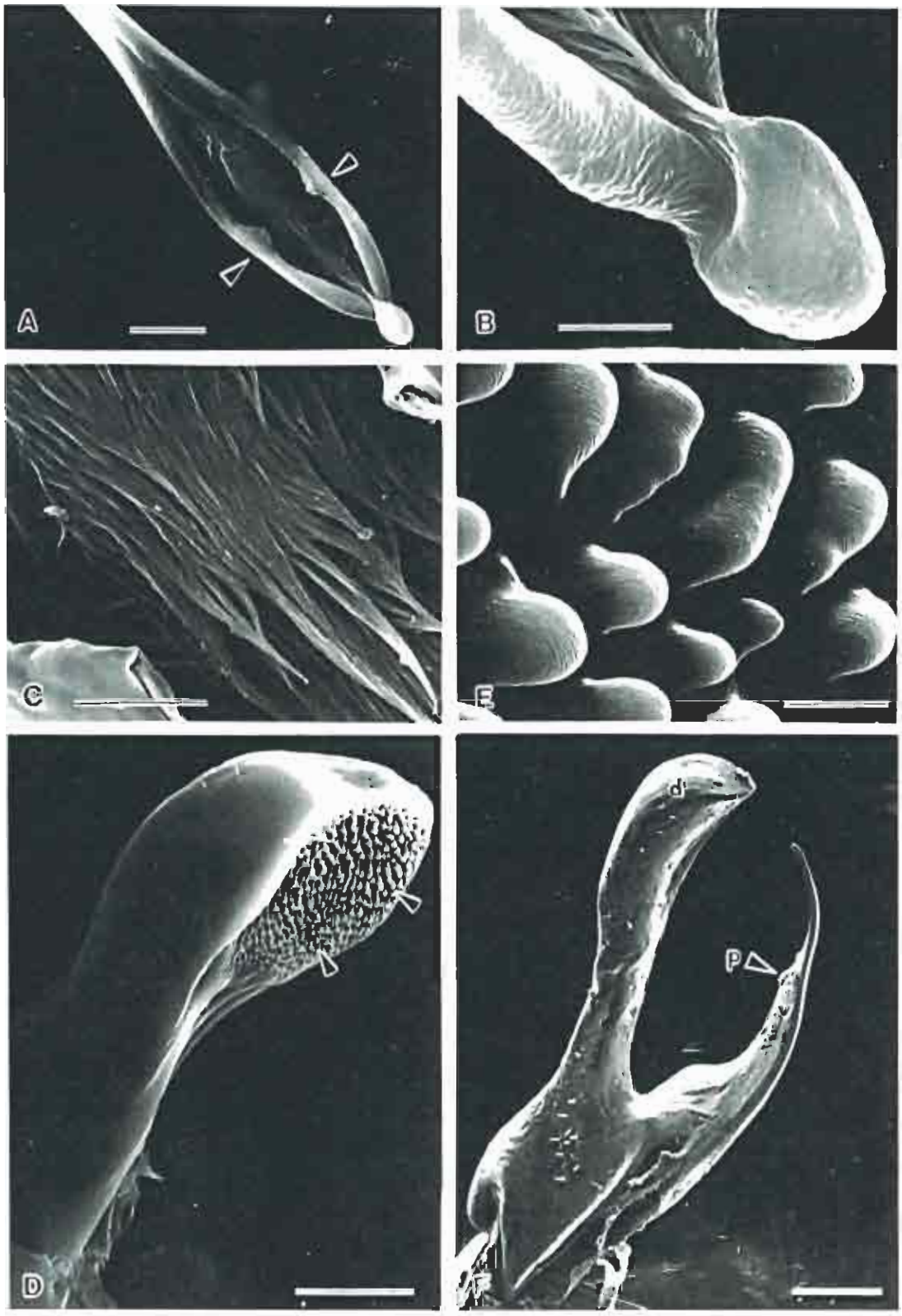
GUANA REPORT FOR 1996



Scanning Electron Micrographs of Beetle Parts

The Conservation Agency

Exploration, Education, and Research

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6 Swinburne Street
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15 April 1997

Dr. Henry Jarecki
10 Timber Trail
Rye, NY 10580

Dear Henry:

Herewith my works and progress report for the year 1996. A lot got published and there are two papers hot off the press already in 1997. It has been a huge year for termites, at long last: three papers published and two more in press. Our first lizard physiology paper is out (in 1997), another is in press, as are two short notes on lizard diets. One frog paper just came out and another is in press.

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As of this date, all plans are go for the pigeon lift of 27 or 28 May. I hope a plan to involve BVI youth/students in next October's projects will develop and succeed.
Please comment on 1997 proