIONS:
SEPARATING FACT FROM FICTION

J. H. Poorbaugh

The difficulties of dealing with persons who claim they are being bitten or are infested by unseen insects or mites is familiar to entomologists who have contact with the public. When the cause of the injuries or complaints is an actual insect or mite, the bite reactions and physical circumstances, such as time of day or location, will most times be evident to entomologists familiar with the various biting arthropods in their local vicinity and region of the country. In California the possibilities are rather limited, and the attack syndrome is characteristic. For example, cat fleas generally leave a row of bites as they probe the human skin in vain for a blood meal. Rat mites leave hard discrete bite reactions near the belt or stocking line and are usually seen by the victim. Pet fur mites (Cheyletiella sp.) are tiny but characteristic in attack syndrome, and the victims notice bites on the arms or exposed skin right after holding the infested dog or cat in their lap. Real scabies is diagnosed by the physician and modern treatments are effective.

When the cause of the supposed infestation is not immediately evident and the victim makes elaborate claims about the supposed organism's behavior that do not fit the realities of any known arthropod's morphology and capability, then the investigator should be able to conclude that the cause of the problem lies with something other than an arthropod. It is at this point that entomologists will many times state that they don't know what the problem is, but that the affected person should keep on the lookout for some unknown creature that remains hidden from discovery. This lack of confidence in an opinion that an arthropod is not involved is understandable. The entomological and medical literature is pervaded with cautions about cryptic or hidden arthropod infestations and creatures which may persist in the human body despite repeated treatments. This uncertainty on the part of the entomologist makes it difficult for the dermatologist or the physician to determine the actual cause of the problem, whether physical, or as is many times the case, delusional.

What is the basis of this concept of cryptic infestation by rare mites? The story begins in Russia in 1864 when a pioneer acarologist, A. Bogdanow, was examining mites collected from debris from the skin of a crusted scabies patient and found a "new" mite, which he implied could be pathogenic because of its association with the pathological condition. He named it Dermatophagoides scheremetewskyi, a "skin-eating like" creature. Subsequently, this "rare" mite was reported from human urine and sputum samples and other unusual sources, such as rodent and bird nests. Compilations in the 1930s through the early 1950s devoted considerable space to these rare "parasitic" mites. Then in 1951, one of the most often quoted papers in the medical entomological literature was published, in which a biologist (Jay Traver, 1951) described in detail the invasion of her body by this rare mite and her incredibly determined effort to prove she was infested despite opinions to the contrary by entomologists and physicians. This paper has served as the basis for developing the feeling of uncertainty in otherwise competent entomologists that we could be wrong, that there is a creature lurking out there that defies observation and control.

Let us put this information into the perspective of the facts as known today. What is the basis of this mysterious infestation of Ms. Traver with this rare mite, and how could it be that the creature could not be controlled with chemicals? Here the reader is referred to the 25 page description of her problem published at the request of Dr. E. W. Baker, a paper which must be one of the most remarkable mistakes ever published in a scientific entomological journal. It is a detailed documentation of a classic case of delusory parasitosis, which has continued to cloud the recognition of this condition to this day. The unfortunate woman describes her invasion and suffering and her utter frustration when physicians and dermatologists told her she was not infested. She used at least 16 different harsh chemicals and poisons on her body repeatedly to rid herself of these imaginary creatures, some of which were new synthetic organic insecticides furnished to her by Gaines W. Eddy of the USDA. Jay Traver died in 1974 and never was given the proper advice and treatment for her real disease, delusory parasitosis.


2California Department of Health Services, CA 95814, U.S.A.; Present Address: 8697 Holmes Ln., Winters, CA 95694, U.S.A.
How could these circumstances come about that a competent biologist could be misled by the expert acarologist of the day into believing that these cryptic mites existed? The information is finally clarified in the most scholarly work on human ectoparasites that exists today, that of John O'Donel Alexander in his 1984 book entitled *Arthropods and Human Skin*. Dr. Alexander points out that *D. scheremetewskyi* is synonymous with the common house dust mite and that the contemporary acarologist, Alex Fain, has examined the specimens found by Jay Traver and identified them as *D. pteronyssus*. Now the reader must remember that the very existence of house dust mites as the most common arthropod inhabitant of the human household environment did not become apparent until 1966 as a result of the work of Fain. The specimens reported by Bogdanow and in human laboratory samples were simply contaminants of the equipment. Old mattresses were full of these mites. If Bogdanow had looked in the patient's mattress he would no doubt have found thousands of his new and "rare" mites. The equipment closets where clean urinals and sputum sample container vessels were kept were dusty, and rodent and bird nests were collected in dusty paper sacks.

It becomes obvious that the cotton swabs used by Jay Traver occasionally contained a mite which she eventually discovered after long hours of searching. If she had examined her mattress dust or rugs she would surely have found this common but as yet "undiscovered" mite. How ironic that she was probably virtually surrounded by dust mites whose existence remained unknown to biologists for at least ten more years.

In conclusion, it is now evident that *D. scheremetewskyi* is a classic mythical beast. No factual publications exist to show that unknown or rare mites lurk about and occasionally infest humans and account for real mites being involved instead of the victims' delusion. There is no substantial evidence that house dust mites can bite or invade the human body, and therefore no basis for the admonition to students that we always need to be cautious in concluding that an arthropod is not involved because Jay Traver in 1951 "proved" that a rare cryptic mite attacked her despite expert opinion to the contrary.

The entomologist should have confidence in an opinion that no arthropod is involved when circumstances so warrant. The affected person should then be informed of this opinion and referred to a family practice physician. Information about delusory parasitosis should be made available to the affected person, as appropriate, and definitely to the physician if the opportunity exists.

In my experience, a confident opinion by the entomologist is very important. It only serves to deepen the suspicion of the affected person when the entomologist says something to the effect that, "Didn't find anything. I don't think an arthropod is involved, but, just in case, I want you to take this sticky tape and these sample bottles and if you do find something, you can then bring it to me." Such advice only serves to exacerbate the problem, not lead to a solution. My approach to finalizing an opinion to a person whom I have concluded is not being affected by an arthropod is to state, "It is my opinion that an insect or mite is not responsible for your problem, but this happens to people. It can seem very real to you that it's a mite or parasite of some kind but our skin and bodies can fool us. I suggest you see a family doctor and if he or she does find something unusual, they can submit it to me. Please let me know when you get the appointment and who it is with and I will furnish your doctor with information." I then contact the physician, as appropriate, and provide information about delusory parasitosis and my opinion that a real arthropod is not involved. This serves to orient the outcome of the case in the right direction.

**ANNOTATED BIBLIOGRAPHY**


The most complete and scholarly work on the subject available today. He has published separately on the mite content of house dust. In regard to Traver, he states that:

"The clinical description of the eruption and its behavior over the years was that of a classic neurodermatitis upon which was superimposed a delusion of cutaneous parasitosis."


A work which is still used for reference and strongly builds a case for cryptic pathogenic mites which we now know is based on false assumptions about the common house dust mite. It predates the "discovery" of house dust mites by about ten years.


A work developed for the urban entomologist and
professional pest control operator in California, and required reading for the medical entomology student. On page 617 the following caution has done much to perpetuate the myth of cryptic arthropods and confuse the entomologist who otherwise might have confidence in an opinion that no arthropod is involved:

"An example of how obscure a real pest problem can be is illustrated by a report by J. R. Traver (1951) on an infestation of mites, Dermatophagoides scheremetewskyi Bogdanow, on her own person, resulting in itching red papules on scalp, eyes, ears, nostrils, shoulders, under the arms, beneath the breasts, on the chest, both upper and lower back, and occasionally around the umbilicus. Other members of her family were likewise infested. The ailment was initially diagnosed by a physician as "psychoneurotic." Fortunately, the victim was a zoologist, had access to a microscope, and found the causative agent. Many attempted treatments failed to eradicate the infestation. However, attacks by the implicated mite species are extremely rare."


We can attribute to Fain the discovery of the common house dust mite and its status as the source of the main allergens in human asthma. Also in this paper he reported his opinion of the true identification of the mites found by Traver and blamed the dusty environment in her home laboratory for the contamination of her cotton swabs or scalp.


Fisher was consulted on the Traver case and states here that the mites were probably casual contaminants. Also we now know that house dust mites feed mainly on the fungus that in turn grows on human skin scales. This supports his assertion here that the Dermatophagoides found in scaly skin of scabies patients were simply browsing for food and not involved in the pathology.


The "bible" for the pest control operator. On page 757 of this sixth edition is found the following caution for the professional reader:

"Traver (1951) reports a scalp and head infestation by the rare mite, Dermatophagoides scheremetewskyi Bogdanow. Local physicians were unsuccessful in discovering the causal agent and diagnosed the ailment as "psychoneurotic." Also, see Baker et al. (1956). Traver, a zoologist, using a microscope, finally found the "imaginary" mites."


Chosen as an example of how a supposedly scholarly publication can perpetuate a myth based on misinformation in older works. This publication devotes a whole subchapter to Dermatophagoides scheremetewskyi and a photograph of one of the mites found by Traver. Should the reader believe the information is true, the following quote indicates how the entomologist and physician would be very cautious of cryptic mite infestations."

"In attacks on humans, Dermatophagoides scheremetewskyi burrows into the dermis, forming runways. The skin reacts by becoming thickened over the infected areas and by developing small, itchy red papules. In a well-documented case of a woman with mites (Traver, 1951), they moved to all parts of her body, but concentrated and persisted for 31 years until the patient's death in 1974, on the head, especially the scalp, the nostrils, eyes, and pinnae. Besides itching, the woman reported a general feeling of something crawling over her, which she attributed to the mites moving in their dermal "highways." In a few other case reports, pathogenic fungi accompanied the mites."


A zoologist can have delusory parasitosis as well as anyone, only she had a microscope and was able to document her imaginary infestation in an otherwise reliable scientific journal. Readers are encouraged to obtain this paper to gain an insight into the incredible syndrome of delusory parasitosis.
THE CLINICAL PRESENTATION, DIAGNOSIS AND TREATMENT OF
DELUSIONS OF PARASITOSIS—A DERMATOLOGIC PERSPECTIVE

C. S. Koblenzer

Our contact with patients who have delusions of parasitic infestation, is not unlike the Indian mythical tale of the four blind men and the elephant. Each of use—dermatologist, psychiatrist, and entomologist—sees the problem from our own perspective, and perhaps a little differently, but each has much to learn from the other. Our discussion today can only help us to deal more empathetically and more effectively with this very troubled group of individuals. So one-sided does one tend to be, that it wasn’t until Don Kushon undertook the survey, that he has just presented (see this volume), that I had any idea whatever of the extent to which these patients impact the professional life of the entomologist.

Because the skin serves important emotional functions in infancy (Greenacre 1958, Hartman et al. 1946) and because it is so very visible, accessible, and available for use in the formation of unconscious symbols, we find it rather frequently participating in psychosomatic phenomena (Koblenzer 1990). A number of primarily psychiatric disorders present with cutaneous symptoms. But because patients with these particular problems, at an unconscious level, have a need to deny psychiatric illness, they often will consult first the entomologist or the dermatologist, while the psychiatrist is their last resort (Gould and Gragg 1976). It is for this reason that those of us who are non-psychiatrists, must be prepared to recognize the nature of the disorder; we must also develop some skills, if not actually in treating the patients, at least in ensuring that the patients can permit themselves to consult someone who is competent to provide effective treatment. In this, the entomologist and dermatologist are often very much the same boat; we recognize that something is very seriously wrong but we are not always certain how best to proceed. I will describe delusions of parasitosis in this broader context.

Other psychiatric conditions that may present to the dermatologist are: factitious disorders, or dermatitis artefacta, dermatologic expressions of obsessive-compulsive pathology, and some depressive equivalents (Koblenzer 1992). In dermatitis artefacta, the patient produces damaging lesions on his or her own skin. For example:

A patient applied a lighted cigarette to nickels on her skin to produce a puzzling, blistering eruption.

A man rubbed in a caustic.

A man poured on a caustic liquid (the linear streaks are the give-away).

While a little girl dug at her skin.

Patients with this condition do not acknowledge that they are responsible nor do they consciously understand why they do it. Unconscious needs and wishes motivate them.

The second group of patients are those with dermatologic expressions of obsessive-compulsive pathology. These patients may have an uncontrollable and incomprehensible need to pull out their hair or eyebrows, pick their skin, or they may have uncontrollable and intrusive worries.

If those worries concern the possibility of an infestation, then we must make a very clear distinction between this problem, and a true delusion of infestation.

A delusion is defined as a false belief, that is not consistent with the patient’s intelligence, educational level or cultural background, and that cannot be corrected by reasoning (Yager 1989). When patients present with delusions, those patients suffer from a psychosis, or “thought disorder.”

So, the patient who comes to you with a false conviction that their house, or their person, is infested with parasites, is psychotic. No argument that you can bring to bear will convince them that there is no infestation.

Patients may present to the dermatologist with a variety of different delusional beliefs, for example, that their skin is degenerating, that their hair is growing inwards instead of outwards, or that offensive or toxic odors are emanating from their bodies. These all represent a monosymptomatic hypochondriacal psychosis (Munro and Chimara 1982).

A lady, for example, had the absolute, unshakable conviction that the odor from her feet permeated her entire high-rise office building,


21812 Delancey Place, Philadelphia, PA 19103, U.S.A.
causing people to shun her, and quit their jobs.

Another lady’s concern was laser beams from the adjacent apartment that distorted her facial contours. She had mapped out the lines of force, and their effects.

But of all delusional beliefs, those of parasitic infestation are the most common.

In 1894, Thieberge, a French dermatologist, first described the Syndrome as a “phobia” (Thieberge 1894). Not being a psychiatrist, he was confused about terminology. Patients do have phobias about parasitosis, but those patients are terrified of bugs; they will do anything to avoid contact with bugs, or situations where bugs might be found. They will refuse to shake hands, lest they become contaminated, avoid crowded places, shun public toilets, and ultimately perhaps, even refuse to leave the safety of their own home (Koblenzer 1987). They do not, however, believe that they, themselves, are actually infested. It was left to Ekibom, a German psychiatrist, in 1938, to define the Syndrome more accurately, and give it the name by which it is now known: Ekibom’s Syndrome (Ekibom 1938).

Anika Skott, in a comprehensive monograph in 1978 (Skott 1978), described acute and chronic phases of the disorder. In clinical practice, we tend to see patients in the acute phase. The more chronic patients generally have come to terms with their symptoms and have learned to live with them.

Clinical Picture

The clinical picture is one with which entomologists as well as dermatologists are all too familiar (Koblenzer 1987).

The patient, most characteristically a female, is anxious to the point of agitation. Rather surprisingly, apart from her specific complaint, she appears to be entirely rational, and she functions adequately in other areas of her life. She approaches with a logic that is consistent, the steps she feels necessary to protect her family, and eradicate her problem. One patient of mine had burned all her clothing including an expensive mink coat. She had thrown out her upholstered furniture, and was living in a single room, with a cot, and one hard wooden chair and table—furnishings that she thought un receptive to infestation. She had retained two sets of clothing only; one of these was immediately sealed in a plastic bag after laundering, and kept sealed until she needed it, when the other set was being in the wash. The patient believed herself to have been infested with a cloud of mites that blew from the air conditioner of her car; for this reason, she had completely given up driving.

The sensations that patients describe are more frequently “crawling” under the skin, tickling, sharp bites, or stings, than itching (Lyell 1983). Reimer has proposed the term “haptic hallucinosis” for this hallucinatory experience (Reimer 1970); it may be felt on, within, or beneath the skin. The patient invariably has intimate knowledge of her own specific parasites, which are referred to in proprietary and familiar terms. Three possible life-styles commonly are elaborated: the organisms may “burrow” into the skin; they may be trapped under the surface from whence they struggle violently to emerge; or they may descend upon the patient as an overwhelming plague.

Such things as lice, scabies, pediculi, beetles, flies, mites, cockroaches, undefined “things,” new and as yet undescribed species of bugs, and worms are among the variants described. One patient of mine about whom we shall hear later, harbored an organism shaped like a flounder: he brought a microscope to my office in order to show it to me. Sometimes there is a complaint of “things” falling out of beard or hair, or jumping on the surface of the skin. Another patient harbored worms around her eyes; these would travel to her mouth when she was eating, to compete for her food. Apparently, they were very finicky eaters, and would move around violently if they did not like what she offered them; her diet was governed by the need not to displeasethem, and she was thin as a skeleton.

A consistent and diagnostic feature is the patient’s absolute conviction that he or she knows exactly what is going on; consistent, also, is the patient’s anger and contempt for an incompetent physician who cannot even see, let alone eradicate the pests the patient “knows” to be present. Behind the evident anxiety, hostility and aggression are barely concealed.

Cutaneous symptoms may precede the patient’s dawning interpretation that he or she is infested, and in a percentage of cases there may have been a valid infestation in the recent or distant past. “Specimens” are frequently brought in, as we see here, in tins, glass jars, paper tissues, or stuck between two layers of adhesive paper tape. These consist of fragments of skin, hair, fiber, lint, paper, crusts, amorphous debris, or tiny flies or other insects; one patient was kind enough to bring for me her own three-day-old feces, in which she detected creatures and their eggs. The history has always a persuasive, if idiosyncratic, logic, and the patient may be so convincing, that in as many as one third of cases, one or more family members acquire the same symptom in a folie a deux, or folie partagee, a shared delusion.

It is not unusual for a complex life-cycle to be ascribed to the parasite: complicated and detailed diagrams may be provided, with seemingly rational explanations as to why conventional methods of
treatment, and of pest control have not been successful. Common explanations are that the parasites hide under the surface to escape eradication; that they come out only at night; that although adult forms are killed, the eggs escape; or that the species is new, as yet unknown and undescribed, and therefore resistant to current methods of eradication (Lyell 1983).

Group outbreaks have been reported from a number of occupational facilities. These have been attributed to "phantom biters" and are believed to have a specific though often undetermined cause. Lyell notes that such outbreaks most commonly follow periods of high temperature and high humidity, and may be caused by fragments of carpet fiber, or glass fiber (Lyell 1983). I recall one patient who may have been a victim of just such a circumstance; she claimed that she and many of her co-workers were bitten by mites that infested the fiberglass covering of the telephone cables, in the exchange where she worked.

How do we know that this is a delusion?
How do we know that there is NOT, in fact, an infestation?
When a patient has a valid infestation, no matter what the parasite, initially there are no symptoms and no lesions.

It is only when the host has mounted an immunologic response to the saliva, or other products of the parasite, that symptoms develop. These symptoms result from that immunologic reaction (Rook 1986).

In a valid infestation therefore, if the patient is symptomatic, there will be a primary cutaneous eruption. The intensity of that eruption will vary, according to the level of immunologic reactivity, in the host.

Early lesions take the form of transient urticarial wheals. Later, we see the characteristic dermal papules of the delayed type of hypersensitivity reaction - so-called "papular urticaria". When the reaction is intense, bullae, will form.

The location and arrangement of lesions, helps to identify the culprit. Pet fleas may attack the extremities, but often they get under clothing, to produce grouped pink papules—often in groups of three—the so-called "breakfast-lunch-and dinner" configuration.

In scabies, lesions are concentrated on the web-spaces of hands and feet, axillae, and genital area (Rook 1986).

Burrows can be identified, and mites, eggs, and feces, visualized microscopically in the scrapings. The patient with scabies is intensely itchy, and extensively excoriated.

With head or pubic lice, the parasites themselves can be visualized with the naked eye, localized itching is intense, and nits can be identified on hair or eye-lashes (Rook 1986).

In contrast, when the infestation is delusional, visible cutaneous lesions vary according to the particular activities attributed to the organisms. Where insects "descend like a plague," or "burrrow into the skin," there may be no lesions, minor scratch marks, or a nonspecific dermatosis that is attributed to the infestation. Attempts are often made to "let out," those that live under the surface, and lesions may consist of circular excoriations, deeply gouged pits, or even knife slashes. Not infrequently, in an attempt to demonstrate the creature in the office, the patient will pull out stringy fragments of follicular epithelium with a pair of tweezers for the edification of the physician. Lesions secondary to frenetic and obsessional attempts at cleansing and decontaminating the skin, are seen in irritant or chemical dermatitis. A recent patient had scrubbed her skin daily with bleach and steel wool for many months, while another frequently applied gasoline.

**Incidence**

No figures are available for the actual incidence of delusional parasitosis, but the condition is said to be uncommon.

Although the onset may occur at any age, from the late teens into old age, the highest incidence is in the decades between 50 and 80, with a peak in the late 60's.

Women are most commonly affected. The male to female ratio is 1:1 under the age of 50, and 1:3 over 50 years. Many patients are found to be isolated and lonely, and some degree of auditory or visual impairment is not uncommon (Koblenzer 1987).

So in our experience, the usual picture is of an elderly, slightly deaf old lady who lives alone, seldom ventures out, withdraws from family and friends, and will find any excuse not to pick up her brand new grandchild, much to the distress of her family.

Even under normal circumstances, there is a tendency for the skin in the elderly to be dry, flaking, and predisposed to itching or "sticking" sensations; static electricity is reported to cause similar dyesthesias. Certain medical conditions such as pellagra (Aleshire 1954), Vitamin B12 deficiency (Pope 1970), and amphetamine or cocaine addiction (Grinspoon and Bakalar 1985) can also cause similar cutaneous sensations. Against a psychotic matrix, these feelings may be incorporated into a complicated and convoluted delusional belief. A careful history will often reveal that there has been recent psychosocial stress, while psychologically, the specific delusional belief represents the projection onto the external parasite of unconscious
aggression and guilt (Fenichel 1945). Fear of contaminating friends and family will commonly have led to poor self-esteem, depression, withdrawal, and social isolation. Suicidal thoughts are not uncommon, but the incidence of attempted or successful suicides is not known.

**Differential Diagnosis**

The differential diagnosis from a dermatologic standpoint is not too difficult. The characteristic story, the clinical presentation, the negative specimens, and the normal laboratory findings all support the diagnosis.

From the psychiatric standpoint, it is perhaps a little more complex because we need to differentiate between intense obsessional worries and true delusions on the one hand and the different delusional syndromes on the other. I will leave elucidation of these for Don Kushon in a later paper of this symposium.

**Treatment**

A great deal has been written about the difficulty in engaging these patients, and the importance of the dermatologist not immediately referring the patient to psychiatry (Grould and Gragg 1976, Torch and Bishop 1981). These patients are extremely sensitive and readily feel rejected. They also find the dermatologist, who fails to find the bugs they know are there, to be incompetent.

The therapeutic approach that I have found most effective, and with which I am most comfortable, is to empathize with the patient’s distress and frustration, acknowledging the steps they have taken to resolve their problem, and the extent to which the symptoms interfere in their everyday life. I examine the specimens they bring, firstly because it would seem disrespectful not to, but also because sometimes there truly is an infestation, and one would feel pretty foolish to have missed it (Koblenzer 1987).

I explain to the patient that I fully believe what they tell me about their experience, but that unfortunately I have not been able to confirm what they have described. I also remind them of the numerous steps they have taken, and all of the lab studies that have revealed nothing. When one asks how they explain these inconsistencies, wild and wonderful elaborations and theories invariably ensue. It is pointless to argue or try to persuade a delusional patient; there is no way he or she can be swayed, and the physician will only bring contumely upon his or her head. The delusional belief serves an unconscious purpose for the patient, and he or she dare not give it up. Painful though it is, the false belief is often less frightening to the patient than the reality-based pain that it replaces (Koblenzer 1987).

Sometimes the inexperienced will be tempted to agree with the patient, thus, colluding with the delusional belief. Such a step leads only to trouble because the logical sequelae will be ever greater, more excessive and more destructive attempts at eradication, and ever more arcane and extensive tests for elucidation. Only those tests predicated by the story, or the clinical picture, should be undertaken, and then only one time each (Koblenzer 1985).

**Dermatologic Treatment**

Because the patient has great emotional investment in the skin, I usually allow him or her to maintain that focus, but I substitute positive healing measures for their prior destructive rituals. Supervision of topical treatments through frequent, even quite short office visits, serves to allow a supportive and accepting relationship to develop. Hopefully, this will gradually allow the patient to accept either oral medication or a referral to a psychiatrist (Koblenzer 1987). I will leave discussion of these topics to Don Kushon, in the next presentation (this volume).

**Prognosis**

It is very clear that unless we can find a way to enable these patients to accept appropriate psychiatric treatment, conditions are not going to change. Over time, the condition will become chronic, the patient will cease to search for solutions, and gradually adjust to their idiosyncratic and restricted life-style. With treatment, 80 percent will experience symptomatic relief, though 10 percent may have a post-psychotic depression (Munro and Chimara 1982).

**REFERENCES CITED**


DELUSIONS OF PARASITOSIS: A SURVEY OF ENTOMOLOGISTS FROM A PSYCHIATRIC PERSPECTIVE

D. J. Kushon, J. W. Heitz, J. M. Williams, K. M. K. Lau, L. Pinto, and F. E. St. Aubin

Problems involving the overlap between arthropods and mental illness have long been known to the entomological profession (Pierce 1921, 1944, Smith 1934, Davis 1944, Miller 1954, Gage 1957, Pomerantz 1959, Howell 1960, St. Aubin 1981, Pennington 1986). In the medical literature Wilson and Miller (1946) first proposed the term "delusions of parasitosis" (DOP), to describe the particular mental illness in which patients have an unshakable belief that live organisms, such as mites or insects, are present in the skin. Waldron (1962), an entomologist, shortened this term to "delusory parasitosis," which is the name most familiar to pest control operators for this condition.

A variant of DOP involves group outbreaks of imagined arthropods in work settings usually among female employees. In their extensive monograph, The June Bug, Kerckhoff and Back (1968), describe such an epidemic involving 200 mill workers. Waldron (1972) introduced the term "illusions of parasitosis" into the literature to distinguish those cases in which a misperception or misinterpretation of real external sensory stimuli such as a chemical or mechanical irritant is the most prominent characteristic. In its various forms DOP remains a problem that regularly confronts entomologists (Pennington 1986) who often find themselves with only their own limited resources with which to work (Keh 1983).

DOP has mainly been described in sporadic case reports based on the narrow segment of patients who present to health care professionals. Wilson and Miller (1946) reviewed the 51 cases of the syndrome then published in the literature and described six original cases. Skott (1978) has estimated that about 401 cases of DOP have been reported worldwide. One of the largest series was reported by Schut and Waldron (1963) who saw 100 cases in five years, but unfortunately they were not systematically described. The purpose of this paper is to describe our study which was designed to tap into the population of subjects identified by 200 randomly selected entomologists, hypothesizing that they may see a population of patients not otherwise accounted for in the medical literature, and to determine the incidence of DOP encountered by entomologists. Demographics and phenomenology of the syndrome and the approaches taken by entomologists were also explored.

Estimated Incidence of DOP

In a period of 12 months, 156 cases were reported by the 86 entomologists in the present sample, that is 1.8 cases per entomologist per year. Based upon estimates from the Entomological Society of America (ESA), there are 1,736 entomologists who have contact with the public, either by working in the medical-veterinary field or as extension entomologists. Of the 156 cases reported in the present study, 94 percent were new cases within the past year. If this finding is extrapolated for the 1,736 entomologists cited above, then approximately 2,954 new cases of DOP are seen each year (11.8 new cases/1 million population/year). At least one case of DOP was seen by 52 percent of all the entomologists surveyed in the past year and 90 percent saw at least one case in their career. It is important to note that according to the ESA less than 10 percent of all pest control firms have an entomologist on staff. Therefore, it is likely that the 156 cases in our study represents 1 percent or less of the DOP cases actually seen by pest control operators.

The results challenge the opinion that DOP is a rare syndrome. Dohring (1960) first suggested that many cases of DOP may be moving unrecorded between nonmedical authorities such as environmental or hygiene agencies. This study supports this position and finds entomologists are often the first professional to have contact with these individuals. Most cases in the literature report duration of symptoms for at least one year (Ekblom 1938, Reilly and Batchelor 1986, Skott 1978). In contrast, the cases presented in this study were more acute which may suggest that entomologists may be seeing DOP earlier than physicians.

Demographic characteristics of DOP

The age of presentation varied widely. Women predominated with a ratio of 2:6:1. Among group outbreaks at work, one entomologist reported that usually the workers were all women. Caucasians accounted for


2Department of Mental Health Sciences, Hahnemann University, Philadelphia, PA, U.S.A.
the largest racial group. Most were married, living with family, employed, and at a lower middle class socio-economic level. Clerical or service workers were the most common occupation. Although no cases involving professionals were reported within the last year, cases involving a pest control firm owner, a retired surgeon, a dermatologist, two nurses, and a Ph.D. psychologist were seen in the past. An occupational risk was suggested by one entomologist who wrote, "I, myself, decided once that I must have scabies and it was difficult for me to accept an authoritative, reliable, negative diagnosis."

The Typical Description of DOP

The most common symptoms were tactile misperceptions of arthropods biting, burrowing, or crawling and visual misperceptions of arthropods jumping or crawling on the skin, scratching, and skin lesions. Bizarre beliefs defined as totally implausible, absurd ideas were reported relatively seldom. Examples include the belief that insects are coming out of common household items like toothpaste or all of one's orifices are being invaded by the alleged arthropod. Skin lesions included bite or blemish-like irritations. Besides scratching, other skin damaging behaviors included frequent washing or application of caustic agents. Shared beliefs were considered present when other people who were in contact with the presenting individual also complained of infestation. Half of the cases involved a shared belief in which family or coworkers at an office setting were most frequently involved. In one group outbreak a female supervisor reported to the entomologist that there was a relationship between the phase of the menstrual cycle and the number of bites that female employees were having. One entomologist reported encountering long lists of employee complaints about the working conditions and noted that the outbreaks were worse on Mondays and Fridays. The cases were mostly acute or subacute with 65 percent of cases less than six months in duration. Abnormal personality traits most commonly identified were exclusiveness and suspiciousness.

The Approach of the Entomologist

An inspection of the dwelling was performed in 87 percent of the cases. A true infestation was discovered in 13 percent of these cases. These included 2 cases each of scabies, head lice, crab lice, and fleas; and 1 case each of pigeon mites, sarcoptic mange, and psocids. Environmental factors were identified in 53 percent of cases including a variety of airborne particulate matter, heat, humidity, and static electricity. These findings were similar to that of Pinto (1989), Blum (1986), and Waldron (1972). Work settings encountered most commonly included office settings, computer rooms, dispatch rooms, production lines, and paper processing areas. Concerning work related group outbreaks, one entomologist observed that almost all women afflicted still wear nylon stockings during the summer. It was speculated that the stockings do not get washed enough and probably contain environmental and other types of irritants that cause itching. A psychosocial stressor was identified in 49 percent cases. The most common stressor noted was deterioration of health. One amputee and one blind person were noted. Other stressors reported were recent death in the family, marital or family discord, employment problems, loneliness, and financial problems. One case involved the death of a pet dog, and another case involved a mother-in-law's visit.

Of the entomologists surveyed, 59 percent provided the client with a straightforward, positive statement that no insects or arthropods were present while 8 percent complied with the clients belief as a palliative gesture. Despite the lack of an identified organism, 13 percent of cases were treated with pesticides such as Dursban®, Ficam®, Whitmire PT 565®, pyrethrin space spray, and Diazinon®. In addition, prior to seeing the entomologist, 56 percent of the cases had 1 to 6 previous pesticide treatments. No entomologist reported using only water in their spray. One entomologist who explained his decision to spray stated, "they wanted to smell something." Another entomologist said he may treat with a pesticide as a preventative measure. Several entomologists wrote that they were opposed to the use of placebos and cited that it was illegal. Some entomologists supplied their clients with literature from technical releases of the National Pest Control Association (NPCA). In that literature it is recommended to reassure the client that the sensations they feel are real while at the same time to not reinforce the delusional belief. Also this literature recommended reassuring the client that the condition is not communicable, that there is no reason to restrict social and family activities, and to not use pesticide sprays, harsh chemicals, or medications which are not specifically prescribed by a physician.

The cases treated with a pesticide in the absence of any identified arthropod in essence received a form of placebo therapy. The successful use of placebo therapy has been reported in the medical literature (Frankel 1983) but overwhelmingly the consensus is that it is temporary in effect, difficult to do, requiring great reinforcement and a strong personality in the health care provider, and would be likely to compromise the professional-patient relationship (Lyell 1983, Duke 1983). Lyell (1983), however, noted that for group
outbreaks there is a response with spraying even with plain water if done in office hours. Koblenzer (1987) has recommended letting the patient know you believe their symptoms are real without entering into collusion with them as to etiology. Musalek and Kutzer (1990) stressed that by confirming the patients’ belief in infestation, further treatment by another therapist is then rendered impossible because the patient will always refer to the “specialist” who confirmed their parasitic infestation.

Making a Physician Referral

In 61 percent of the cases an entomologist saw the individual before any physician had become involved. The entomologists referred 71 percent of the cases to physicians. Of the cases referred, 58 percent were to dermatologists, 22 percent to allergists, 17 percent to general practitioners, and 3 percent to psychiatrists. Some entomologists reported referring work related group outbreaks to an industrial or environmental hygienist if the problem persisted. One entomologist wrote, “I could lose my job if I referred a client to a psychiatrist even if I thought they needed one.” In contrast to the NPCA recommendation to institute a frank reply stating that no arthropods are present which could cause the symptoms, Webb (1991) reported an approach where the individual’s infestational belief was generally supported while recommending concurrent medical treatment and psychiatric counseling for stress resulting from the alleged infestation. This approach evolved through the recognition that a frank report that no arthropods were present followed by the entomologist disengaging himself from the case tended to result in the individuals continuing their quest with another pest control firm in the hopes of finding someone who could “understand” their problem. This approach attempted to get treatment for these individuals who would otherwise avoid physicians and resent suggestions that they see a psychiatrist.

Pooburgh (1991) reported another approach that divided afflicted individuals into two groups. One group consisted of individuals who the entomologist considered open minded enough to entertain the possibility of a psychological explanation for their problem. For these people the entomologist spent time explaining the phenomena of DOP, provided literature on DOP, and recommended a physician referral. The other group consisted of individuals who appeared more completely absorbed by the infestation. In this group the primary goal of the entomologist was to connect the individual with a primary care physician and then consult with the physician to educate them on DOP.

Regarding the few referrals to psychiatry, the lack of more floridly psychotic symptoms may partially explain this. Also, the stigma associated with psychiatric treatment perceived by both the individual with DOP and the entomologist result in few referrals. It is possible that not all individuals seen as having DOP by entomologists are actually delusional. Some, for instance, may have a preoccupation with the fear of having an infestation (entomophobia) and be able to acknowledge the possibility that their fear is unfounded. These individuals may be open to the entomologist’s statement of negative findings along with a psychological explanation.

The Need for an Interdisciplinary Approach

After referral to a physician, the medical outcome was known in 36 percent of the cases. Several entomologists indicated that better communication was needed between them and physicians. Of the entomologists surveyed, 59 percent found physicians to be helpful sometimes, 29 percent most of the time, 7 percent always, and 5 percent never.

Several entomologists reported that physicians are too quick to blame a patient’s problem on insects just from examining the skin without ever seeing any specimens. On the other hand one entomologist reported a case where a client was told by her physician that she was mentally unbalanced only to discover that she was actually infested with scabies. Several entomologists reported frustration about having no network of professionals to work with to resolve the difficult cases. Of the entomologists surveyed, 92 percent indicated that they would find a interdisciplinary approach including a dermatologist, psychiatrist and an entomologist a useful resource. One entomologist reported a case involving a woman whose husband recently separated from her. He related, “ . . . the sad thing about this is that I never knew what happened to this woman. Suicide was a definite possibility. I had no one to send her to or any other means of helping her.”

Closer cooperation between entomologists, dermatologists, and psychiatrists in the form of a specialized clinic for DOP was considered by most entomologists surveyed to be a way of providing more efficient treatment. Musalek (1990) has stressed this as the essential precondition for efficient therapy of patients with DOP. Also since individuals with DOP are reluctant to accept psychiatric treatment and entomologists are reluctant to make psychiatric referrals, such a clinic opens up the opportunity for psychiatric contact which is acceptable. Musalek (1990, 1991) described operating such a clinic in Vienna, Austria since 1986 under the name D.P.P.—" Out-patient Clinic for Dermatological
and Parasitological Problems" or "Liaison out-patient Clinic for Dermatology, Parasitology and Psychiatry"—
and has evaluated 126 patients with DOP over the past five years. He concluded that both the widespread cooperation among professionals involved with the special outpatient clinic and the high number of patients seen indicate that the majority of patients with DOP living in his region were recorded. To the authors' knowledge, there are no known clinics of this type in existence in the U.S.

REFERENCES CITED


CASE HISTORIES OF INDIVIDUALS WITH DELUSIONS OF PARASITOSIS IN SOUTHERN CALIFORNIA AND A PROPOSED PROTOCOL FOR INITIATING EFFECTIVE MEDICAL ASSISTANCE

J. P. Webb, Jr.

DELUSIONS OF PARASITOSIS

During the last 10 years the Orange County Vector Control District (OCVCD) laboratory has on average seen at least two cases of Delusions of Parasitosis (DOP) each month. Visits to the OCVCD lab by DOP individuals have increased during the last several years, probably due to the fact that the county’s health care facilities and pest control firms were aware of our DOP assistance program and referred suspected DOP victims to us. Among the DOP people that come to our laboratory for aid, most would fall into the category of older post-menopausal women who have recently undergone a life-altering event, such as the loss of a loved one through divorce or death. A small proportion of the DOP individuals who are also seen at the lab may be classed in the toxic reaction (drugs, alcohol) group. Assistance is relatively easy to procure for these individuals because there are local agencies (e.g., Orange County Health Care Agency) with active programs that focus on treating patients for drug or alcohol addiction problems. On the other hand, it has become clear over time that there is no standard method available to medical entomologists to help psychogenically based DOP victims obtain medical assistance. These people have been exposed to a wide variety of experiences when seeking help from entomologists at large including, at one end of the spectrum, the statement that the samples they brought to the laboratory had no arthropod specimens, it might very well be that they were imagining that they were infested with bugs, and nothing more could be done for them. At the other end, the entomologist attentively listens to the afflicted person, carefully examines the samples, and, if nothing is found, encourages the individual to seek medical care. More often than not the encounter by the complainant approximates the conditions nearer to the end of the spectrum given in the first example. This may occur because the entomologist is not familiar with an established method of conduct when dealing with DOP people and may be embarrassed when interacting with a person (often a woman) who sometimes may exhibit bizarre behavior. We have had ladies come into our laboratory and during the discussion of the infestation begin to disrobe to show us their “bug” bites. Others have brought in underpants and other intimate apparel items for us to examine for “bugs.”

Assistance, unfortunately, is also quite often not provided by attending physicians, general practitioners, or dermatologists. Many, if not most, of the DOP cases that come to our laboratory have already been to at least one health care provider, usually their own family physician or a referred dermatologist. They have been “examined” by this doctor and either told there is no evidence of an infestation and they should make an appointment with a psychiatrist or that they probably have scabies or lice and to use Kwell® cream or lotion, a prescription ectoparasicide. It should be noted that Kwell® has become the “aspirin” of the dermatologists who regularly prescribe Kwell® in a manner suggestive of the expression, “Take two aspirin (sub., “Use Kwell®”) and call me in the morning.” The effect in either case is one that is usually deleterious to the DOP individual. When referred to psychiatric treatment, the patient more often than not rebels against the implication that he or she is crazy and continues on their aimless odyssey to find assistance. This is not to say that all or most of the health care providers follow this “easy out” method of treating patients who are generally considered to be “time consuming” and “troublesome.” Yet, the limited, but consistent, consensus of interactions between DOP patients and their physician has indicated a sense of frustration by the doctors who have an established, more traditional medical practice and are unable to provide the time and effective treatment for these DOP victims. Nearly all of the physicians I have contacted regarding the DOP person have been sympathetic with the situation and willing to follow any method that might provide a positive resolution of the condition. These doctors cannot be perse blamed for these failures. In this area of medicine there is a large gap in the Health


2Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92643, U.S.A.
Care System in the United States (perhaps worldwide) and the DOP afflictees are failing through it in large numbers. The gap is not caused by a lack of psychiatric treatment methods and medications but rather by the fact that entomologists, GPs, and dermatologists are not following any standardized guidelines that will get the patient into psychiatric therapy.

During the past decade at the OCVD laboratory, a protocol of assistance for DOP individuals has gradually evolved. Early on it became evident that attempting to convince a DOP person that their samples contained no evidence of arthropods was futile and served no useful purpose. This approach of confronting the individual with negative results, however, is apparently successful with one cohort of individuals (identified originally by Steve Bennett in our lab) as young to middle-aged males who work in the construction industry. Although we have no statistically verified scientific evidence for the conclusion, Steve has, on a number of occasions, demonstrated microscopic sized fibers to the complainant which he tells them may be linked to workers who have contact on the job with wood shavings and/or metal filings. Furthermore, Steve adds that these fibers may be causing them cutaneous sensations that give the impression of crawling and biting bugs. These individuals would technically fall into the category of suffering from Illusions of Parasitosis (Waldron 1972, Keh 1983, Pinto 1989) considered by Kushon (this volume) to be a variant of DOP. Rational discussions with these people and a demonstration of the fibers (which appear similar from case to case) under the magnifying scope seem to provide them with an answer to their problem of itching and “bug” bites.

The majority of the other DOP cases that we see, however, are in a cohort characterized by older, post-menopausal women, who have had a relatively recent traumatic experience. Also, most of these ladies have been to at least one physician who has told her that the infestation is imaginary or he has prescribed Kwell® for her which, according to the patient, has not eliminated the “bugs.” Additionally, she often believes that the “bugs” come from a specific source (e.g., neighbor’s cats, contaminated carpet or furniture) and sometimes the exact day and events may be linked to the initial “bug” infestation. Quite often the entire house, apartment, and/or yard are also claimed to be “breeding grounds” for these creatures. In a few instances the victim’s automobile has also been involved as a refugium for the “bugs.” Other traits that are correlated with DOP cases include the manner in which they bring “bug” specimens to our laboratory for identification. Index cards (3" x 5") are one of their favorite means of bringing in the “bug” specimens. Each card has a piece of cellophane tape affixed to it under which are the variety of “bugs” that were collected off the floor, sink counter, toilet seat, carpet, couch, or even off some part of their body. Many times written information is included on the card denoting the exact time and place where the sample was taken. Bringing vacuum cleaner bags to the lab is another common characteristic of some individuals of this DOP cohort. Plastic pill vials and baby food jars are also commonly brought to the laboratory by DOP afflictees but these are also common specimen conveyance methods used by others as well.

As stated earlier, it became clear during the first two or three years that attempting to demonstrate to a DOP victim that her/his samples contained no “bugs” and advising them to seek medical care on their own were of little value in helping to ameliorate the DOP condition. Many of these persons would return many times to the lab with more samples accompanied by adamant verbal as well as written information that “documented” their claim of being infested. All of this in an endeavor to obtain a validation of their credibility that would attest to their sanity. It is interesting to note that except for this obsessional conviction that they were plagued with a parasitic invasion, a number of these people seemed to have normal mental health and many were able to function at acceptable levels in society, including the maintenance of their job. A recent case that exemplifies this situation is that of a family practice physician who visited our lab several times and on each occasion he would bring many cellophane tape samples of “bugs” that he would collect from his body, particularly his forehead (“the bugs were easy to catch because they would emerge out of the pores of my forehead each morning while I was shaving”) and his perianal area. He believed that the initial infestation was acquired from a pair of imported sandals that he had purchased several months before he noticed the “bugs” on his person. He brought these sandals to our lab for inspection in addition to a new pair of shoes which he also asked us to examine. As far as we could determine his “infestation” had been ongoing for nearly two years during which time he was apparently able to adequately operate and conduct his medical practice.

Because it seemed that the primary health care providers and the entomologists were not guiding these cases in the right direction, we developed some preliminary guidelines that would, we felt, increase their chances of securing appropriate medical services. So instead of examining their specimens and telling them they had no “bugs” and they should seek medical help, we opted for telling them to leave their specimens with us for processing and identification (taking 1 to 2 days) and we would send a report of our findings to their
attending physician. This approach is used, of course, if you have made a determination that the individual in question really does suffer from DOP. Using the characteristic traits already discussed above (see also Fig. 1) usually serve as good reference points for making this preliminary diagnosis. Once they have left the lab, we then immediately examine the specimens under the microscope. If there is no evidence of "bugs," then this serves as another bit of information supporting the DOP diagnosis. If we find evidence of arthropods being present, however (e.g., fleas, mites, lice), then we back away from the DOP hypothesis and call the person and advise her/him of our findings and possible courses of action for resolution of the problem.

In the event that no "bugs" were found during the microscope examination, we have then called the patients's physician; the address and phone number of the individual's physician is requested at the time when the samples are brought to the laboratory. We then tell the victim's doctor that his patient has recently brought us samples of "bugs" which upon close examination revealed no arthropod specimens. In addition, certain behavioral traits (see Fig. 1) have led us to suspect that this individual may be suffering from DOP. Nearly 100 percent of the people who we diagnosed during the last decade were confirmed as being delusional by their attending physician. The doctor is then told that a report of our findings will be sent to him and he may give a copy to this patient, if one was requested. The format of the report (Fig. 2) was designed to "double-talk" the results so that the patient would not be bluntly told that there was nothing in her/his samples. In addition, the results are footnoted with a subtle suggestion that the stress induced by their "infestation" has caused a significant component of their medical problem and this condition may be ameliorated with psychiatric therapy. In addition, with the physician's approval and cooperation, we suggest that a placebo approach of prescribing a mild broad spectrum antibiotic would assist in the endeavor to diminish the delusion sufficiently to convince the patient that psychiatric treatment was warranted.

To date, this approach has had a variety of outcomes due probably to the differences in specialties and decision making bent of each doctor. The variation in results, however, is far from the optimal goal, the focus of which is to consistently get the patient into appropriate medical hands. This end point is best achieved by having the patient referred by their own doctor directly to the psychiatrist we have recommended, who has a specialty in handling DOP cases. Finding a medical professional with this background may be difficult and when one is found it is proposed that the medical entomologist and the psychiatrist establish a rapport and a working protocol for providing effective and consistent treatment for DOP victims. We are currently canvassing medical

The individual may:

A. Complain of "mites" or "bugs" on or in them.

B. Say that "bugs" are causing itching, rashes, and/or lesions.

C. Have seen a number of physicians who have said "there are no bugs."

D. Have had the house sprayed for bugs by a professional pest control company a number of times in a relatively short period of time.

E. Have set off "bug bombs" in the house an inordinate number of times.

F. Be an older woman.

G. Be an afflicted person who lives alone.

H. Often be an individual who has suffered a recent loss of a loved one—divorce, death, rejection.

I. Bring in "specimens" on scotch tape/index cards or vacuum bag dust.

Figure 1. Characteristics that may suggest the condition of Delusional Parasitosis.
TO: Dr. I. Kan Kuryew  
FROM: James P. Webb, Jr., Ph.D.  
RE: Specimen identification for Mrs. Anita Helpp

On 28 October 1991, materials were received by the OCVCD Laboratory from Mrs. Helpp. The following represents the results of our examination of these items:

1. Examination of the scotch tape and vacuum bag samples revealed an infestation*, ** of specimens that (in detail) heretofore have not been seen by scientific investigators. Study of these entities will continue and, if necessary, other experts contacted to ascertain their detailed identification.

2. In addition, cast skins of carpet beetle larvae were found. It is known, however, that these forms are never involved as parasites of vertebrates.

*A limited number of case histories of infestations of this type has indicated that a broad spectrum antibiotic (or other appropriate medication) is effective in eliminating the problems within two to three weeks after beginning the per os regimen. Pesticides such as Ficam-W or Precor (Methoprene) were shown to be effective in the home, yard, and/or automobile within one to two weeks.

**Evidence from these same case histories also indicated that the trauma and discomfort caused by the infestation was best ameliorated by psychiatric counseling. Stabilization by this counseling seemed to shorten recovery time from the infestation. Under certain circumstances, concurrent antibiotic and psychological therapy may be appropriate.

JPW/jk

Figure 2. Format of Results Report sent to the physician of DOP patients.

professionals to find a psychiatrist who has a background in dealing with DOP patients.

With retrospect to our evolving DOP assistance system a number of criticisms have arisen. One in particular has focused on the aspect of our protocol that seemed to confirm the DOP person’s contention of infestation. In evaluating the report and interview formats there is no evidence that we tell the patient that we agree that they indeed have bugs, mites, or any other parasites, thereby strengthening the delusion. In fact we make every effort to tell them nothing about their samples except to say they will be processed and identified and the findings will be sent to their physician. Additionally, a strong effort is made not to tell them that there is nothing there because once this idea has been conveyed to them, they lose confidence in our program, leave our laboratory, and continue their directionless meandering in search of help.

Another criticism has been that we practice medicine without proper credentials and licensing. Again, we make every attempt to treat the DOP individual with a professional approach without trespassing over the boundary into medical procedures. As a point in fact, we have had to remind some persons that we were not physicians and physical examinations of their rashes and bites as well as prescribing medications were out of our venue. Furthermore, in our protocol we request at the outset from the patient their doctor’s name, address, and telephone number so that the report of our findings can be sent to him quickly. At times, because a DOP person may be insistent that we look at their bites and their samples immediately, we again assure him or her that he or she has a medical condition that can only be evaluated by a physician. We further counsel them that the results of our examination of their samples will be sent to their doctor. This approach has usually been acceptable to the DOP individual and we have followed up on our promise to submit our report to their health care provider. An emphasis is noted here that we try to establish a rapport with the DOP victims which will
transfer with them when they enter medical treatment. Relevant accounts of this *modus operandi* have been clearly outlined and discussed by Gould and Gragg (1976), Keh (1983), and Lyell (1983).

The evaluation of this mode of assistance has been difficult and follow-up information on each DOP case has been practically impossible to obtain. Most of the feedback that we have received has been from the DOP individuals' physician to whom we sent our report with the suggested treatment protocol. A significant number of the contacted doctors have not heard from their patient after the initial office visit and others indicated to us that our suggested protocol was not successful with their DOP patient and the individual continued to complain of a "bug" infestation. In this latter situation, the doctor indicated that eventually they told the patient that they had no "bugs" and they imagined the infestation, and soon after they, not surprisingly, lost contact with that person. On several occasions during the past 10 years, DOP individuals have come back with their "bugs" to our laboratory. One case in particular comes to mind of a lady in her middle fifties who came into our laboratory one afternoon. She looked familiar so I asked her if she had been to our laboratory previously. She answered that she had brought specimens for us to identify one or two years before and we had sent a copy of our results report to her doctor. I asked her if he had recommended any treatment for her and she said he had prescribed Kwell® lotion for her and since then has renewed her prescription for this medication whenever she called his office. In another positively contrasting instance, an older lady who lived in Leisure World had been beset by a persistent "infestation of bugs." She sent specimens to us by mail and we sent a report of our identification and suggestions to her doctor with whom we had confirmed the lady's diagnosis of having DOP. Approximately six weeks later I received a phone call from this lady and she thanked me profusely for our help in ridding her of her bug problem. She continued on by saying that our laboratory's people were the only ones who listened to her, believed what she said, and provided a way of helping her to eliminate her annoyance. Interestingly, at the end of our conversation, she added that even though the "bugs" were now gone, every now and then she still had the feeling there were still a few lurking around.

The success rate, therefore, has been uneasy to ascertain due primarily to the difficulties in doing follow up patient evaluations. With the advent of referrals to a designated psychiatrist/dermatologist, a more scientifically based determination of this approach to assisting the unfortunate sufferers of DOP may be documented. More importantly, a greater number of DOP victims, who normally would not receive any essential psychiatric assistance, will be guided to appropriate medical care.

**VERIFIED ARTHROPOD INFESTATIONS**

In the preceding discussion the focus of attention was on the individual who was considered a likely Delusions of Parasitosis (DOP) case for a number of reasons including the consistent failure by medical entomologists to identify any tangible evidence of parasites. It was also stated that in the event that specimens were found that were known to cause untoward cutaneous effects or other pathology, the complainant was notified and advised about methods to resolve the problem. The medical entomologist, however, is sometimes placed in the compromising position of assisting a person who generally fits the DOP criteria (see Fig. 1) and, at least initially, no specimens of an offensive nature are seen in the samples brought in by the complainant. In addition, the affected individual has not yet been to see a physician thereby eliminating the corroborative support for a diagnosis of DOP from a medical authority. Under these conditions, then, it is even more imperative that specimens of medical importance be sought and accurately identified. To that end, an example of an investigation form is presented in Appendix A.

Recently, just such a situation occurred in our laboratory when a woman called complaining of being bitten by mites that she couldn't see. She knew they were mites, however, because several years before our laboratory had identified specimens she had retrieved in her home that had recently undergone rat (*Rattus rattus*) removal treatment. After relating this story to me, the next day she brought a baby jar sample of cotton balls covered with dust and debris on which she thought were some mite specimens. Furthermore, she said that she had not yet been to see her doctor regarding the bites on her person. Because of her general demeanor that was suggestive of some of the characteristics of a DOP individual, I was leaning toward diagnosing her as a DOP so I told her that I would process her specimens, identify them, and send the results to her physician. She accepted this procedural proposal and left the laboratory after giving us the name and phone number of her doctor. After examining the cotton balls, I mounted some suspicious material from her sample on a microscope slide and examined it under the microscope. The specimen that was seen immediately in the field of view I identified as a house dust mite (*Dermatophagoides* sp.). I called the lady on the telephone and told her that the only specimen we found in her sample was a dust...
mite and these mites are not considered to be significant skin parasites although they have been established as a cause of allergies and asthma. I then asked her to try to find some crawling specimens and bring them in for identification. I had decided that if she failed to bring any verifiable specimens to the laboratory that were of medical significance, then I would call her physician and send him a copy of the results and our suggested treatment protocol. The next day I reexamined the prepared specimen on the slide, at which time I found a second mite specimen in addition to the dust mite. It was a blood-engorged female Ornithonyssus bacoti, the tropical rat mite. Needless to add, I immediately called the lady, told her my finding, apologized for my oversight, and recommended that she call our District Office for a service request to reinspect her property for roof rats.

Episodes like the preceding one make it mandatory that medical entomologists/parasitologists remain alert and updated about medically important arthropod (and other) species that may be relevant to potential cases of human or domestic animal infestations in their own geographic area of responsibility. In the greater Los Angeles area of southern California, the tropical rat mite (O. bacoti) is the most commonly found cause of topical skin pathology (e.g., bites, rash, pruritus) in the cases in which an arthropod specimen has been identified.

Keh (1957) has outlined more information on O. bacoti including information on dermatitis in humans, description and life cycle, collection procedures, and disease transmission potential.

A number of other mites are occasionally found in association with humans and/or their pets in California and are capable of producing a dermatitis condition and this includes Ornithonyssus sylviarum (fowl mite), commonly associated with wild birds and poultry (Evans et al. 1961), and Dermanyssus gallinae (Chicken or poultry mite), a parasite of wild birds and caged birds (Evans et al. ibid). In addition, Cheyletiella yasguri (on dogs), C. blakei (on cats), and C. parasitivorax (on rabbits) have also been correlated with human attacks (Hewitt et al. 1973). A good reference for identifying specimens of Cheyletiella is provided by Smiley (1970).

Another mite species that we have seen, although not commonly, is the straw itch mite, Pyemotes triici (= ventricosus). This acarine is a predator of grain insects and has been reported biting workers handling grain, hay, chaff, and straw (Evans et al. 1961). Records are documented of this mite producing an intensely pruritic skin rash with a number of the bite victims being hospitalized (Moser 1975). Fine and Scott (1965) have also provided epidemiological reports of a number of case histories of straw itch mite caused dermatitis involving P. triici found in association with infestations of the common furniture beetle (Anobium punctatum). This mite has also been found in association with termite infestations. Cross and Moser (1975) have published a key to the species of Pyemotes including P. triici.

Certain mite species of the genera Acarus, Tyrophagus, and Glycyphagus are associated with grains and other stored food products. These mites have been implicated with contact dermatitis in humans (e.g., grocer’s itch) as well as other allergic reactions. Hughes (1961) has produced a monograph regarding mites of stored food that includes keys to the species and information on worldwide distribution records, habitat and food products infested, and dermatitis and other annoyance problems to humans.

Mites that occasionally may be important in the United States as the cause of bites on people are the chigger mites of the genus Eutrombicula, including E. belkini, E. alfredugesi, E. splendens, E. batatas, in addition to Euschoengastia numerosa (Wrenn, unpublished data). Descriptions, keys, and case histories involving Eutrombicula spp. may be found in Loomis (1956), Gould (1956), Bennett (1977), Bennett and Loomis (1980), Webb et al. (1983), and Bennett and Webb (1985). Hoffmann (1990) published records of Eutrombicula spp. and of Paraschoschoengastia nunezi, another human dermatitis causing species, from Mexico. A case of severe trombiculosis in horses was reported by Brennan and Yunker (1964) that was caused by still another chigger species, Euschoengastia latchmani, from northern California.

On rare instances during the past decade we have had specimens of the mite Pellenonysus passeri sent to us for identification. The specimens had been recovered by physicians from patients complaining of itchy skin and rashes. This acarine species is a parasite of English Sparrows (Passer domesticus) and is found in their nests (Clark and Yunker 1956), many of which are built in structures co-inhabited by people.

An interesting case of persistent dermatitis suffered by an 18-month-old boy was traced to bat mites (Chiroptonyssus robustipes) that were infesting their usual hosts, Brazilian free-tailed bats (Tadarida brasiliensis), found in large numbers in the walls of the home where the boy lived (Keh 1974).

Fleas, lice, ticks, and spiders may also be incriminated with dermatological maladies and investigators should, therefore, be aware of local species in each of these groups. Keys and descriptions for the flea species may be found in Hubbard (1968) and Lewis et al. (1988). A key and description of sucking lice of North America is provided by Kim et al. (1986). References, keys, and descriptions of tick species for the United States include Cooley (1938), Cooley and Kohls.
Acknowledgments

Appreciation is extended to Mr. G. L. Challet, Manager, Orange County Vector Control District (OCVCD) for providing the opportunity, laboratory space, and supplies in developing the protocol for assisting DOP individuals. Gratitude is also given to Mr. S. G. Bennett (OCVCD), Mr. M. B. Madon, Environmental Management Branch, California State Department of Public Health, Dr. Ron Barr (UCI Medical Center, Orange), and Dr. W. J. Wrenn (University of North Dakota) for comments, suggestions, and unpublished information used in this paper.

REFERENCES CITED


Kaston, B. J. 1978. How to Know the Spiders. W. C.


## Investigate Form
**Medically Important Arthropods**
(Mites, Fleas, Lice, Etc.)

### Date: _____________________________

**Name:** ___________________________  **Referred by:** _____________________________

**Address:** ___________________________  **Physician’s Name:** _____________________________

**Phone:** ___________________________  **Phone:** _____________________________

**Sex:** Male □  Female □  **Age:** _______

**Medical History:**

1. Description of Problem:
   - Bites □  Itching □  Rash □  Other □  **(Specify)**

2. Problem Occurring on:
   - Hands and Arms □  Legs □  Eyes and Nose □  Chest □  Waist □  Ankle □  **(Specify)**

3. Suspected Cause of Problem:
   - Mites □  Lice □  Fleas □  Chiggers □  Other □  **(Specify)**

4. Problem Began _____________________________  **(Date)**

5. Specimens Recovered From:
   - Body □  House □  **(Specify)**
   - Apartment □  Automobile □  **(Specify Room)**
   - Other □  **(Specify)**

6. Physicians Seen:
   - Personal MD □  Referred MD □  **(Specify)**

7. Diagnosis:
   - Scabies □  Lice □  Other □  **(Specify)**

8. Recommended Treatment:
   - Kwell® □  Other □  **(Specify)**
   - 9. Other Medication □  **(Specify)**

10. Pre-existing Medical Condition: □  **(Specify)**

11. Date Diagnosed _____________________________  **(Specify)**

### Environmental Factors:

1. Recent visitors □  from where?
2. Recent carpet or furniture purchases □
3. Recent travel □  to where?
4. Rat infestation □  Mice infestation □  Birds □
5. Central heating/cooling system □
6. House/apartment treated by pest control company □  Number of times _____________________________
7. Bug bombs used in house/apartment □  Number of times _____________________________
Appendix A

SOCIAL FACTORS:


SAMPLING:

<table>
<thead>
<tr>
<th>Samples taken from</th>
<th>Date</th>
<th>Results</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RECOMMENDATIONS:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
THE USE OF AN INSECT ACTIVITY MONITOR IN BEHAVIORAL STUDIES
OF THE FLEA, *XENOPSYLLA CHEOPIS* (ROTHSCHILD)¹

F. Clark², M. T. Greenwood³, and J. S. Smith³

ABSTRACT: A flea activity monitor of potential use to vector ecologists in the testing of insecticides and in the study of flea behavior has been developed. The monitor is designed to detect both ‘running’ and ‘jumping’ activity of fleas in response to a variety of environmental and host-derived cues. With a modular, highly adaptable computer driven monitor, it allows various experiments to be conducted simultaneously.

INTRODUCTION

The automatic recording of flea activity using an insect monitor was first attempted by Greenwood et al. (1991) using bird fleas of the genus *Ceratophyllus*. In the present study, the oriental rat flea, *Xenopsylla cheopis* (Rothschild), the principal vector of *Yersinia pestis*, has been used as a model in a new updated design of the monitor to test if changes can be measured in the activity patterns of fleas in response to light. Once a baseline profile for each species is determined, the species-specific profiles may be used to measure responses to a variety of stimuli and allow comparisons to be made among species, giving a further insight into their behavior and ecology.

MATERIALS AND METHODS

In Siphonaptera, running and jumping gaits can be detected and used as a measure of activity. The original monitor reported by Greenwood et al. (1991) was based on only one experimental chamber, a feature that restricted experimental design and the repetition of trials. In the updated version of the monitor, a module of four chambers was used. Three of these were experimental chambers (containing fleas) and one was used as a control (no fleas). Any extraneous vibration or disturbance that was detected and recorded by the monitor could then be accounted for and the profiles adjusted. The chambers were initially made of clear plastic tubing but these produced a considerable amount of static electricity that often caused the fleas to adhere to the walls of the chambers. Therefore, the plastic was replaced with glass tubing (8 cm in length; 2 cm in diameter).

A thin membrane of stretched plastic formed the base of the chamber. Each chamber fitted into a socket within the monitor so that the plastic membrane rested on a microphone situated at the base of the socket. When the fleas jumped, the resulting vibrations on the membrane were detected by the microphone. These vibrations were transmitted by an operational amplifier after amplification of the voltage, and shaped into a pulse by a Schmitt trigger circuit. Trials performed with the monitor connected to an oscilloscope showed that for every take off and landing only one pulse was recorded. Pulses were counted on a two-bit binary coded decimal counter, which was decoded and displayed on a computer screen according to the numbers of jumps in each chamber.

The running activity was detected by two infrared beams, which crossed over at the base of the chamber. These were produced by two infrared emitters approximately 1 mm in diameter and were detected by phototransistors. When running occurred, the infrared beams were broken and a voltage signal from the phototransistors was amplified using an operational amplifier and a pulse produced using a Schmitt trigger circuit. This was displayed on the computer screen as the number of runs per chamber. The length of time for each trial could be programmed and set for any unit from 1 minute to 24 hours duration. The number of runs and jumps could be recorded at intervals of one, to several minutes, to hours.

Fleas were removed from culture using an aspirator attached to a vacuum line. Carbon dioxide was then blown into the aspirator to anesthetize the fleas. They were then removed from the aspirator onto a plate made of porous polyethylene through which CO₂ was bled, keeping the fleas anesthetized as they were manipulated.

²Department of Zoology, University of Leicester, Leicester, ENGLAND
³University of Technology, Loughborough, ENGLAND
into the activity chambers. This was done using a pair of soft-nosed stork’s bill forceps that appeared not to damage the fleas.

The flea cultures used in the trials were kept without light and all procedures were carried out under red light (620 nm). This minimized the number of stimuli to which the fleas were exposed prior to any experiment. Red light was chosen because in a previous study of spectral efficiency, Crum et al. (1974) showed that X. cheopis was least sensitive to the longer wavelengths. All trials were conducted at 25±1°C in a darkened constant temperature room, the temperature at which the colony of X. cheopis was maintained. When the mouse nest box was removed for subculturing every 21 days, all adult fleas were removed leaving only larvae. After a further 14-21 days emerging, fleas were used in the trials described below. An approximate age of the fleas could therefore be estimated but it was not known if they were mated or unmated.

RESULTS

Density Effects

Figures 1a to 1d illustrate examples of typical results from trials conducted with 1, 2, 5, and 10 individuals per chamber. When single fleas were used, few jumps were recorded, whereas, at higher densities of 10 fleas, only jumps with few runs were detected. All subsequent experiments were conducted with five individuals per chamber as the number of runs and jumps were of similar magnitude. This insured that when using different stimuli, only one gait may be affected and detected by the treatment.

Differences in the Sexes

Evidence suggests that for some flea species, one sex may be more active than the other (Marshall 1981). Trials using each sex separately and in a random mix as from culture were undertaken. A one-way analysis of variance (ANOVA) for total activity (runs and jumps) on \( \log_{10} \) transformed data showed no significant difference between the three treatments (TABLE 1), and all trials were therefore conducted on batches of five fleas taken randomly from culture.

Length of Trials

Trials of different duration were tested from five minutes to two hours duration and with various recording intervals, from one to ten minutes. From these an experimental time of one hour was chosen, recording jumps and runs every minute. Each experiment began with a settling period of 15 minutes with no light. There was evidence that the performance of the fleas diminished with time, and one hour appeared to be long enough to detect any effects produced from the stimulus being studied.

Effects of CO\(_2\)

Xenopsylla cheopis were anesthetized with CO\(_2\) before they were introduced into the monitor and their activity was compared with that of fleas that had not been anesthetized (three replicates of five fleas each). A t-test between the two treatments performed on \( \log_{10} \) transformed data showed that there was significantly more activity in the fleas that had not been anesthetized than those given CO\(_2\) (mean + SE with CO\(_2\) = 1.0560±0.040; without CO\(_2\)=1.449±0.023; t=8.47 DF=2 P<0.001).

Variations Within Treatment

The data for runs and jumps per minute from ten trials are shown in Figure 2. Although there is considerable variation between replicates within a treatment at each time unit, when the total jumps or runs were taken for each replicate and log-transformed, the error was sufficiently small that these data could be used for comparison with data from other treatments. This may be seen in the ANOVAs for running and jumping between the two sexes run separately and in combination (TABLE 1).

| TABLE 1. ANOVA (Log\(_{10}\)) using total activity between five males, five females, and a mixture of five males/females Xenopsylla cheopis in combination, in no-light treatment at 25°C (10 replicates each). |
|---------------|----------------|------|------|------|
|               | F       | EMS  | DF   | P     |
| Total Activity| 0.31    | 0.0418| 2.27 | NS    |
| Runs          | 0.45    | 0.0160| 2.27 | NS    |
| Jumps         | 0.95    | 0.0134| 2.27 | NS    |
Figure 1 continued on next page.
Figures 1a-1d. Activity profiles using Xenopsylla cheopis at densities of 1, 2, 5, and 10 individuals per chamber.
Figure 2a. Activity profile of *Xenopsylla cheopis* (runs per minute) of 10 replicates: mean + 1. SD.

Figure 2b. Activity profile of *Xenopsylla cheopis* (jumps per minute) of 10 replicates: mean + 1. SD.
Figure 3a-3c. Activity profile of *Xenopsylla cheopis* in a no-light regime; in all-light; and in a treatment where light is switched (15 mins. L: 15 mins. D). Duration = 60 mins.
TABLE 2. Pearson correlation between runs and jumps for males, females, and combinations of males/females *Xenopsylla cheopis* in combination in no-light treatment at 25°C.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>-0.184</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>-0.321</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Males and Females</td>
<td>0.522</td>
<td>8</td>
<td>NS</td>
</tr>
</tbody>
</table>

We looked for a relationship between running and jumping using a Pearson product moment correlation, but no significant correlations were obtained for either sex or for the combination of sexes (TABLE 2). Why there should be a shift from weak negative correlation when the sexes are run separately to a positive correlation when run in combination was not clear. The result for the separate sexes is more logical because while running, no jumping can occur, and vice versa.

**Activity as a Measure of Response to Various Stimuli**

Light was chosen as a stimulus to assess the sensitivity of the monitor because it was one of the easiest stimuli to introduce into the chambers and because both negative and positive phototaxis have been reported in a number of flea species including *Xenopsylla* (Marshall 1981). Light was carried via optic fibers into the top of each chamber from a Schott light source. As shown in Figure 3, *Xenopsylla* is most active without light (Fig. 3a) and least active in light (Fig. 3b). This activity can be controlled when the light is switched on and off (Fig. 3c).

**DISCUSSION**

Changes in activity can be used as a measure of response to light and the monitor has proved efficient at detecting and quantifying these responses. The results from the experiments using light as the stimulus support previous studies that *X. cheopis* is negatively phototactic.

The modular nature of the monitor also allows for replication of treatments so that many trials can be conducted in a short period of time and a data base established. Pesticides are generally tested using gross knockdown procedures. However, they also may have sublethal effects that affect behavior, which may be of interest to vector biologists. This monitor appears to be sensitive enough to allow this level of detection to take place. Although the amount of activity was significantly reduced after exposure to CO₂, the levels of activity obtained from CO₂ treated fleas was sufficient to allow comparisons to be made between treatments, although more subtle changes in activity may have been lost.

**REFERENCES CITED**


CRITICAL ISSUES IN VECTOR-BORNE DISEASE

A. R. Barr

I believe that the most important problem facing the world today is the increasing prevalence of people. Malthus pointed out almost two centuries ago (1798) that animal populations tend to increase faster than their natural resources. As we modify the factors that regulate the density of human populations, we inevitably increase pressure on the environment that sustains us, to the point that it no longer is sufficient. Sri Lanka is a case in point. The control of malaria and other infectious diseases reduced the annual death rate by approximately half. The birth rate, however, did not change substantially, so the population of the island doubled within a couple of generations, which produced increased overpopulation, shortage of food, and increased poverty. What is the answer to this situation? Obviously, cutting down of the forests so that more rice can be grown to support the increased population so that it may increase even more, leading once again to food shortage and a further decrease in the standard of living. Sooner or later we must acknowledge that human populations will always outdistance their resources unless we regulate their size. I believe that overpopulation, if it is not checked, will inevitably lead to increased hunger, poverty, aggression, a reduction in the quality of life for all, and a devaluing of human life. The consequences for vector-borne diseases I believe are obvious.

A related problem is the economic condition that at present prevails in most of the world. We see this most clearly in the United States, but it affects most of Latin America, Africa, Australia, Europe, and Asia as well. Poverty is a risk factor for most vector-borne diseases: malaria, Chagas' disease, African trypanosomiasis, onchocerciasis, and filariasis are examples. It will be difficult to progress in the control of most of the important vector-borne diseases until the economic climate improves considerably, and that does not appear likely in the near future. The problem that we are experiencing with introduction of malaria into California is a function of the economic condition in Mexico and undoubtedly will continue as long as there is such a great disparity in the economies of Mexico and the United States. The shortage of funds for public agencies to control vectors in this country today imperils our ability to protect the public from vector-borne diseases. The situation probably will deteriorate even further before it improves.

An important movement that is taking place in much of the world today is what has in the past been called "balkanization," the breaking up of larger political entities into smaller ones. The former Soviet Union has now divided into 15 countries; Yugoslavia apparently has become three or more states. It is likely that Czechoslovakia and Poland also will divide. The driving force behind these movements is ethnic diversity-cultural conflict. The result is smaller, less viable countries, with weaker economies, less prepared to deal effectively with sanitation problems. In the worst cases, as in Yugoslavia, there is social disorder, open hostility, and aggression. Similar events are taking place among some of the now independent countries of the former Soviet Union. What effect these events will have on public health and disease transmission is not yet apparent but we would not be surprised to see the reappearance of epidemic typhus this winter or the reintroduction of malaria with the return of warm weather.

A fourth problem is environmental pollution. We are constantly becoming more conscious of the deterioration of our environment caused by our own activities, and in some cases, by agencies that were only recently discovered. It now appears that certain types of vegetation, for example, liquidambar or sweet gum trees contribute significant amounts of chemicals that must be considered to be atmospheric pollutants. The recent accidental spillage of an enormous quantity of herbicide in a river in the Sacramento Valley of California apparently eliminated virtually all life in the river. We were told that years will be required for the river to recover. As we all know, fear of environmental pollution, whether reasonable or not, places important restrictions on the control of vectors and vector-borne diseases.

Another fear that many have, perhaps not in the immediate future but in the years to come, is global warming. There appears to be a fair amount of agreement

---


2Department of Epidemiology, School of Public Health, University of California, Los Angeles, CA 90024-1772, U.S.A.
among scientists that global warming is a reality, although it is not clear how soon or how much. The effects of global warming are not at all clear beyond the basic idea of an increase in temperature throughout the world. It is clear that this would allow tropical organisms to expand their distributions toward the polar regions of the world. Since the most important vector-borne diseases are largely tropical in distribution, we would expect that afflictions such as leishmaniasis, falciparum malaria, trypanosomiasis, and filariasis would increase in prevalence in areas in which they presently do not occur or are not at present very prevalent. The restriction of the most important vector-borne diseases, largely to the tropics, primarily is because of the tropical distribution of their vectors. What is not clear is what effect the increase of temperatures in presently temperate zone regions would have on other aspects of weather. What would be the effect on precipitation, prevailing winds, and other elements of the weather? Certainly these factors are as important as temperature in limiting the distributions of tropical vectors and the etiological agents they maintain. At any rate, global warming seems to be in our future although its effects on vector-borne diseases, it seems to me, are largely unpredictable at present.

Something of more immediate concern to our profession is the shortage of medical entomologists, which apparently will become even more acute in the future. Our ranks are being thinned by retirement and lack of replacement. There is a perception by many in responsible positions that infectious disease is no longer very important and that resources should go toward the amelioration of chronic diseases. This in spite of the discovery of legionellosis, AIDS, babesiosis, Lyme disease, and other important infectious diseases in recent years. Infectious agents are being postulated even for many chronic diseases, such as nasopharyngeal carcinoma, primary hepatocellular cancer, cervical cancer, and stomach cancer. Some believe that even chronic fatigue syndrome has an infectious etiological agent. Several universities have discontinued training in medical entomology or parasitology. At UCLA two of us who trained students in medical entomology have now retired and neither will be replaced with a person with this expertise.

It is not clear to me why medical entomology as a discipline has fallen into disfavor. Perhaps it is in part due to the lack of a current textbook. It is likely that courses would be given in medical entomology in some institutions where it is not currently given if an adequate textbook were available. A survey made by LTC Harlan pointed out the need for such a text but none is available yet.

The curtailment of training in traditional systematics is also of concern. Admittedly, there is some interest in newer methods that have been applied to systematic problems, such as electrophoretic methods, cytogetenics, restriction fragment analysis, and DNA analysis; but these merely complement and cannot supplant traditional taxonomic methods. Enormous advances have been made in recent years in ferreting out cryptic species, which has clarified our understanding of previously puzzling epidemiological findings. Where will the next generation of systematic biologists come from? Who will identify our disease vectors in the future? The answer to these questions is not clear.

A final problem that I would like to address concerns information storage and retrieval. Most of the scientists in the entire history of the world are alive today and a large proportion of them are still producing new information. In many cases, they are unknowingly rediscovering things that have been known and forgotten. Frequently it is easier to do something than it is to find out whether it has already been done. A case in point involves work on cytoplasmic incompatibility in mosquitoes done in my laboratory. After the basic facts of cytoplasmic incompatibility were known it was clear that the phenomenon could be caused by an infectious agent, but no suitable infectious agent was known to be present in the mosquitoes under study. One of my students discovered bacteria in the eggs of our mosquitoes, which apparently could explain the phenomenon. We then learned that these organisms had been described in 1924. We, and others, had been studying cytoplasmic incompatibility for 20 years, realizing that it could be caused by an infectious agent, but not knowing that such an agent had been described almost 30 years earlier. The incident is cited to point out the difficulty with the retrieval of scientific information from the enormous literature that exists. Much of the information that is being produced today is computerized in ways that facilitate its retrieval, but there is the great mass of earlier literature that still is difficult to access. One of our highest priorities should be the systematizing of the older literature in such a fashion that we can more easily determine what has been done.

I would now like to turn to some problems dealing specifically with vector-borne diseases. Malaria is still our most important concern. During the ill-fated campaign for the global eradication of malaria much progress was made in the eradication or control of vivax malaria from many areas but there was little progress in the eradication of falciparum malaria, especially in areas where malaria was stable. The primary tools for malaria control are chemoprophylaxis and the control of vectors, and there are problems with both of these
Drug resistant strains of *Plasmodium falciparum* are now widespread and multiply-resistant strains have become common. Some strains are resistant to almost all chemotherapeutic agents in use. Prophylaxis of malaria has become very difficult in many areas. Recently, resistance of *Plasmodium vivax* to chloroquine has been reported in Indonesia so drug resistance is no longer restricted to falciparum malaria. The vectors of malaria also continue to show tolerance or resistance to the commonly used insecticides. The malaria problem in developing countries is exacerbated by political instability, tribal warfare, disrupted social conditions, and especially the lack of sufficient funds to deal with the problem. Control of malaria in many areas could be accomplished by modification or elimination of breeding places of the vectors but such methods, while technically feasible, are quite expensive.

An enormous amount of money has gone into the development of malaria vaccines in recent years but it seems unlikely to me that this approach, even if successful, could have much effect on malaria endemicity because of the relative ineffectiveness of immune processes and the high expected cost of such a vaccine.

We note that malaria continues to be introduced into the United States. Presumably mosquito-transmitted cases have been reported not only from California but also from Florida and New Jersey. It should be noted that there are those who promulgate infection with malaria for the treatment of Lyme disease, as was once done for neurosyphilis. For this reason malarial blood has been imported into the United States, or patients with Lyme disease have traveled to Mexico to be infected. One wonders whether the presumably autochthonous vivax infections recently reported in New Jersey might have been transmitted from such an imported infection. *A propos* of the introduction of strains of malaria, it now appears that a malaria of simians in Latin America, *Plasmodium brasilianum*, actually is *Plasmodium malariae*, which transferred to simians after its introduction in people some time after colonization of the New World by Europeans. If true, not only does this confirm the possibility of introduction of malaria into new areas but it also indicates that human quarten malaria is now zoonotic, which makes its eradication much more difficult. There are anecdotal accounts of zoonotic infections of *Plasmodium falciparum* also in Latin America.

Another area of concern is the continued risk of introduction of malarial vectors into the United States from endemic areas elsewhere in the world. One has only to remember the exacerbation of malaria transmission that took place in Brazil when *Anopheles gambiae* was introduced there to realize what can result from the introduction of an exotic vector. It is fortunate that the introduction of the same mosquito into the Philippine Islands was detected before it became established there. The introduction of *Anopheles subpictus* onto Guam, which was discovered in the late 1940's, fortunately did not result in the importation of malaria. Global warming might be expected to result in the introduction of Latin American malaria vectors into the United States.

Leishmaniasis is another disease that I believe is very much in our future. It can no longer be considered to be exotic. Autochthonous cases of cutaneous leishmaniasis in humans, dogs, and rodents have now been detected in Texas and Oklahoma. Although species of sandflies are not numerous in the United States, the introduction of cases of *Leishmania tropica* from the Middle East is worrisome, especially in view of the visceral manifestation of some of these infections with strains that usually produce cutaneous leishmaniasis. One of the more important effects of global warming might be the extension of the ranges of several species of sandflies into the United States. A recent study indicating that strains of *Leishmania chagasi* are indistinguishable from those of *Leishmania infantum* suggests that visceral leishmaniasis was indeed introduced from the Old World into the New and has subsequently become endemic.

We are only now beginning to recognize human cases of ehrlichiosis in the United States. *Ehrlichia sennetsu* has been known to cause human disease in the Far East for some years but the manner of transmission of the organism has not been discovered. The etiological agent in the United States, *Ehrlichia chaffeensis*, has been described and named only recently. It apparently is tick-borne although the epidemiology of the disease has not yet been worked out. The recognition of this bacterium as an etiological agent of human disease complicates the diagnosis of Lyme disease and rickettsial diseases.

*Rickettsiae* also are very much in our future. Recent work on the rickettsiae that cause Rocky Mountain Spotted Fever indicate that not a single species of bacterium is involved but a spectrum of strains that vary serologically and in virulence, pathogenicity and natural history. A strain of *Rickettsia typhi* has been found infecting fleas and opossums in Los Angeles; human cases have been neither seen nor looked for as yet but very likely occur. Tick-borne typhus in Israel apparently is caused by a rickettsia similar to but different from *Rickettsia conorii*. Changes in the economy in Malaysia have caused a conversion of what were plantations of rubber trees to plantations of oil palms, which has resulted in an
exacerbation of scrub typhus. The relationship of Rickettsia tsutsugamushi to its trombiculid vectors still is not at all clear and requires additional study.

It is not clear why yellow fever has never been introduced into Asia, or if it was, why it died out before being detected. Commercial traffic between Africa and Asia has taken place for at least a thousand years; and, in the early years at least, ships commonly had open vessels of potable water that would suffice for the breeding of mosquitoes. Aedes aegypti itself was introduced into Asia in this manner, although it is not clear when, probably around the turn of the century. There were vectors capable of transmitting yellow fever in Asia even before the introduction of Ae. aegypti so it isn’t clear why the virus was never introduced. It is true that most of the traffic was from the east coast of Africa where yellow fever is not very common, but that seems to be a rather weak explanation for its failure to colonize Asia. At present, traffic between Africa and Asia, even West Africa, is much more common and much more rapid. The introduction of yellow fever virus into Asia should be much more probable today and we would expect would be calamitous. The demonstration that the virus may be transmitted vertically in mosquitoes would seem to facilitate such an introduction. One can only guess what would be the effect of global warming on the distribution of the vectors and vertebrate hosts of the virus.

The possibility of introduction of other arboviruses and their vectors into new areas by modern means of transport is not unlikely. Culex triaeniorhynchus was detected on Guam for the first time during the late 1940’s and Japanese encephalitis has subsequently been reported on the island. It apparently did not occur there before the introduction of the vector. Introductions of dengue have been recorded abundantly onto islands in the South Pacific, onto islands in the Caribbean Ocean, into North America, and elsewhere. They probably are as common as were introductions of yellow fever in the past, although they were less commonly recorded. The recent finding of Potosi virus in the United States suggests that it may have been introduced in Aedes albopictus although it is not known from elsewhere in the range of that species.

There is much interest in Chagas’ disease in this country at the present time because of the threat to our blood supply. Chronic infection with Trypanosoma cruzi is very difficult to detect and cases of transfusion with infected blood have occurred. The methods of detecting chronic infection with this organism are quite imperfect. Serologic detection lacks specificity and xenodiagnosis lacks sensitivity. There is hope that DNA hybridization after intensification by a polymerase chain reaction will provide a sensitive, specific test for detecting infection but this method is not yet fully developed and tested.

Chagas’ disease is an excellent example of a disease whose prevalence is dependent on economics. Chagas’ disease is associated with poverty—with substandard housing. Vector control measures are grossly inadequate to deal with this problem in areas with poor housing. There is hope that the vector can be eradicated in areas where it is thought to be strictly domestic, that is, in areas where Triatoma infestans is the important vector. In most areas, however, the vectors are not strictly domestic, and control of the disease will have to rest on improvements in housing preceded by an improvement in economic conditions.

We might expect that global warming would allow vectors of Chagas’ disease in tropical areas to expand their distributions northward with a subsequent exacerbation of the disease in Middle and North America.

Another disease that we expect will continue to concern us is onchocerciasis. Although we now have adequate methods of controlling the blackfly vectors and a reasonably effective chemotherapeutic agent, ivermectin, the control of this disease will require an enormous expenditure of money and manpower. The problem is complicated by political instability and poverty in both Africa and Latin America. I am afraid that our ability to control the vectors will slip away from us before widespread control of the disease is accomplished.

African trypanosomiasis is another problem whose solution is not yet on the horizon. Although the prevalence of these infections in humans has declined, there is at present no feasible way of dealing with the diseases in domestic and wild animals. Control of tsetse flies and other biting fly vectors is not yet feasible. Immunization is not yet and may never be possible, and chemotherapy at present is not possible on any scale.

Not wishing to sound like a Jeremiah, I would like to point out some of the encouraging developments that are currently taking place in our field.

The application of biotechnology to the control of vector-borne diseases, I believe, has enormous possibilities. Although the program for development of an efficacious malaria vaccine has not been successful, the advances made in genetic engineering in the program have been enormous. It now appears possible to develop vaccines for immunization to many more etiological agents than was previously possible. Biotechnology also is providing methods for development of diagnostic techniques of greater sensitivity and specificity than was previously possible. Improved methods of identification of vectors, blood meals, and sources of
vectors are expected to result from application of biotechnological methods.

I am also hopeful that models of disease transmission and vector population dynamics will improve our understanding of the relative importance of the various parameters of transmission of disease by vectors and increase our ability to alter them. The development of models already has made us cognizant of much that we did not know and forced us to examine aspects of population dynamics and disease transmission of which we previously were not conscious.

Remote sensing, I believe, is an important development that can contribute to the understanding and control of many vector-borne diseases.

Our inability to control *Ae. aegypti* and dengue transmission has led us to reconsider an old approach, namely, community-based control. Educating the community and encouraging it to participate in sanitation appears to be the only way to deal effectively with dengue at the present time. This approach could also prove to be of value for the control of other vector-borne diseases.

In conclusion, I would stress that the perception of the lack of importance of the field of medical entomology, which is current among many administrators of training institutions today, probably is the most critical issue that we, as professionals, face. The abolition by the World Health Organization of the Vector Biology and Control Division, I believe, is a sign of the times. Programs that formerly were designed and executed by entomologists are now no longer in existence or are administered by workers whose expertise is more in clinical medicine than in field biology. I believe that most vector-borne diseases can be controlled only by sanitary measures and vector control. Clinical medicine is no substitute for preventive medicine.
PLEXIGLAS™ BOX SYSTEM FOR MOSQUITO REARING

J. Olejnicek

ABSTRACT: A rearing system for mosquitoes and other insect species with similar demands for laboratory rearing is described and illustrated. The advantages of this system include improved handling, less space, and lower demands for humidity control of the rearing laboratory.

INTRODUCTION

Mosquito species are reared in laboratories all over the world, using equipment ranging from cages of various dimensions (e.g., Beier et al. 1986) to buckets (Münstermann and Wasmuth 1985). Mosquitoes are mostly reared in insectaries or special airconditioned rooms. After testing various types of equipment for the laboratory rearing of mosquitoes, we have developed the following system using Plexiglas boxes. This system has been well-tested during four years of operation.

MATERIALS AND METHODS

The rearing boxes (Fig. 1) were made from 4 mm thick Plexiglas plates. On the front wall of the boxes, three to four holes for handling are cut out. Two small holes allow the insertion of a sucrose drinking device made from plastic screw-cap vials, with the hole plugged with filter paper, and for the addition of mosquitoes with an aspirator. A fabric sleeve, held tight with a rubber band, is attached to the large holes with the help of a frame from the metal plate. Rails for inserting a box into the stand are attached on the sides of the boxes, (Fig. 2).

Figure 1. Two dimensions of Plexiglas boxes used for mosquito rearing, and the polystyrene boxes used for larvae.

1Institute of Parasitology, Czechoslovakia Academy of Sciences, Branisovska 31, C. Budejovice 370 05, CZECHOSLOVAKIA
Boxes used in our laboratory have the inner dimensions of 25 x 40 x 40 cm (40 l volume), and 12.5 x 26 x 15.4 cm (5 l volume). Through the bottom handling hole, dishes with water are introduced. The dishes are used for egg laying and adult emergence (Olejnicek 1988) and are able to maintain a high relative humidity within the box (to 90%) even in an extremely dry room. For blood feeding, an anesthetized mouse can be placed on a Petri dish that can be pushed into the box through the square handling hole. Other insect species with similar demands for space (various Diptera, some Orthoptera, Polistes wasps, etc.), which are not able to bite through the fabric sleeve, could also be successfully reared in these boxes.

REFERENCES CITED


PREVENTION OF MOSQUITO PRODUCTION AT AN AQUACULTURE WASTEWATER RECLAMATION PLANT IN SAN DIEGO USING AN INNOVATIVE SPRINKLER SYSTEM

R. Ebipane¹, E. Heidig¹, and D. W. Gibson¹

ABSTRACT: A preliminary study was conducted to determine the effectiveness of a sprinkler system to prevent mosquito production at an aquaculture wastewater reclamation plant in San Diego, California. Pulsating garden sprinklers were employed to simulate rain conditions over treatment ponds to discourage mosquito oviposition. Reductions in mosquito counts during 1989 and 1990 were highly significant (P<0.0005 Mann-Whitney U Test) when compared to larval count data from 1988.

INTRODUCTION

The City of San Diego has been experimenting with an innovative aquaculture system of wastewater reclamation and reuse as part of its Total Resource Recovery Program since 1981. In 1984, Aquaculture II began operations on a 13 acre site in Mission Valley, San Diego, California. Because of Aquaculture II's urban location, effective control of mosquitoes was considered an important design criterion.

Mosquito breeding was a significant problem in the early operational stages of the project. The Aquaculture project was required by the San Diego County Vector Surveillance and Control Division and the California State Health Services Department to prevent mosquito production by biological means (Tchobanoglous et al. 1989). Despite the presence of Gambusia affinis and other predators, larval mosquito populations were unacceptably high from 1984 through 1988. During this time, the requirements of San Diego County and State vector control agencies were not met and regular treatments with Teknar™ (BTI), a liquid concentrate of Bacillus thuringensis var. israelensis, and Golden Bear 1111™ (GB1111) larvicide oil were necessary to prevent adult emergence.

In 1986, the Aquaculture staff began experimenting with garden sprinklers for supplemental surface aeration, frost protection, and larvicide application. During operation of the sprinkler system, observations indicated a possible reduction of mosquito oviposition and plans were made to operate sprinklers for vector prevention.

Culex spp. mosquitoes oviposit on still bodies of water and require 20 to 35 minutes to complete oviposition (de Meillon and Thomas 1966). Disturbing or killing female mosquitoes with showers of water before they can land or shortly thereafter should result in fewer eggs laid on the ponds. This study, therefore, examined the effectiveness of the garden sprinklers in reducing immature mosquito populations in the aquaculture ponds.

MATERIALS AND METHODS

Domestic wastewater is drawn from a 600 mm sewer interceptor line located near the project site. Primary wastewater treatment removes 44.6 percent of the solids before the resulting effluent is gravity-fed into six aquaculture secondary sewage treatment ponds. Effluent enters the ponds in equal volumes of 5000 gpd at 15.3 m intervals (Tchobanoglous et al. 1989).

The aquaculture pond purification system uses water hyacinth plants, Eichhornia crassipes (von Mart.) Solms-Laubach, and a diverse community of microorganisms to treat filtered primary wastewater to secondary quality effluent. Each pond measures 10.1 m wide by 126.8 m long and 1.2 m deep. The surface area of each pond is 918.7 m² with an approximate volume of 200,000 gallons (Tchobanoglous et al. 1989). The ponds are continuously aerated to maintain a minimum dissolved oxygen level of 1 ppm. Hyacinths are harvested regularly and the accumulated bottom sludge removed yearly.

Six 3.0 horsepower (3450 RPM) Magnetek™ pumps, one per pond, were utilized to pump secondary effluent to the sprinklers. The sprinklers employed in the study were pulsating garden sprinklers (Rainbird™

¹Aquaculture II Project, Water Utilities Department, City of San Diego, San Diego, CA 92108, U.S.A.
Figure 1. Pond sprinkler system schematic.
Model 2045 PS Maxi-Bird). Each had a base diameter of three-quarters of an inch and produced a spray pattern adjustable from a coarse mist to a fine stream with an effective radius of 3 m to 11 m, respectively. Ten sprinklers were mounted on each side of the ponds in alternating sequence 14 m apart (Fig. 1). Sprinklers were set to cover a 180 degree arc with a radius of 9 to 10 m, covering the pond in overlapping, coarse-spray patterns. When installed, the average sprinkler travel time to complete an arc was 20 seconds. Sprinklers were operated continuously every night from one hour before sundown to one hour after sunrise. The sprinklers were checked and adjusted weekly. Sprinklers that failed or that had worn out so that the visually approximated travel time exceeded 40 seconds per rotation were replaced.

The ponds were monitored weekly for mosquito egg rafts, larvae, and pupae. A minimum of 40 samples, five per cell, were taken at intervals of about 3 m from each side of each pond (80 samples total), using a "plunge" sampler described by Townzen and Wilson (1983). The plunger was designed to penetrate the dense canopy of water hyacinths. It consists of an inverted pyramidal collecting container (10 x 10 x 28 cm) attached to a 1.5 m pole arm. The plunger collects approximately 850 ml of pond water, and is designed so that the area of the aperture equals that of the standard dipper. Immature mosquitoes collected in the samples were counted and recorded. Third and fourth-instar larvae were preserved in 70 percent ethyl alcohol and identified to species. During all years of operation, when a plunge sample contained more than five mosquitoes, additional samples (not included in the analysis) were taken to delineate the area to be thinned of hyacinths and/or treated with larvicides.

Because of budget constraints and the pre-established compulsory requirement to prevent mosquito production (Tchobanoglous et al. 1989, Eldridge and Martin 1987), a standard experimental design utilizing proper controls and replications could not be employed. Consequently, the data generated in 1989 and 1990 were compared with that of 1988 to indicate the effectiveness of the sprinklers in preventing oviposition. Mosquito counts per plunge were compared using the Mann-Whitney U test.

RESULTS AND DISCUSSION

Beginning in August, 1988, a garden sprinkler system was used to spray pond effluent over one pond (Pond 3) nightly when Culex spp. mosquitoes would most likely to be ovipositing (Bentley and Day 1989, Schreiber et al. 1988). This trial resulted in almost complete elimination of mosquito larvae from that pond while neighboring ponds continued to support large mosquito populations. In March, 1989, all six ponds were equipped with identical sprinkler systems and the average monthly counts per plunge for all ponds for that year were reduced by 94 percent over 1988 average monthly totals (Fig. 2). The Mann-Whitney U test comparing count data per plunge for the two years was highly significant ($U_{12,12} = 136, P < 0.0005$). In 1990, the average monthly totals were reduced by 97 percent over 1988 averages and the Mann-Whitney U test was equally significant ($U_{12,12} = 137, P < 0.0005$).

Mosquito outbreaks in the aquaculture ponds prior to 1989 were frequent and characterized by large numbers of larvae and pupae of all stages present in areas greater than 10 m$^2$ throughout the peak breeding season of May to September. In contrast, the outbreaks in the summers of 1989 and 1990 were infrequent, noticeably smaller in numbers, composed primarily of single cohorts, and typically restricted to areas less than 2 m$^2$.

Control was usually achieved by manually thinning the hyacinths in that area to improve conditions for larval predation by mosquitofish.

Larvicide demand was sharply reduced during the study. In 1988, 18.25 l of GB1111 and 17.25 l of Tekna™ were needed to meet minimum vector control requirements. After March 1989, with all six ponds equipped with sprinkler systems, the total volume of larvicide required was reduced to 1.01 l of Bti and 0.25 l of GB1111. Larvicide demand was similar in 1990 with only 2.0 l of Bti and 20 Bactimos™ briquets (solid preparations of Bti used experimentally for two weeks in one pond) to control mosquito outbreaks. No GB1111 was used that year.

Several problems were identified in the sprinkler system during the study. Summer hyacinth and pondside weed growth sometimes blocked or jammed sprinkler heads, temporarily limiting their ranges. Also, clogged pump intakes and occasional low water flow sometimes resulted in sprinkler downtime for as long as two hours. Additionally, sprinklers suffered considerable wear during continuous operation resulting in stuck sprinklers and lengthened travel times. Consequently, mosquito outbreaks continued, although at a significantly reduced level.

In July, 1990, an extensive mass of culicine egg rafts (>500 rafts) was found in stagnant, organically polluted water in the influent box of a drained pond.
(Pond 3). This basin was available for oviposition for only two nights and the larvae found with the rafts were very early first-instar; indicating that a large gravid population of female culicine mosquitoes was active in the area at that time. Samples of these larvae and rafts were reared and found to consist of *Cx. pipiens* and *Cx. stigmatosoma*. This observation is significant in light of the fact that San Diego was experiencing a drought during the study, which may have reduced the number of gravid culicines attempting oviposition. These outbreaks indicated that gravid mosquitoes were in fact ovipositing where conditions were favorable.

The level of mosquito prevention achieved during 1989 and 1990 utilizing the sprinkler system to prevent oviposition greatly exceeded that of control strategies employed before 1989. Those strategies were directed at reducing existing larval populations using conventional larvicides and predation by *Gambusia affinis* to prevent emergence of adult mosquitoes.

As a mechanical form of mosquito prevention, the sprinkler system is not subject to the same limitations as conventional control techniques where heavy vegetation and water quality can be severely limiting. *Gambusia affinis* and larviciding oils have been shown to be less effective in heavily vegetated bodies of water (Linden and Cech 1990; Mian et al. 1986; Townzen and Wilson 1983; Eldridge and Martin 1987). The effectiveness of Teknar™ is reduced in heavily polluted waters characteristic of aquaculture wastewater reclamation (Dupont and Boisvert 1985, Lacey and Undeen 1986).

However, as a mechanical system, the sprinklers are subject to breakdowns and physical interference. Mosquito outbreaks still occur but they are minor in magnitude and more easily controlled when compared to previous outbreaks at Aquaculture II and other aquaculture systems. The sprinkler system approach met the original mandates for vector prevention and greatly reduced larvicide demand and human investments for monitoring, specimen identification, larvicide application, and follow-up. Additional benefits of the sprinkler system also include frost protection of the hyacinths and increased aeration of the surface water, which may improve conditions for mosquitofish and insect predators (Tchobanoglous et al. 1989).
Eldridge and Martin (1987) and Townzen and Wilson (1983) recommended that future water reclamation projects should be designed and operated in a way to prevent mosquito breeding. If successful over several more years as a component of an integrated vector prevention program, the sprinkler system could open the doors to the establishment of other aquaculture water reclamation projects, an option attractive in light of the six-year drought that ended in February 1993 in California.

Acknowledgments

Sincere appreciation is extended to Pete Silva, Deputy Director, City of San Diego Water Utilities Department, and Ken Thompson, Superintendent, Aquaculture II Project. Special thanks are also extended to Theodore Smith, California State Health Services (ret.), Moise Mizrahi, San Diego County Vector Surveillance and Control Division, and Dr. Ronald Monroe, San Diego State University. Our gratitude is also extended to the staff of the Aquaculture II Project and to the anonymous reviewers whose suggestions substantially improved the clarity of this paper.

REFERENCES CITED


NEW JERSEY'S APPROACH
TO ENCEPHALITIS PREVENTION 1

W. J. Crans 2 and L. J. McCuiston 2

ABSTRACT: Since 1975, the state of New Jersey has developed a cooperative program to monitor and control eastern equine encephalitis (EEE) virus. The agencies involved in the program include: the New Jersey Agricultural Experiment Station, the New Jersey State Department of Health, the New Jersey State Department of Agriculture, the New Jersey Department of Environmental Protection, and seven county mosquito control agencies that benefit directly from the service. Funding is provided by the New Jersey State Mosquito Control Commission on an annual basis. The program monitors EEE and its mosquito vectors at a number of established study sites in the state where human or equine cases have caused concern in the past. Culiseta melanura is used as the main indicator of virus activity in nature. Deviations in population levels from the long-term mean are calculated by computer and Minimum Field Infection Rates (MFIR) in Cs. melanura are updated weekly. All information is made available to state and county mosquito control agencies on a regular basis during the encephalitis season. Equine cases are used as an indicator for accelerated control of Culex tarsalis perturbans and associated vectors in inland areas of the state. The physiological age of Aedes sollicitans populations is used as the main indicator of risk for transmission to humans. During periods of risk, chemical control is directed toward localized, physiologically "old" populations of Ae. sollicitans. Since the inception of this program, the number of confirmed human cases of EEE have declined markedly and broad-scale emergency airsprays have not been needed.

INTRODUCTION

In 1975, the New Jersey Agricultural Experiment Station developed the New Jersey Vector Surveillance Program, a monitoring effort directed toward eastern equine encephalitis (EEE) and its mosquito vectors in coastal areas of the state (Crans et al. 1976). Since that time, the program has grown and is now a cooperative effort that involves the New Jersey Department of Health, the New Jersey Department of Agriculture, and the New Jersey Department of Environmental Protection, as well as seven county mosquito control agencies that benefit from the service. The program is funded annually by the New Jersey State Mosquito Control Commission, a commission that is appointed by the Governor of New Jersey to oversee the expenditure of an allotment of state funds to be directed toward responsible mosquito control.

The objectives of the program are two-fold: 1) to determine if EEE virus is present in nature in a variety of established study sites where the disease has been detected in the past, and 2) to determine the risk of transmission to humans. In the event of risk, the New Jersey State Mosquito Control Commission utilizes its State Airspray Program to control the epidemic vectors and minimize the potential for transmission to humans.

New Jersey's efforts to monitor and control EEE virus have been extremely successful. In the 15 years prior to the monitoring program, the State had 49 confirmed human cases of EEE. In the 15 years since its inception, the State has had only three confirmed cases, one of which was a visitor from Massachusetts that was never attributed to New Jersey in the official records (Crans and Schulze 1985). This paper summarizes New Jersey's approach to encephalitis monitoring and control with the hope that other states will modify the procedures used and develop a program that is applicable to their particular geographic area.

1 New Jersey Agricultural Experiment Publication D-40101-01-91 supported by Hatch Act Funds and funding from the New Jersey State Mosquito Control Commission.

2 Mosquito Research and Control, Department of Entomology, Rutgers University, P.O. Box 231, Cook College, New Brunswick, N.J. 08903, U.S.A.
METHODOLOGY OF THE SURVEILLANCE EFFORT

Establishment of Permanent Study Sites

Eastern equine encephalitis virus is monitored within its mosquito vectors at a series of study sites that were selected on the basis of: 1) a history of known virus activity based on past human cases, 2) a focus on equine cases of EEE over the years, or 3) a recent history of either human or equine cases that appear to defy our present hypotheses on disease transmission. Some of the sites have been monitored since 1975; others are rather recent and have been monitored for less than five years. The program tries to keep a balance between coastal sites that threaten human health and the economy of the resort industry and inland sites where equine cases are common. Unlike many states on the eastern seaboard, New Jersey has never had a confirmed human case at an inland area. Human cases are a coastal phenomenon that have only been confirmed from coastal residents or in persons from inland areas that visited the New Jersey coast within two weeks of disease onset (Goldfield and Sussman 1968). In coastal areas, the program monitors Culiseta melanura, the primary epionithic vector, (Burbatis and Jobbins 1957, Chamberlain et al. 1958) and Aedes sollicitans, the only mosquito in New Jersey that has been implicated in the transmission to humans to date (Crans et al. 1986a). At inland study sites the program monitors C. melanura and Coquillettidia perturbans, the only mosquito that has been documented as an epizootic vector in New Jersey (Crans and Schulze 1986). Other mosquito species are collected and tested for EEE virus for research purposes if funds are available, but are not considered a part of the main surveillance thrust.

Culiseta melanura is Used as the Main Indicator of Virus in Nature

Culiseta melanura is selectively collected at each of the permanent study sites each year to determine: 1) population levels of the species as the season progresses, 2) deviations in population levels from the long-term mean, and 3) the levels of EEE virus in the bird populations that it is feeding on. Specimens are collected from a line of 25 resting boxes at each study location. The specimens are introduced to glass vials (one vial per box for statistical purposes), frozen on dry ice at the collection sites, and transported to the laboratory where they are frozen at -57°C until they are sorted. The resting box is the collection method of choice because it collects a broad spectrum of stages in the gonotrophic cycle. Resting box collections include: 1) UNENORGED mosquitoes that are in the process of locating a host, 2) ENGORGED mosquitoes that have recently acquired a blood meal, 3) BLACK-BLOODED mosquitoes that have partially digested a recent blood meal, and 4) GRAVID mosquitoes that have digested a prior blood meal and contain fully developed eggs. We believe that this broad cross section of stages in the gonotrophic cycle increases our chances of locating infected mosquitoes in nature. We also believe that the light trap is much less efficient in detecting the infected portion of the mosquito population because it only attracts unengorged mosquitoes in numbers.

The resting box collections are identified to species on a chill table (to preserve living virus particles), pooled in groups no greater than 50 specimens within each of the aforementioned categories, triturated in mosquito diluent, and refrozen for virus isolation attempts. The New Jersey State Department of Health performs the virus isolation attempts at their laboratories and notifies the Vector Surveillance Program of the results.

All information is entered into a database for rapid collation, analysis, and graphics. The program (written in dBase language) assimilates the information by Pool Number, divides data by Site, and calculates Minimum Field Infection Rates (MFIR values) by Site, Week, or Month. The MFIR values (isolation rates per 1,000 specimens tested) gives us a numerical rate that can be used to compare the intensity of virus transmission in different areas of the state. The computerized program allows us to compute this information as soon as the results of virus isolation attempts are known and gives us an immediate picture of EEE amplification over a broad geographic area. The information is phoned weekly to the participating county mosquito control agencies, mailed weekly in hard copy to all agencies, and published bimonthly in a "New Jersey Vector Surveillance Report."

Equine Cases are the Indicator for Accelerated Control of Coquillettidia perturbans at Inland Areas of the State

The New Jersey State Department of Agriculture requires veterinarians to report all suspect horse cases as soon as possible. They also require the veterinarians to submit a series of blood samples taken during the clinical course of infection and the brain of euthanized animals whenever possible. All suspect animals are tested for EEE. The results are made available to the New Jersey Vector Surveillance Program as soon as possible. County mosquito control agencies are contacted by phone, given the address of the equine case, and urged to conduct mosquito trapping for virus
isolation attempts. Control by ground ULV is strongly recommended in the area of a confirmed equine case and all county mosquito control agencies are notified of confirmed, presumptive, and pending cases. Data from prior years implicate *Cq. perturbans* as the main vector to equines (Crans et al. 1986b). We do not have evidence that *Aedes vexans* and other floodwater mosquitoes are involved but most agencies engage in accelerated control that reduces the overall vector populations.

**In Coastal Areas, the Physiological Age of *Aedes sollicitans* is Used as the Main Indicator of Risk for Transmission to Humans**

*Aedes sollicitans* populations are monitored twice weekly from June through October at numerous stations along the New Jersey coast. Field technicians record multiple minutes landing rates to determine the mean number of mosquitoes coming to bite per minute. They also collect and freeze specimens for physiological age determinations according to the procedures outlined by Detinova (1962). In the laboratory, the ovaries of no fewer than 20 specimens from each collection station are dissected in distilled water, dried on a microscope slide, and examined for parity. The presence of tracheolar skeins in the ovaries indicates a nulliparous mosquito (a mosquito that has not yet laid eggs); extended tracheoles indicate a parous mosquito (a mosquito that has laid at least one batch of eggs and has, therefore, had at least one prior blood meal). The data are used to calculate the percentage of parous mosquitoes in the biting populations at each station.

The number of parous mosquitoes coming to bite per minute is obtained by multiplying the landing rate, collected at the field site, by the parous rate determined by dissection. This yields a Vector Potential Index (parous landing rate) which is used as the main indicator of risk for transmission to humans. Control of *Ae. sollicitans* is recommended if the parous landing rate exceeds ten per minute in an area where EEE virus is known to be circulating in *C. melanura*.

Monitoring physiological age to determine transmission risk is a technique that was developed by Russian scientists and put into practice by the British, and others, to reduce the rate of malaria transmission in malarious regions (Detinova 1962). In those studies, the aim was to assure that no mosquitoes lived longer than nine calendar days, the amount of time required for the malaria parasite to reach infectivity in the vector. Control methodology involved spraying the inside of domiciles with a persistent pesticide because the vector entered houses, fed on sleeping humans, and rested on the walls before leaving to deposit its eggs. Over a nine-day time span, the average anopheline mosquito would feed three times, thus, the probability that the mosquito would contact the pesticide during its first nine days of life was high. The technique kept the age of the biting population “young” and the percentage of mosquitoes that were capable of transmitting malaria dropped markedly.

*Aedes sollicitans* is a brooded mosquito and the logic developed by the British can be applied to reduce vector potential for EEE. In a brooded mosquito, high mosquito populations are usually an indication of a fresh emergence. Annoyance can be considerable but the adults are mainly nulliparous, thus, vector potential is low. Over time, annoyance declines but the adults become parous and the vector potential of the biting population increases. The technique of measuring vector potential by measuring the number of parous mosquitoes coming to bite yields the index that New Jersey uses to institute control for the prevention of human disease. When EEE is known to be cycling in *C. melanura*, chemical control is directed toward “old” populations of *Ae. sollicitans*. This results in spot treatments in designated areas of the State rather than broad scale chemical coverage during emergency periods.

**CONCLUSIONS**

New Jersey’s approach to encephalitis control has worked well over the years and we no longer see the massive air sprays that characterized the 1950s and 1960s. The program works because the state and county agencies that are responsible for mosquito control are kept aware of the status of EEE and its mosquito vectors every season. The system also works because it is based on science and supported by ongoing research. The New Jersey State Mosquito Control Commission has funded much of the research that is now put into practice and continues to support research that is needed for the future. Eastern equine encephalitis is still a threat to coastal residents and the economy of the resort industry in southern New Jersey. Encephalitis prevention, however, is now a cooperative effort in New Jersey; and it is doubtful that the State will experience the panic and hysteria that accompanied the 1959 outbreak and the EEE episodes that characterized the 1960s.

**REFERENCES CITED**


A COMPARISON OF ABDOMINAL SCALE PATTERNS
IN THE MOSQUITO Aedes aegypti

R. E. Duhrkopf1, W. K. Hartberg1, and R. Novak2

ABSTRACT: Extensive variation in abdominal scaling pattern exists in natural populations of Aedes aegypti. Data on the abdominal scaling patterns were collected from females of 50 different populations of Aedes aegypti (13 African, 19 Caribbean/Central and South American, 14 United States, and 4 Asian and South Pacific). The populations were compared via cluster analysis. No relationships between banding pattern and geographic location or habitat were detected. A polygenic model involving three loci has been proposed for this trait. The equilibrium frequencies for the alleles at each of the loci were empirically estimated by computer modeling. These were also analyzed via cluster analysis. The results indicate patterns of extensive underlying genetic variation but no systematic relationship to either geographic region or habitat type.

INTRODUCTION

The abdominal scaling pattern of the adult mosquito Aedes aegypti (L.) is highly variable. The abdominal tergites are commonly blackish brown with white scales occurring as basal bands and lateral spots. Additional patches of white scales may extend from the basal bands. There is much variation in the extent of such additional scaling, ranging from almost nothing to white scales covering almost all of the abdominal tergites. This variation allows quantification of scaling primarily on the basis of the distal-most location of extra white scaling (McClelland 1960). McClelland (1974) later expanded his system to one involving 30 groups in which his earlier classes (F through Q) were supplemented with subclasses.

Abdominal scaling has been used to classify Ae. aegypti into three subspecies (Mattingly 1957). Forms with no additional scaling have been designated as subspecies formosus. Forms with extensive additional white scaling have been designated as variety queenslandensis, and intermediate forms are placed into the type form - aegypti. The differences in banding pattern have also correlated with differences in geographic region and habitat. In some parts of Africa, only the formosus variety is present. In other parts of Africa, both the formosus and aegypti forms overlap. In those places where the two forms overlap, they occupy different larval and adult habitats with the formosus variety existing in sylvan habitats and the aegypti variety living in domestic habitats. Saul et al. (1980) showed that the habitat differences were caused not just by differences in adult behavior, but by differences in the fitness characteristics of the larvae. On the other hand, Mogi et al. (1984) found no correlation between scaling pattern and habitat in samples of the aegypti type form taken from the Philippines. Mogi et al. (1989) later showed that scaling pattern remained seasonally consistent in a locality in northern Thailand.

In more recent work on abdominal scaling, Hartberg et al. (1986) proposed a simplified system of classification based upon McClelland's 30 groups. In this system (designated CKM), only the number of tergites with additional white scaling is considered. The extent of white scaling on the tergites is disregarded. This allows for classification using eight different classes. Class 0 has no extra white scales. The adults have white scales which appear only as basal bands and lateral spots. Class 1 has extra white scales only on the first tergite. Class 2 has extra white scales on the first and second tergites. This continues until class 7, which has extra white scales on all abdominal tergites.

McClelland (1960) proposed a multifactorial system of inheritance. However, he suggested that the trait was controlled by only a few pairs of alleles. VandeHey et al. (1978) speculated that scaling pattern was controlled by a polygenic system involving one or two major genes with modifiers.

Based upon results of over 30 single-pair matings involving parents with different scaling patterns, Hartberg et al. (1986) proposed a model for the inheritance of scaling patterns. The model involved the

1Department of Biology, Baylor University, Waco, Texas 76798, U.S.A.

2Illinois Natural History Survey, Champaign, Illinois 61820, U.S.A.
activity of three independently segregating loci. The first locus was called "tergite-white" (Tw). It has two alleles, Tw and Tw+, which determine whether white scales can or cannot appear on the tergites. The recessive Tw+ allele prevents white scaling on the tergites while the dominant Tw allele allows white scaling. The second locus, called "white-scaling," is occupied by a series of multiple alleles. Three alleles, L1, L2, and L, were designated. This locus controls the extent of white scaling, if present. The L1 allele promotes greater amounts of white scaling. The L2 allele promotes less white scaling, and the L allele produces no white scaling. This locus follows the classical additive polygenic model with scaling increasing from no white scaling in individuals with an l/l genotype to large amounts of scaling in individuals with an L1/L1 genotype. The third locus, called "white-intensifier," acts to intensify the effects of the second locus. It has two alleles. The L allele, which is completely dominant, acts to intensify the effects of the L1 and L2 alleles, and the recessive l allele does not intensify the effects of the L1 and L2 alleles.

The present study had two objectives. The first was to compare abdominal scaling patterns in different populations from diverse habitats and geographic locations. The second was to attempt to use those data to generate estimates of allelic frequencies for the three genes involved in the polygenic model proposed by Hartberg et al. (1986).

MATERIALS AND METHODS

Fifty collections of Ae. aegypti were used. The materials used, their origins, and methods of collection are presented in TABLE 1. The sampled populations represented a variety of habitats from several geographic locations. For each strain, larvae were reared under standard procedures (Craig and VandeHey 1962) in an insectary with a temperature of 26°±0.5°C. Larvae were fed on a suspension of liver powder in tap water (12 ml/liter). Pupae were collected, segregated by sex, and allowed to emerge individually.

Within 26 hours after emergence, 100 adult females were classified for the pattern of abdominal scaling based upon the CKM system (except for 8 populations where fewer females were classified). McClelland (1974) and Hartberg et al. (1986) both concluded that consistent data are obtained by scoring only females. These data were subjected to cluster analysis using the unweighted pair-group method as described in Weir (1990). This method defines the distance between populations by averaging all the pairwise distances between members of the populations. Clusters are then established based upon distances between populations.

The genetic model does not allow for differentiation between CKM classes 0 (no additional white scaling), and 1 (small amounts of additional white scaling on the first tergite). Because of this, the numbers of individuals in CKM classes 0 and 1 were combined for estimation of the allelic frequencies. Gene frequencies were empirically estimated via computer simulation. The model predicts 54 possible genotypes. Each CKM class is associated with a unique array of genotypes.

Theoretical equilibrium frequencies for each of the CKM classes were determined by the Castle - Hardy - Weinberg Theory. For example, if p, q, r, s, t, u, and v represent the frequencies of the Tw+, Tw, I, L2, L1, and i alleles, the equilibrium frequency of the genotype Tw+/Tw L2/lI/l would be 2pq+2st+2uv. Such genotypic frequencies were then combined to generate expected equilibrium phenotypic frequencies for each of the CKM classes (TABLE 2).

Given the three locus model, if allelic frequencies are incremented from 0 to 1.0 in units of 0.1, there are 7,986 different, possible combinations of allelic frequencies. The program iterated through all those allelic frequencies and then calculated the expected phenotypic numbers for each of the CKM classes. The program compared the generated expected numbers with the observed data via a Chi-square goodness of fit test (with appropriate corrections for cells with expected numbers less than 1). In almost all cases, there were several different arrays that showed non-significant differences. Those arrays were then analyzed to determine the most parsimonious array. This was determined by identifying that array with the lowest Chi-square statistic and the greatest number of degrees of freedom. These frequencies were then used for a second cluster analysis of genetic similarities between the populations.

As an example of the method used, we can begin with the data from the Bryan-College Station, Texas population. That population had 99 females of CKM classes 0 and 1, and 1 female of CKM class 2. The program tested the different frequencies of the seven alleles. The method can be seen by looking at two of those arrays. If the frequency of the Tw+ allele is 0.9, the frequencies of the I and L2 alleles are 0.8 and 0.1, and the frequency of the 1 allele is 0.9, the expected phenotypic numbers would be 93 females in CKM classes 0 and 1, two females in CKM class 2, and two females in CKM class 3 (\(\chi^2= 2.887, 2 \text{ d.f.}\)). If the frequencies of the Tw+ allele is 0.9, the frequencies of the I and L2 alleles are 0.9 and 0, and the frequencies of the 1 allele is 0.7, the expected number of females in CKM classes 0 and 1 would be 96, the expected number
<table>
<thead>
<tr>
<th>Location</th>
<th>Strain Desig.</th>
<th>Stage</th>
<th>Locale Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFRICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bansang, Gambia</td>
<td>Bansang I</td>
<td>Larvae*</td>
<td>Domestic (Ext)</td>
<td>58 Containers</td>
</tr>
<tr>
<td>Bansang, Gambia</td>
<td>Bansang-S</td>
<td>Ovitraps</td>
<td>Sylvan</td>
<td>15 Ovitraps</td>
</tr>
<tr>
<td>Bansang, Gambia</td>
<td>Bansang II</td>
<td>Larvae*</td>
<td>Domestic (Int)</td>
<td>30 Containers</td>
</tr>
<tr>
<td>Diourbel, Senegal</td>
<td>Diourbel</td>
<td>Larvae*</td>
<td>Domestic (Ext)</td>
<td>30 Containers</td>
</tr>
<tr>
<td>Kalifi</td>
<td>Kalifi</td>
<td>Larvae*</td>
<td>Domestic (Ext)</td>
<td>No. Unknown</td>
</tr>
<tr>
<td>Kari, Upper Volta</td>
<td>Kari-T</td>
<td>Larvae*</td>
<td>Sylvan</td>
<td>8 Treeholes</td>
</tr>
<tr>
<td>Kari, Upper Volta</td>
<td>Kari-W</td>
<td>Larvae*</td>
<td>Domestic (Int/Ext)</td>
<td>14 Containers</td>
</tr>
<tr>
<td>Kongoliken, Upper Volta</td>
<td>Kongo</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>34 Containers</td>
</tr>
<tr>
<td>Kedougou, Senegal</td>
<td>Kedo</td>
<td>Larv*/Ovit.</td>
<td>Sylvan/treeholes</td>
<td>No. Unknown</td>
</tr>
<tr>
<td>Mintah Kundi, Gambia</td>
<td>Mintah</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>25 Ovitraps</td>
</tr>
<tr>
<td>Roca, Kenya</td>
<td>Roca</td>
<td>Ovitraps</td>
<td>Peridomestic</td>
<td>25 Ovitraps</td>
</tr>
<tr>
<td>Ton, Upper Volta</td>
<td>Ton</td>
<td>Larvae*</td>
<td>Sylvan</td>
<td>7 Treeholes</td>
</tr>
<tr>
<td><strong>CARIBBEAN/SOUTH AND CENTRAL AMERICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anguilla, BWI</td>
<td>Ang</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>48 Containers</td>
</tr>
<tr>
<td>Anguilla, BWI (June)</td>
<td>Ang-Rock</td>
<td>Larvae</td>
<td>Sylvan</td>
<td>15 Rockholes</td>
</tr>
<tr>
<td>Anguilla, BWI (Nov)</td>
<td>Ang-Rock-I</td>
<td>Larvae</td>
<td>Sylvan</td>
<td>10 Rockholes</td>
</tr>
<tr>
<td>Antigua</td>
<td>Antiq</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>56 Containers</td>
</tr>
<tr>
<td>Barbados</td>
<td>Barbados</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>15 Ovitraps</td>
</tr>
<tr>
<td>Caguas, P.R. (June)</td>
<td>Caguas-I</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>89 Containers</td>
</tr>
<tr>
<td>Culebra, P.R.</td>
<td>Culeb</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>48 Containers</td>
</tr>
<tr>
<td>Rexville, P.R. (May)</td>
<td>Rex</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>75 Containers</td>
</tr>
<tr>
<td>Rexville, P.R. (Sept.)</td>
<td>Rex-I</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>128 Containers</td>
</tr>
<tr>
<td>Martinique</td>
<td>Martinique</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>55 Containers</td>
</tr>
<tr>
<td>St. Kitts</td>
<td>St. Kitts</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>36 Art. Containers</td>
</tr>
<tr>
<td>St. Lucia</td>
<td>St. Lucia</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>33 Containers</td>
</tr>
<tr>
<td>St. Martin</td>
<td>St. Mart</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>38 Containers</td>
</tr>
<tr>
<td>Tortola, BVI</td>
<td>Tortola-N</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>21 Containers</td>
</tr>
<tr>
<td>Tortola, BVI</td>
<td>Tortola-I</td>
<td>Larvae</td>
<td>Domestic (Int)</td>
<td>9 Containers</td>
</tr>
<tr>
<td>Trinidad</td>
<td>Trinidad-I</td>
<td>Ovitraps</td>
<td>Domestic</td>
<td>25 Ovitraps</td>
</tr>
<tr>
<td>Paramaribo, Surinam</td>
<td>Param</td>
<td>Ovitraps</td>
<td>Sylvan</td>
<td>10 Ovitraps</td>
</tr>
<tr>
<td>Malaga, Columbia</td>
<td>Malaga</td>
<td>Ovitraps</td>
<td>Domestic</td>
<td>25 Ovitraps</td>
</tr>
<tr>
<td>Tapachula, Mexico</td>
<td>Tapas</td>
<td>Ovitraps</td>
<td>Domestic</td>
<td>35 Ovitraps</td>
</tr>
<tr>
<td><strong>ASIA/SOUTH PACIFIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiji</td>
<td>Olongapo City, Phil</td>
<td>Larvae*</td>
<td>Domestic/Perid</td>
<td>No. Unknown</td>
</tr>
<tr>
<td>Olongapo City, Phil</td>
<td>Olong</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>No. Unknown</td>
</tr>
<tr>
<td>Hainan, China</td>
<td>Hainana</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>10 Ovitraps</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>Sri Lanka-Old</td>
<td>Laboratory Col.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>UNITED STATES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Orleans, LA</td>
<td>NO-Air</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>25 Containers</td>
</tr>
<tr>
<td>Almonaster</td>
<td>NO-Almon</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>25 Containers</td>
</tr>
<tr>
<td>Chef Menteur</td>
<td>NO-Chef</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>35 Containers</td>
</tr>
</tbody>
</table>

TABLE 1 continued on next page.
TABLE 1. The strains of *Aedes aegypti* analyzed for CKM banding pattern - continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Strain Desig.</th>
<th>Stage</th>
<th>Locale Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. George</td>
<td>NO-St. G</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>35 Containers</td>
</tr>
<tr>
<td>Dante</td>
<td>NO-Dante</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>15 Containers</td>
</tr>
<tr>
<td>Magazine</td>
<td>NO-Mag</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>No. unknown</td>
</tr>
<tr>
<td>Miami, FL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downtown</td>
<td>Miami-D</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>40 Containers</td>
</tr>
<tr>
<td>Coconut Gr.</td>
<td></td>
<td></td>
<td>Domestic (Ext)</td>
<td>45 Containers</td>
</tr>
<tr>
<td>San Antonio, TX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downtown</td>
<td>San A-D</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>35 Containers</td>
</tr>
<tr>
<td>Villa Cornado</td>
<td>San A-Villa</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>20 Containers</td>
</tr>
<tr>
<td>Losaga</td>
<td>San A-Los</td>
<td>Larvae</td>
<td>Domestic (Ext)</td>
<td>No. unknown</td>
</tr>
<tr>
<td>Bryan-College Station TX</td>
<td>B-C S</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>No. unknown</td>
</tr>
<tr>
<td>Corpus Christi TX</td>
<td>C C TX</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>No. unknown</td>
</tr>
<tr>
<td>Savannah, GA</td>
<td>Savan</td>
<td>Ovitraps</td>
<td>Domestic (Ext)</td>
<td>15 Ovitraps</td>
</tr>
</tbody>
</table>

* Collected as larvae reared to adult, blood feed, and sent as eggs. Each egg paper that was hatched produced over 1,000 female *Ae. aegypti*.

Note: All ovitrap collections yielded > than 1,000 females for collection.

Note: All material used for morphotyping and/or electrophoresis did not exceed the F2 generation.

of females in CKM class 2 would be 1, the expected number of females in CKM class 3 would be 0, and the expected number of females in CKM class 4 would be 1 ($\chi^2= 1.094$, 2 d.f.). Since the second $\chi^2$ statistic is smaller, that array of allelic frequencies would be more parsimonious.

RESULTS

The observed values for the CKM banding patterns of the different strains are presented in TABLE 3. There was a wide variety in frequency distributions in CKM classes. The strains varied from those in which only one of the banding classes was present to those in which each of the banding classes were represented.

Figure 1 shows the cluster analysis for the scaling pattern data. The different populations assort into two groups. The first group of 12 populations includes those in which relatively large numbers of females fell in the middle and lighter CKM classes (CKM classes 3 to 7). These populations had very few or no females in the CKM 0 class. This large group assorted into two internal clusters. The first contained seven populations. These had higher numbers of females in the CKM 2, 3, and 4 classes. This group included populations collected from domestic, outdoor containers (St. Kitts and Antig), sylvan tree holes (Koum and Ton), domestic ovitraps (Mintah and Fijji), and sylvan rock holes (Ang-Rock). The second cluster contained five populations. These populations had more females in the CKM 4, 5, 6, and 7 classes. This group included populations collected from domestic, indoor containers (Tortola-I), domestic, outdoor containers (Olong and San A-D), and domestic ovitraps (Malaga and Trinidad I).

The second large group included 37 populations. These had larger numbers of darker females. In general, 70-90 percent of the females in these populations were in CKM classes 0, 1, or 2. This large group assorted into three clusters with two isolated populations. The first cluster contained five populations. These populations had almost all females in the CKM class 0 with the rest of the females in CKM class 1. This group included populations collected from domestic, indoor containers (Bansang II), domestic, outdoor container (Bansang I), sylvan tree holes (Bansang S and Kari-T), and peri-domestic ovitraps (Roca). The second internal group had only four populations. These had significant numbers, but fewer females in CKM class 0, with a majority of females in class 1. A few females were distributed in lighter CKM classes. This group included populations collected from domestic, outdoor containers (Kalifi and San A-Los), domestic, outdoor ovitraps (Kongo), and sylvan tree holes (Kedo). The third internal group included 27 populations. This group had populations in which few of the females were in CKM class 0. Almost all of the remaining females were in CKM class 1 with a few females in CKM classes 2 to 7. This group included populations...
TABLE 2. Expected Equilibrium Frequencies of CKM Classes.  

<table>
<thead>
<tr>
<th>CKM Class</th>
<th>Expected Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1</td>
<td>$p^2 + r^2(1 - p^2)$</td>
</tr>
<tr>
<td>2</td>
<td>$2rsu^2(1 - p^2)$</td>
</tr>
<tr>
<td>3</td>
<td>$u^2(1 - p^2)(2rt + s^2)$</td>
</tr>
<tr>
<td>4</td>
<td>$(1 - p^2)[2rs(1 - u^2) + 2stu^2]$</td>
</tr>
<tr>
<td>5</td>
<td>$(1 - p^2)((1 - u^2)(2rt + s^2) + 2u^2)$</td>
</tr>
<tr>
<td>6</td>
<td>$(1 - u^2)[2st(1 - p^2)]$</td>
</tr>
<tr>
<td>7</td>
<td>$t^2(1 - p^2)(1 - u^2)$</td>
</tr>
</tbody>
</table>

$p, r, s, t, and u$ are the frequencies of the $Tw^+$, 1, L_2, L_1, and I alleles, respectively.

collected from domestic, indoor containers (Kari-W), domestic, outdoor containers (Diourbel, Ang, Caguas-I, Culeb, Martinique, Rex, Rex-I, St. Lucia, St. Mart, NO-Air, NO-Almon, NO-Chef, NO-Dante, Miami-Coca, Miami-D, San A-Villa, and NO-St. G), domestic, outdoor ovitraps (Hainana, Barbados, B-C S, C C TX, Savan, and Tapa), sylvan ovitraps (Param), rock holes (Ang-Rock-I), and a laboratory colony (Sri Lanka-Old).

There were two populations in the second large group that did not fit closely in any of the internal clusters. The first of these was the NO-Mag population. It was similar to the 27 populations of the third group. However, it had more females in the CKM 4 and 5 classes than any of the members of the group. The second independent population was Tortola-N. It had a large majority of its females in the lighter classes, but far fewer females in the darker CKM classes than either of the broad groups.

The estimated equilibrium gene frequencies for the 50 populations are presented in TABLE 4. The accurate determination of the gene frequencies for 12 of the 50 populations was impossible due to the epistatic nature of the system and the modeling methods used in this study. For example, in the NO-Air strain, all the adults analyzed were in CKM classes 0 and 1 (no extra white scaling). Lack of extra white scaling is a result of being genotypically $Tw^+/Tw^+$ or $T^i$. A population in which all individuals were in CKM classes 0 and 1 would result from fixation for either the $Tw^+$ or the $T^i$ allele. In TABLE 4, the frequencies of these two alleles are shown as 1.0 for each. Given the design of this study, determining which of the two genes was fixed was impossible. If $Tw^+$ was fixed, then the L_1 and L_2 alleles at the second locus would have no phenotypic effect. Since their effects are masked, there would be no means for determining their frequencies. The same argument holds for fixation of the $T^i$ allele. If that allele is fixed, the frequencies of the $Tw$ and $Tw^+$ alleles could not be determined because their presence could not be differentiated resulting from the lack of the L_2 or L_1 alleles necessary to show the presence of the $Tw$ allele. Similarly, due to fixation, there would be no way of determining the frequency of the $T^i$ or $T$ alleles. The effects of those alleles are masked by homozygosity of either the $Tw^+$ or $T^i$ allele.

In other strains, where $Tw^+$ and $T^i$ were not fixed, allelic frequencies for all loci could be estimated. In those strains, there is significant variation in the estimated frequencies of all the alleles. The estimated frequencies of the $Tw^+$ allele varied from 0.1 to 1.0. The estimated frequencies of the $T^i$ allele varied from 0 to 1.0. The estimates for the L_2 allele varied from 0 to 0.6, and the L_1 allele varied from 0 to 0.9. The estimated frequencies of the $I$ allele varied from 0 to 1.0.

Analysis of the gene frequency data by cluster analysis (Fig. 2) began by separating those 12 populations in which either the $Tw^+$ or the $T^i$ allele is fixed. These represent a group that cannot be connected with any other populations. This group contains populations collected from sylvan tree holes and ovitraps (Bansang-S, Kari-T, and Kedo), domestic, outdoor containers (NO-Air, NO-Chef, NO-Dante, Miami-D, and NO-St. G), domestic ovitraps (Hainana, Savan, and Tapa), and peridomestic ovitraps (Roca).

The remaining 38 populations assorpt into two groups. The first group contained 16 populations. All these populations had relatively low estimates of the frequency of the $I$ allele ($u <= 0.7$). This group contained
TABLE 3. CKM Banding Patterns in the Experimental Strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>CKM Class 0</th>
<th>CKM Class 1</th>
<th>CKM Class 2</th>
<th>CKM Class 3</th>
<th>CKM Class 4</th>
<th>CKM Class 5</th>
<th>CKM Class 6</th>
<th>CKM Class 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFRICAN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bansang I</td>
<td>83</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bansang-S</td>
<td>92</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bansang II</td>
<td>84</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Djourbel</td>
<td>1</td>
<td>84</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kalifi</td>
<td>19</td>
<td>58</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kari-T</td>
<td>94</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kari-W</td>
<td>9</td>
<td>49</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kongo</td>
<td>35</td>
<td>58</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Koum</td>
<td>1</td>
<td>50</td>
<td>22</td>
<td>15</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Kedo</td>
<td>42</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mintah</td>
<td>3</td>
<td>50</td>
<td>16</td>
<td>7</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Roca</td>
<td>72</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ton</td>
<td>0</td>
<td>21</td>
<td>28</td>
<td>18</td>
<td>26</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>CARIBBEAN/SOUTH AND CENTRAL AMERICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang</td>
<td>4</td>
<td>89</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ang-Rock</td>
<td>0</td>
<td>40</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ang-Rock-I</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Antiq</td>
<td>0</td>
<td>34</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Barbados</td>
<td>0</td>
<td>82</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Caguas-I</td>
<td>1</td>
<td>91</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Culeb</td>
<td>1</td>
<td>92</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rex</td>
<td>2</td>
<td>92</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rex-I</td>
<td>2</td>
<td>97</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Martinique</td>
<td>1</td>
<td>81</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>St. Kitts</td>
<td>0</td>
<td>56</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>St. Lucia</td>
<td>4</td>
<td>85</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>St. Mart</td>
<td>3</td>
<td>86</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Tortola-N</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>58</td>
</tr>
<tr>
<td>Tortola-I</td>
<td>0</td>
<td>50</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Trinidad-I</td>
<td>0</td>
<td>59</td>
<td>2</td>
<td>7</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Param</td>
<td>1</td>
<td>96</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malaga</td>
<td>0</td>
<td>42</td>
<td>3</td>
<td>0</td>
<td>15</td>
<td>6</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Tapa</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>SOUTH PACIFIC/ASIAN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiji</td>
<td>0</td>
<td>24</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Olong</td>
<td>0</td>
<td>32</td>
<td>3</td>
<td>1</td>
<td>18</td>
<td>17</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Hainana</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sri Lanka-Old</td>
<td>1</td>
<td>91</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>UNITED STATES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO-Air</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 3 continued on next page.
two internal clusters. The first had populations in which the estimated frequency of the I and Tw+ alleles were relatively high \( r \geq 0.4, p \geq 0.9 \). This group contained populations collected from domestic, indoor containers (Bansang II) and domestic, outdoor containers (Caguas-I, Rex, Rex-I, St. Mart, NO-Mag, Miami-Coca, and San A-Villa). The second cluster had populations in which the estimated frequency of the I allele was much lower \( r \leq 0.3 \), and the estimated frequency of the Tw+ allele was somewhat lower \( 0.4 \leq p \leq 0.9 \). This group contained populations which were collected from domestic, indoor containers (Tortola-I), domestic, outdoor containers (Martinique, Olong, Tortola N, and San A-D), and domestic ovitraps (Malaga, Trinidad-I, and C C TX).

The second large group contained 22 populations, all of which had relatively high estimated frequencies of the I allele \( u = 0.7 \). It also included two internal clusters. The first contained 12 populations characterized by high estimated frequencies of the Tw+ and I alleles \( p \geq 0.7, r \geq 0.6 \). They were collected from domestic, indoor containers (Kari-W), domestic, outdoor containers (Bansang I, Diourbel, Kalifi, Ang, Culeb, St. Lucia, and NO-Almon), domestic ovitraps (B-C S and Kongo), sylvan ovitraps (Param), and laboratory colony (Sri Lanka-Otd). The second cluster contained 10 populations, all with lower estimated frequencies of either the Tw+ or I allele \( p \leq 0.9, r \leq 0.7 \). The members of this group were collected from sylvan tree holes (Koum and Ton), domestic, outdoor containers (St. Kitts, Antig, and San A-Los), domestic ovitraps (Mintah, Fiji, and Barbados), and rock holes (Ang-Rock and Ang-Rock-I).

**DISCUSSION**

*Aedes aegypti* has long been treated as a polytypic species (Mattingly 1957) because of the differences noted in abdominal scaling patterns. More recently, McClelland (1974) argued in favor of the simpler concept of a polymorphic species. Hariberg, et al. (1986) supported the polymorphic position. The results of this study further support that argument. No geographic or habitat pattern in either phenotype (scaling pattern) or estimated genotypic frequencies is apparent. What is obvious is extensive variation in the pattern of scaling.

The present study involved 50 different populations from various geographic regions. Of the 50 populations sampled, 13 were African, 19 were Caribbean/South and Central America, 14 were from the United States, and 4 were from Asia and the South Pacific. Many different comparisons between these populations are possible. One such comparison is geographic, but the data do not support any geographic associations. The 13 African populations were assorted in all banding pattern clusters and all gene frequency clusters. The 19 Caribbean/South and Central American strains were assorted in all banding pattern clusters. Although there were no Caribbean strains in which either the Tw+ or I allele were fixed, the strains were assorted in the rest of

<table>
<thead>
<tr>
<th>Strain</th>
<th>CKM Class</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-Almon</td>
<td>6</td>
<td>90</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO-Chef</td>
<td>23</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO-St. G</td>
<td>1</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO-Dante</td>
<td>7</td>
<td>93</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO-Mag</td>
<td>6</td>
<td>84</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Miami-D</td>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miami-Coca</td>
<td>7</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>San A-D</td>
<td>1</td>
<td>52</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>San A-Villa</td>
<td>11</td>
<td>70</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>San A-Los</td>
<td>27</td>
<td>51</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>B-C S</td>
<td>8</td>
<td>91</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C C TX</td>
<td>0</td>
<td>71</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Savan</td>
<td>11</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
the gene frequency clusters. The 14 United States populations were assorted in all banding pattern and gene frequency clusters. The four Asian and South Pacific populations also were assorted widely in the banding pattern and gene frequency groups.

The closest one can come to making any geographic associations is to say that all of the populations in which a majority of the females are in CKM class 0 come from Africa. However, we do not take this as support for the polytypic concept. Even in those populations in which a majority of females are class 0 there are some females with additional white scaling. No population was exclusively class 0. The failure to find non-African populations in which the majority of females are dark is more likely related to sampling bias. As *Ae. aegypti* spread out of African, those populations which were most likely to be carried elsewhere were those that were more closely associated with humans, i.e., forms with lighter scaling patterns.

The estimated frequencies of each of the alleles vary in each location. The frequency of Tw+ varies from 0.1 to 1.0 in the African populations (with all except one population having frequencies between 0.6 and 1.0), from 0.4 to 0.9 in the Caribbean populations,
Figure 2. Cluster Analysis of Allelic Frequencies for the 50 Populations.

and from 0.7 to 1.0 in the North/South American populations. The frequency of the L2 allele varied from 0.5 to 1.0 in the African populations, from 0 to 0.9 in the Caribbean populations, and from 0.1 to 1.0 in the North/South American populations. The frequency of the I allele varied from 0.3 to 1.0 in the African populations, from 0.3 to 1 in the Caribbean populations, and from 0 to 0.8 in the North/South American populations. All regions had similar degrees of variation. In each region, there were populations that differed significantly from other populations within the region and populations that did not differ significantly from others in different regions.

A second comparison is between sylvan and domestic collections. Differences in breeding sites could cause different selection pressures resulting in
<table>
<thead>
<tr>
<th>Strain</th>
<th>pl</th>
<th>r2</th>
<th>s3</th>
<th>u4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFRICAN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bansang I</td>
<td>0.90</td>
<td>0.90</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>Bansang-S</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Bansang II</td>
<td>0.90</td>
<td>0.90</td>
<td>0.10</td>
<td>0.50</td>
</tr>
<tr>
<td>Diourbel</td>
<td>0.80</td>
<td>0.70</td>
<td>0.30</td>
<td>0.90</td>
</tr>
<tr>
<td>Kalifi</td>
<td>0.70</td>
<td>0.70</td>
<td>0.20</td>
<td>0.80</td>
</tr>
<tr>
<td>Kari-T</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Kari-W</td>
<td>0.90</td>
<td>0.60</td>
<td>0.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Kongo</td>
<td>0.90</td>
<td>0.70</td>
<td>0.30</td>
<td>0.80</td>
</tr>
<tr>
<td>Koum</td>
<td>0.60</td>
<td>0.50</td>
<td>0.40</td>
<td>0.90</td>
</tr>
<tr>
<td>Kedo</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Mintah</td>
<td>0.60</td>
<td>0.60</td>
<td>0.30</td>
<td>0.80</td>
</tr>
<tr>
<td>Roca</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Ton</td>
<td>0.10</td>
<td>0.50</td>
<td>0.40</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>CARIBBEAN/SOUTH AND CENTRAL AMERICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang</td>
<td>0.90</td>
<td>0.70</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>Ang-Rock</td>
<td>0.70</td>
<td>0.40</td>
<td>0.30</td>
<td>0.80</td>
</tr>
<tr>
<td>Ang-Rock-I</td>
<td>0.90</td>
<td>0.10</td>
<td>0.50</td>
<td>0.80</td>
</tr>
<tr>
<td>Antiq</td>
<td>0.50</td>
<td>0.40</td>
<td>0.50</td>
<td>0.80</td>
</tr>
<tr>
<td>Barbados</td>
<td>0.90</td>
<td>0.20</td>
<td>0.40</td>
<td>0.70</td>
</tr>
<tr>
<td>Caguas-I</td>
<td>0.90</td>
<td>0.40</td>
<td>0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>Culeb</td>
<td>0.90</td>
<td>0.80</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Rex</td>
<td>0.90</td>
<td>0.90</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>Rex-I</td>
<td>0.90</td>
<td>0.90</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>Martinique</td>
<td>0.90</td>
<td>0</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>St. Kitts</td>
<td>0.50</td>
<td>0.70</td>
<td>0.20</td>
<td>0.80</td>
</tr>
<tr>
<td>St. Lucia</td>
<td>0.90</td>
<td>0.70</td>
<td>0.30</td>
<td>0.80</td>
</tr>
<tr>
<td>St. Mart</td>
<td>0.90</td>
<td>0.50</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Tortola-N</td>
<td>0.40</td>
<td>0.00</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Tortola-I</td>
<td>0.80</td>
<td>0.10</td>
<td>0.20</td>
<td>0.30</td>
</tr>
<tr>
<td>Trinidad-I</td>
<td>0.70</td>
<td>0.20</td>
<td>0.30</td>
<td>0.70</td>
</tr>
<tr>
<td>Param</td>
<td>0.90</td>
<td>0.80</td>
<td>0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>Malaga</td>
<td>0.60</td>
<td>0.00</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Tapa</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td><strong>ASIA/SOUTH PACIFIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiji</td>
<td>0.50</td>
<td>0.60</td>
<td>0.40</td>
<td>0.90</td>
</tr>
<tr>
<td>Olong</td>
<td>0.50</td>
<td>0.30</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>Hainana</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Sri Lanka-Old</td>
<td>0.70</td>
<td>0.90</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>UNITED STATES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO-Air</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>NO-Almon</td>
<td>0.90</td>
<td>0.80</td>
<td>0.10</td>
<td>0.80</td>
</tr>
<tr>
<td>NO-Chef</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>NO-St. G</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
</tbody>
</table>

*TABLE 4 continued on next page.*
different scaling patterns. Again, no pattern can be detected in the cluster analysis. Domestic populations were assorted in all banding and gene frequency clusters. Feral populations were also widely distributed in the various clusters. We can further note that this finding is consistent with that of Mogi, et al. (1984) for populations collected from the Philippines. In that study, banding pattern frequencies of populations collected indoors were not significantly different from those collected outdoors.

Other specific comparisons can be made. For example, how much variation is found in populations sampled from the same general location or habitat? Three populations were sampled from Bansang, Gambia. Two were collected from domestic habitats, and one from tree holes. All three showed similar patterns of banding and were closely associated in the banding pattern groups. They showed less association in their estimated gene frequencies. One population was fixed for the Tw+ or I allele, and the other two populations differed in their frequencies of the I allele. There were six populations sampled from the New Orleans area. They were all taken from domestic habitats. They were found in a variety of banding pattern and gene frequency clusters. The three collections from Anguilla, British West Indies show a slightly different pattern. They show great differences in banding pattern association and gene frequency clusters.

Another aspect of this can be seen by looking for populations that are matched by the cluster analysis and yet come from different locations or habitats. For the banding frequencies, there are 20 different cases in which two or more populations are closely associated with each other. Of those 20 associations, 7 come from different geographic regions and 7 were collected from different habitats. There are 15 different cases in which the cluster analysis paired two or more populations on the basis of gene frequencies. Of the 15 pairs, 11 come from different geographic regions, and 5 come from different habitats.

Any determination of equilibrium allelic frequencies is based upon the standard assumptions of the Castle - Hardy - Weinberg model. Those are balanced mutation, balanced migration, no selection, random mating, and large population size. This study also assumes old, stable populations. The general Castle - Hardy - Weinberg model holds that equilibrium will be established in a single generation when a single gene is considered. The model as extended to two independently segregating loci as presented in any of the standard texts (Li 1955, Crow 1986) holds that equilibrium can be reached, but only after enough generations of random mating to overcome linkage disequilibrium. We believe this model best explains our data.

As support for some of these assumptions, we can again point to the data. The frequencies of the alleles studied do not appear to follow any particular trend with geographic gradient, nor with habitat. Populations sampled from the same geographic area can show considerable variation. It appears there is no strong selection pressure acting in relation to this system. The data support the concept of a random system. Such a

<table>
<thead>
<tr>
<th>Strain</th>
<th>p1</th>
<th>r2</th>
<th>s3</th>
<th>u4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-Dante</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>NO-Mag</td>
<td>0.90</td>
<td>0.60</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Miami-D</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
<tr>
<td>Miami-Coca</td>
<td>0.90</td>
<td>0.70</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>San A-D</td>
<td>0.70</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>San A-Villa</td>
<td>0.80</td>
<td>0.50</td>
<td>0.20</td>
<td>0.40</td>
</tr>
<tr>
<td>San A-Los</td>
<td>0.90</td>
<td>0.20</td>
<td>0.50</td>
<td>0.90</td>
</tr>
<tr>
<td>B-C S</td>
<td>0.90</td>
<td>0.90</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>C C TX</td>
<td>0.80</td>
<td>0.10</td>
<td>0.30</td>
<td>0.70</td>
</tr>
<tr>
<td>Savan</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>*</td>
</tr>
</tbody>
</table>

1 p is the frequency of the Tw+ allele. The frequency of the Tw allele is q = (1 - p).
2 r is the frequency of the Tw allele.
3 s is the frequency of the L2 allele. The frequency of the L1 allele is t = [1 - (4+s)].
4 u is the frequency of the I allele. The frequency of the i allele is v = (1-u).

* Frequency could not be determined.
model would be consistent with various studies of isozyme loci (Tabachnick et al. 1979, Wallis and Tabachnick 1990). However, although no pattern can be seen in the present data, such a random model may still not be the correct one. Most of the females analyzed in this study had little extra white scaling. Only a small number of populations had extensive white scaling. Further analysis of the system would be aided by finding more populations with larger proportions of lighter individuals. Such populations might divulge a pattern of selection pressure which is not seen in the present data.

The classical polygenic model makes analyses of the genetic structure of a population impossible and uninteresting. The phenotype of an individual results from the coordinated activity of the various loci which affect the trait. In the classical additive model, each locus contributes a small amount to the total expression of the trait. As such, the effect of a single locus is normally considered to be negligible. Information regarding the genetic structure of a population relative to the array of loci would be interesting because it would provide information about the behavior of such genetic systems. However, such information is impossible to obtain because one cannot separate the effects of the individual loci. As such, the frequencies of individual loci are impossible to determine, and the structure of the population for such systems is impossible to study. Similarly, since any one gene has a small effect upon the phenotype, any information about the genetic structure of a population for an individual gene is of little interest because phenotypic expression relies upon the coordinated activity of several loci.

The system presented in this paper is novel because it does allow for the analysis of the genetic structure of populations for the polygenic system. The frequencies of the alleles involved can be studied and are of interest in looking at the dynamics of the populations involved.

REFERENCES CITED


THE IMPACT OF ENVIRONMENTAL FACTORS ON THE EFFICACY OF BACILLUS SPHAERICUS AGAINST CULEX PIPiens

N. Becker1, M. Ludwig1, M. Beck1, and M. Zgomba2

ABSTRACT: Environmental factors can influence the effectiveness of microbial control agents in mosquito control programs. Three of these factors (water temperature, larval density, and sunlight) were studied with Bacillus sphaericus under laboratory and semi-field conditions using third-instar Culex pipiens mosquitoes. Bioassays revealed that larvae are about 52 times more sensitive to B. sphaericus at 25°C than at 5°C. A significant increase in the activity of B. sphaericus was also observed between 5°C and 8°C and between 8°C and 15°C, although not between 15°C and 25°C. The efficacy of B. sphaericus decreased in a linear manner with increasing larval density. In comparison with the LC50 values using 10 larvae/cup, the value with 75 larvae/cup was about four-fold higher, with 150 larvae/cup about nine-fold higher, and with 200 larvae/cup about ten-fold higher. Strong sunlight had a negative effect on the activity of B. sphaericus.

INTRODUCTION

The importance of Bacillus sphaericus as a microbial agent for mosquito control has increased significantly in recent years. It is active against stagnant and floodwater mosquitoes (de Barjac 1990, Davidson 1984, Davidson and Yousten 1990, Lacey 1990), and no affect on non-mosquito fauna. Only mosquitoes, and neither blackflies nor Chironomidae, are affected by B. sphaericus. As with Bacillus thuringiensis subsp. israeliensis, B. sphaericus is environmentally safe, but in contrast, B. sphaericus is able to persist or multiply under natural conditions (Davidson 1984, Karch et al. 1990), which may lead to a desirable long-term efficacy. This efficacy of B. sphaericus against Culex spp. has provided encouraging results from the first field studies with B. sphaericus within the control area of the German Mosquito Control Association (Beck and Becker 1992). There is now a proposal for a wider B. sphaericus treatment against the urban mosquito Culex pipiens in the Upper Rhine Valley.

In order to choose the appropriate dosage of B. sphaericus for routine field treatment under various environmental conditions, it is very important to have a prior understanding of ecological and economic factors that influence the efficacy of B. sphaericus. It has been shown that temperature, larval density, and sunlight intensity can have a strong influence on the efficacy of B. thuringiensis israeliensis (Becker et al. 1992, Burke et al. 1983, Mulla et al. 1990, Wraith et al. 1981). In the work presented here, we have investigated the effect of these same three factors on B. sphaericus in bioassays with Cx. pipiens.

MATERIALS AND METHODS

Egg rafts of Culex pipiens Linnaeus were collected from rain barrels in gardens of the Upper Rhine Valley. Larvae were hatched and reared in water-filled plastic vessels in the laboratory, maintained at 25°C, and fed with commercial fish food (Tetramin®). All bioassays were performed according to WHO guidelines (World Health Organization 1985), with slight modifications to meet the specific needs of this study.

To prepare a stock solution, 50 µl of B. sphaericus (Spherimos FC, 1,000 IU/mg) were added to 10 ml of distilled water and homogenized in a mixing machine (IKA CombiMag Reo) at 700 rpm for 10 minutes, then homogenized in an ultrasonic bath (Branson Instruments) for 15 minutes. One ml of the homogenized solution was added to 99 ml of distilled water. Depending on the concentration required, a range of 15 to 3,000 µl of the homogenized and diluted Spherimos FC suspension was added to the test vessels with a micropipette. Tests were run at six different concentrations with controls, in three runs with at least three replicates each. Mortality was evaluated after 48 hours and corrected according to Abbott’s formula (Abbott 1925). The results were

1German Mosquito Control Association (KABS), Ludwigstr. 99, 6701 Waldsee, GERMANY.

2University of Novi Sad, Institute of Plant Protection, Trg D. Obradovica 8, 21000 Novi Sad, YUGOSLAVIA.
subjected to log-probit analysis (Finney 1971, Raymond 1985). Duncan’s multiple range test and Student’s t-test (Köhler et al. 1984) were used to assess the significance of differences between LC values. Data from the temperature and density tests were subjected to regression analysis.

To assess the effects of temperature, bioassays were run at 5, 8, 15, and 25 ± 1°C. Late third-instar larvae were acclimatized to the required temperature for four hours before being transferred in batches of 25 into 200 ml plastic cups containing 150 ml of distilled water at the required temperature.

The effects of larval density were determined by placing 10, 75, 150, or 200 late third-instar larvae per 150 ml of distilled water. This corresponded to densities of 67 to 1,330 larvae per liter.

Tests to determine the effects of sunlight were carried out in duplicate series with late second-instar larvae. One series was exposed to sunlight for seven hours, and the other simultaneously kept in shade for the same period. The plastic cups containing 150 ml of distilled water with 25 larvae in each were placed outdoors in water baths and kept at a constant temperature of 25 ± 1°C. The sunlight intensity was measured every hour with an exposure meter (Siemens M 09010-A 108). After exposure for seven hours, both series were transferred to a laboratory rearing chamber without sunlight and kept for an additional 41 hours at 25°C.

RESULTS

Effects of Temperature

A comparison of the LC50 values showed that late third-instar larvae of Cx. pipiens were about 24 times more sensitive to B. sphaericus at 25°C (0.0119 mg/l) than at 5°C (0.2895 mg/l). At 8°C (0.1523 mg/l) and 15°C (0.0390 mg/l) the activity of B. sphaericus is two and eight times higher respectively (Fig.1). Comparison of LC90 values showed similar trends. The LC90 value at 25°C(0.0270 mg/l) indicated a 52-fold higher activity than at 5°C (0.1403 mg/l). At 15°C (0.1601 mg/l) and 8°C (0.8323 mg/l) this factor was nine and two, respectively (Fig.1).

Regression analysis revealed that LC50 and LC90 values both increased linearly with decreasing temperature, and Duncan’s multiple range test revealed significant differences between LC50 and LC90 values at the different temperatures (except between 15 and 25°C).

![Figure 1](image-url)  
Figure 1. Impact of temperature on Bacillus sphaericus efficacy against third instar Culex pipiens larvae. Error bars indicate the SD.
Effect of Larval Density

Larval density had a significant impact on the efficacy of *B. sphaericus* applications. Regression analysis showed that both LC$_{50}$ and LC$_{90}$ values rose linearly with increasing larval density (Fig. 2). After 48 hours, with 10 larvae/cup the LC$_{50}$ value (0.0077 mg/l) was two-fold lower than with 75 larvae/cup (0.0168 mg/l), four-fold lower than with 150 larvae/cup (0.0304 mg/l), and seven-fold lower than with 200 larvae/cup (0.0519 mg/l). Comparison of the LC$_{90}$ values showed similar trends: compared with 10 larvae/cup (0.0137 mg/l) it was necessary to increase the dosage from four-fold at 75 larvae/cup (0.0502 mg/l), nine-fold at 150 larvae/cup (0.1235 mg/l), and 11 times at 200 larvae/cup (0.1423 mg/l). Duncan's multiple range test demonstrated that significant differences occurred between all the different larval densities, at both LC$_{50}$ and LC$_{90}$.

Effects of Sunlight

A comparison of bioassays under the impact of strong sunlight and shadow indicated that high light intensity had a negative impact on the efficacy of *B. sphaericus*. After 48 hours, LC$_{50}$ and LC$_{90}$ values for bioassays using late second instar larvae exposed to sunlight for seven hours were 1.6 and 2.0-fold higher, respectively, than those in the shadow (Fig. 3). A comparison of LC$_{50}$ and LC$_{90}$ values by Student's t-test indicated that they are significantly different ($p < 0.05$). Log probit lines support these results (Fig. 4).

DISCUSSION

*Bacillus sphaericus* has great potential as a microbial control agent against a variety of mosquitoes (Davidson and Yousten 1990), especially *Culex* species. In this context, *B. sphaericus* may play an significant role in the control of lymphatic filariasis transmitted by *Culex quinquefasciatus* (Hougard 1990). An important precondition for the wide scale use of *B. sphaericus* is a better understanding of the impact of environmental factors influencing the efficacy and persistence of this agent. Our earlier studies showed a significant effect of environmental factors in various climatic conditions on the effectiveness of *B. thuringiensis israelensis* (Becker and Ludwig 1983, Burke et al. 1983, Mulla et al. 1990, and Retlich 1983). The present study has shown a similarly strong correlation between the evaluated parameters and the larvicidal effectiveness of *B.*
Figure 3. Effect of sunlight of *Bacillus sphaericus* efficacy against *Culex pipiens* (second instars).

Figure 4. Comparison of log probit lines of bioassays conducted in the shadow and under strong sunlight.
sphaericus.

The temperature range under which B. sphaericus was evaluated extended from 5 to 25°C. It resulted in a 52-fold decrease of the LC90 values. A less dramatic but still highly correlated temperature impact on the LC90 value was demonstrated by Mulla et al. (1984, 1990). A substantial temperature effect on the efficacy of B. sphaericus was also shown by Wraight et al. (1981, 1987), who reported a two to six-fold increase in mean LC90 values with an 8 to 10°C decrease in temperature. In terms of a practical application, these findings indicate that greater quantities of B. sphaericus need to be applied as the temperature falls.

According to the results of Mulla et al. (1990), a negative relationship exists between larval density and activity of B. sphaericus. Our investigations show that the relationship is linear, as we also found to be true in an earlier study on B. thuringiensis israelensis (Becker et al. 1992).

Using the figures obtained in the present study it should be possible to create a mathematical model for establishing the optimal dosage for routine treatment. From the regression coefficients obtained in this study it is quite simple to determine an adequate B. sphaericus rate corresponding to any particular larval density within the range studied.

Increased sunlight lowers the efficacy of B. sphaericus, as was earlier shown by Burke et al. (1983). We have demonstrated the same effect with B. thuringiensis israelensis (Becker et al. 1992). In fact, our earlier studies showed that high sunlight intensity caused a greater increase in LC50 values against B. thuringiensis israelensis than we have observed in the present study with B. sphaericus. The lower sensitivity for B. sphaericus may result from the fact that the protein of this bacterium is enclosed in the exosporium, whereas, the delta-endotoxin of B. thuringiensis israelensis is uncoated. It is possible that a coated spore crystal complex is more tolerant to ultraviolet light than the uncoated protein. These findings may be even more important for field situations in tropical parts of the world, where sunlight intensity can reach more extreme levels.

Acknowledgments

This study was financially supported by the "Gesellschaft für internationalen Erfahrungsaustausch bei der Schmetterlingbekämpfung e.V.-GiES," Ludwigshafen, Germany. The authors thank Dr. Roger J. Wood, Department of Entomological Biology, University of Manchester, for reviewing the manuscript and his valuable comments which have improved the paper.

REFERENCES CITED


Davidson, E. W. 1984. Microbiology, pathology and genetics of Bacillus sphaericus: Biological aspects, which are important to field use. Mosq. News 44: 147-152.


AN IXODES SCAPULARIS (DEER TICK) DISTRIBUTION STUDY
IN THE MINNEAPOLIS-ST. PAUL, MINNESOTA AREA

D. F. Neitzel1, J. L. Jarnefeld1, and R. D. Sjogren1

ABSTRACT: In 1990-1991, a deer tick, *Ixodes scapularis* (Say), distribution study was conducted in the Minneapolis-St. Paul metropolitan area of Minnesota. Small mammal trapping and drag cloth sampling were used to collect deer ticks from 78 of 250 woodlots in 1990 and 54 of 270 woodlots in 1991, respectively. In 1991, 75 sites sampled in 1990 were resampled to look for changes in *I. scapularis* distribution. No significant changes in distribution were identified. Although the entire metropolitan area was sampled intensely, deer ticks were found primarily north and east of the Twin Cities. To date, this was the most intensive sampling effort for *I. scapularis* conducted in Minnesota. Small mammal sampling gave us the majority of our tick data (18,409 [98%] of 18,714 total ticks collected). A total of 1,126 *I. scapularis* was collected from 9,217 mammals. The larval *I. scapularis* population peak in June of both years is much earlier than reported from the eastern United States. Of 2,769 drag samples taken, 2,579 (93%) were negative for all tick species. Only 14 *I. scapularis* were collected through drag sampling (1 in each of 14 drag samples). Our data suggest that *I. scapularis* populations are lower than those reported from the eastern United States, and the most effective way to detect these populations is by sampling their small mammal hosts.

INTRODUCTION

Lyme disease is an emerging public health concern caused by the bacterium *Borrelia burgdorferi* (Burgdorfer et al. 1982), and transmitted to humans primarily by *Ixodes dammini* (dear tick) (Steere et al. 1977, Burgdorfer et al. 1982). Oliver et al. (1993) have since indicated that *I. dammini* (Spielman et al. 1979) is synonymous with *Ixodes scapularis*. This paper will use the revised classification.

*Ixodes scapularis* and human cases of Lyme disease have been reported from several counties in Minnesota. Drew et al. (1988) obtained location records for *I. scapularis* from grouse hunters in several parts of Minnesota. Gill et al. (1993) collected *I. scapularis* from white-tailed deer taken during a special hunt at an east-central Minnesota state park. The Minnesota Department of Health has recorded human cases of Lyme disease from across the state (Osterholm et al. 1984, Minnesota Department of Health 1987, 1990). Most of the *I. scapularis* and Lyme disease cases appear to be occurring in east-central Minnesota, north and east of the Twin Cities metropolitan area. However, many confirmed cases of Lyme disease have been reported from within the metropolitan area as well (Minnesota Department of Health 1987, 1990).

In 1990, the Metropolitan Mosquito Control District initiated a Lyme Disease Tick Surveillance Program. The main goals of the program are to determine the distribution and prevalence of *I. scapularis* and *B. burgdorferi* within the Minneapolis-St. Paul metropolitan area. The current study addresses the need for an *I. scapularis* distribution map.

MATERIALS AND METHODS

Sampling Site Selection

The seven county area surveyed for *I. scapularis* consisted of nearly 3,000 square miles (4,827 square kilometers) of rural, suburban, and urban land. Much of the rural land was agricultural property, and suitable wooded or brushy *I. scapularis* habitat was rare or fragmented. Agricultural development was especially intense south and west of the Mississippi River that bisects the metropolitan area. Suitable woodlots in the suburban and urban habitats were also quite often fragmented by development and lakes.

In 1990, 250 woodlots were chosen for study. The square mile sections of land to be sampled were chosen using a systematic random method. The actual location sampled within each section was chosen nonrandomly by searching for the thickest woodlot.

1Metropolitan Mosquito Control District, 2099 University Ave. West, St. Paul, MN 55104-3431, U.S.A.
(heaviest canopy, shrub, and herb coverage). This was done to maximize our chances of collecting *I. scapularis*. Woodlots varied considerably in dominant vegetation types and percentages of ground coverage. More sampling effort was focused on the northern counties of the metropolitan area during the first year of the study because these areas had greater numbers of past Lyme disease cases.

In 1991, sampling emphasized southern metropolitan counties. Woodlots (n=195) were chosen for study by arbitrarily choosing a certain number of sites to sample in each township (based on township size) and then selecting sections at random as in 1990. An additional 75 of the 250 woodlots from 1990 were also selected for repeat sampling to look for changes in *I. scapularis* presence/absence status over several years. Repeat sites were selected from our 1990 site list based on three criteria: representative habitat of an area, locations that were unlikely to be developed, and good small mammal numbers.

The result of our sampling site selection in 1990-1991 was a network of 445 sites (75 of them for repeated sampling) that represent the entire seven county area (TABLE 1).

*Ixodes scapularis* Collection Methods

One 300 foot (91 meter) transect was established at each sampling location. Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, FL) baited with peanut butter and oats were placed along these transects at 50 foot (15 meter) intervals. Any small mammals caught in the traps were euthanized and searched for ticks. All ticks found were removed with forceps and stored in alcohol for later identification. Drag samples were taken along the same transect lines or along 300 feet (91 meters) of edge habitat (trails, brushy edges, etc.) in the site, using a tick drag (1 square yard [0.90 square meter] flannel cloth attached to a pole). All sampling was conducted between 8:00 AM and 12:00 PM by the same staff members to avoid sampling bias. The cloth was inspected for ticks periodically during and after each drag sample was taken. Any ticks found were removed and stored for later identification. Random small mammal and tick samples were kept for subsequent quality assurance checks.

In both 1990 and 1991, sampling was initiated in late April and ended in late October. Each year, the 27 week study was divided into three sampling periods. Every site was sampled for one week (randomly selected) during each nine-week period. Each week consisted of 21 trap nights (7 traps x 3 nights) and three drag samples for each site. Drag sampling was not attempted during the second sampling period of 1991.

**RESULTS**

During our two year study, we sampled a total of 9,217 small mammals and conducted 2,769 drag samples. Of 18,714 ticks collected, 1,140 were *I. scapularis*. At least one *I. scapularis* was collected from 78 of 250 sampling sites in 1990 and 54 of 270 sites in 1991. Most of the positive locations were in the northeastern part of the metropolitan area (Anoka, Washington, and northern Ramsey counties) (Fig. 1). Scattered positive sites were found in other counties.

Small mammal sampling gave us the majority of our tick data. All but 305 of the 18,714 ticks collected were removed from the mammals. Thirteen species of mammals and one bird species were sampled, but nearly 80 percent of the *I. scapularis* were found on *Peromyscus leucopus*, the white-footed mouse (TABLE 2). This species was also the most abundant mammal collected

### TABLE 1. Distribution of sampling sites in the Minneapolis-Saint Paul metropolitan area.

<table>
<thead>
<tr>
<th>County</th>
<th>Land Area (Sq. Miles, Sq. km)</th>
<th>1990 Sites</th>
<th>1991 New Sites</th>
<th>Sites Sampled 1990-1991</th>
<th>Ttl Sites Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoka</td>
<td>447</td>
<td>719.2</td>
<td>90</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>Carver</td>
<td>376</td>
<td>605.0</td>
<td>5</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Dakota</td>
<td>554</td>
<td>891.4</td>
<td>20</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>Hennepin</td>
<td>611</td>
<td>983.1</td>
<td>20</td>
<td>67</td>
<td>9</td>
</tr>
<tr>
<td>Ramsey</td>
<td>177</td>
<td>284.8</td>
<td>20</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Scott</td>
<td>359</td>
<td>577.6</td>
<td>10</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Washington</td>
<td>422</td>
<td>679.0</td>
<td>85</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>2946</strong></td>
<td><strong>4740.1</strong></td>
<td><strong>250</strong></td>
<td><strong>195</strong></td>
<td><strong>75</strong></td>
</tr>
</tbody>
</table>
Figure 1. *Ixodes scapularis* distribution in the Minneapolis-St. Paul metropolitan area. Shaded area represents *Ixodes scapularis* present. Shaded circles represent isolated *Ixodes scapularis* records.

during our trapping efforts. We found many more *I. scapularis* larvae than nymphs (979 to 147, respectively). Most of the ticks were attached to the ears, face, or neck of the mammals. *Dermacentor variabilis* was by far the most common tick encountered (17,184 collected), especially on *P. leucopus* and *Clethrionomys gapperi* (TABLE 2).

The number of larval and nymphal *I. scapularis* collected showed definite seasonal trends. The average number of both larvae and nymphs/mammal/week peaked at the same time during early June in 1990, and were synchronous again in 1991, but the peak occurred two weeks later (Figs. 2 and 3). During these peaks, the average number of *I. scapularis*/mammal was still relatively low (approximately 2.0 for larvae and 0.3-0.4 for nymphs). However, in May of 1990, an eastern chipmunk (*Tamias striatus*) was collected and found to have 102 *I. scapularis* larvae and 31 nymphs. This anomalous mammal was not included in the data analysis for Figures 2 and 3.

Drag cloth sampling was much less successful at sampling *I. scapularis* populations. Of 3,860 potential

<table>
<thead>
<tr>
<th>Mammal Species</th>
<th>Number Sampled (% Tl Collected)</th>
<th><em>Ixodes scalapularis</em> (% Tl Collected)</th>
<th><em>Dermacentor variabilis</em> (% Tl Collected)</th>
<th>Other Tick Species** (% Tl Collected)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peromyscus leucopus</em></td>
<td>7229 (78.40)</td>
<td>894 (79.4)</td>
<td>14956 (87.00)</td>
<td>42 (42.4)</td>
</tr>
<tr>
<td><em>Tamias striatus</em></td>
<td>619 (6.70)</td>
<td>187 (16.6)</td>
<td>34 (0.20)</td>
<td>4 (4.0)</td>
</tr>
<tr>
<td><em>Blarina brevicauda</em></td>
<td>557 (6.00)</td>
<td>16 (1.4)</td>
<td>11 (0.10)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td><em>Clethrionomys gapperi</em></td>
<td>504 (5.50)</td>
<td>21 (1.9)</td>
<td>2128 (12.40)</td>
<td>16 (16.2)</td>
</tr>
<tr>
<td><em>Sorex cinereus</em></td>
<td>227 (2.50)</td>
<td>2 (0.2)</td>
<td>1 (0.01)</td>
<td>14 (14.1)</td>
</tr>
<tr>
<td><em>Microtus pennsylvanicus</em></td>
<td>27 (0.30)</td>
<td>0 (0.0)</td>
<td>36 (0.20)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td><em>Zapus hudsonius</em></td>
<td>20 (0.20)</td>
<td>2 (0.2)</td>
<td>14 (0.02)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td><em>Mustela erminea</em></td>
<td>10 (0.10)</td>
<td>4 (0.4)</td>
<td>4 (0.02)</td>
<td>8 (8.1)</td>
</tr>
<tr>
<td><em>Spermophilus tridecemlineatus</em></td>
<td>6 (0.10)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Glaucomys volans</em></td>
<td>5 (0.10)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Tamiasciurus hudsonicus</em></td>
<td>5 (0.10)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>4 (0.04)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Sorex arcticus</em></td>
<td>2 (0.02)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Trogodytes aedon</em>**</td>
<td>2 (0.02)</td>
<td>0 (0.0)</td>
<td>0 (0.00)</td>
<td>10 (10.1)</td>
</tr>
</tbody>
</table>

**TOTALS** 9217 (100.00) 1126 (100.00) 17184 (100.00) 37 (100.00)

*Primarily *P. leucopus*, but the overlapping morphological characteristics of the few tentatively identified *P. maniculatus bairdii* and *P. maniculatus gracilis* caused us to categorize these species into one grouping.

**Other tick species included *Ixodes muris*, *Ixodes marxi*, *Ixodes cookei*, and *Haemaphysalis leporispalustris*.

***House wren.

drag samples, 1,091 (28.3%) were not taken due to rain, and 2,579 (66.8%) samples were negative for all tick species. Of the 190 (4.9%) samples that had ticks, only 14 samples had any *I. scapularis*. However, in 1990, three sites that were positive for *I. scapularis* were by drag sampling only, not mammal sampling.

The presence/absence status of *I. scapularis* at the 75 woodlots sampled in both 1990 and 1991 changed at 17 (22.6%) of the sites. In particular, we found that 23 sites remained positive for *I. scapularis*, 35 remained negative, 9 changed from negative to positive, and 8 changed from positive to negative.

In 1991, quality assurance measures demonstrated no significant problems with tick removal from the mammals or tick identification. Reinspection of 171 of 5,566 (3%) euthanized mammals for ticks missed on the initial check found additional ticks on 8 of 172 (4.7%) mammals. None of these additional ticks were *I. scapularis*. Ninety-seven percent of the total ticks found on the mice were found on the first check. Ninety-nine percent of 316 ticks checked for proper identification were correct.

**DISCUSSION**

During both years of the study, we found *I. scapularis* primarily in the northeastern part of the metropolitan area. The scattered *I. scapularis* positive sites south and west of the Mississippi River appear to indicate areas where isolated and/or low populations of the ticks occur. We were unable to find *I. scapularis* in Hennepin county on the west side of the metropolitan area despite an abundance of apparently suitable habitat. If the tick is present there, it is either highly localized, or at such low population levels we have not detected them yet. It is apparent after two years of data collection that most of the detectable (by our methods) *I. scapularis* populations are in Anoka, northern Ramsey, and Washington counties.

We were surprised at the peak time of year for *I. scapularis* larvae. In particular, the peak in June of both years is much earlier than reported from the eastern United States (Piesman and Spielman 1979, Main et al. 1982, Wilson and Spielman 1985, and Schulze et al. 1986). Our data suggest that the *I.*
Figure 2. *Ixodes scapularis* larvae 1990-1991. Average ticks per mammal by month.

Figure 3. *Ixodes scapularis* nymphs 1990-1991. Average ticks per mammal by month.
I. 

**scapularis** life cycle may be different for our area. In addition, average numbers of **I. scapularis**/mammal were lower than reported in the eastern United States (Carey et al. 1980, Main et al. 1982, and Schulze et al. 1986).

Drag cloth sampling appears to be a relatively ineffective **I. scapularis** sampling method for our area, in contrast to high numbers of ticks collected this way in the eastern United States (Falco and Fish 1989, Maupin et al. 1991, and Telford et al. 1992). Sampling was often laborious because of the difficulty in keeping the drag cloth as low to the ground as possible for optimal sampling in the thick brush of many sites. Rain or heavy dew conditions also made consistent sampling difficult. If tick numbers are low or localized, as we speculate much of the area to be, the chance of encountering a tick is low unless much more extensive drag cloth samples are taken. We feel that when tick numbers are low, the most effective way to sample them is when they are concentrated on their hosts.

The sampling effort conducted in 75 woodlots both years did not demonstrate major changes in **I. scapularis** presence/absence status. Most of the sites that changed status were locations where very few **I. scapularis** were collected in one or both years. These sites tended to be located in the middle of the metropolitan area, nearer the edge of the current known **I. scapularis** distribution. Repeat sites in the northeastern part of the metropolitan area (where most sites are positive) and the southwestern part (where most sites are negative) did not change status as frequently. These locations will be sampled in upcoming years to look for long-term changes in **I. scapularis** distribution.

The **I. scapularis** distribution data we have collected during this study will be used in several ways. The Minnesota Department of Health will be given these data to use for their risk assessment analysis. This study should help to identify areas that may require public health warnings or areas for future tick control measures as they become available. We anticipate that **I. scapularis** may eventually colonize much more of the metropolitan area. Other than intensively farmed land or urban landscapes, we see no significant barriers to their colonization.

**Acknowledgments**

We would like to thank Bridget Dunnigan, Denise Kukiela, Bruce A. Lindner, and Matthew J. Maida for their valuable assistance with the field data collection. We would also like to thank Dr. Andrew Spielman (Harvard School of Public Health) for his help with the identification of representative tick samples to **I. dammini** and **I. muris**, and Edward Campos (Centers for Disease Control) for his help with general tick identification. In addition, we would like to thank Dr. Susan Palchick (Metropolitan Mosquito Control District) for reviewing drafts of this document. This study was funded by the Metropolitan Mosquito Control District.

**REFERENCES CITED**


Minnesota Department of Health. 1990. Lyme disease:


ADVERTISING

Commercial advertising space is available in the Bulletin; full page (black and white) at $150.00 per issue, half page (black and white) at $90.00 per issue. Inquiries may be addressed to H. B. Munns, Advertising Manager, SOVE, P. O. Box 338, Pioneer, California 95666, telephone (209) 295-3540 or Fax (209) 295-3563.

The publisher reserves the right to approve or refuse any advertisement. The publisher is not responsible for any claims, litigations, or expenses resulting from the advertiser's unauthorized use of any name, photograph, sketch, or words protected by registered trademark or copyright.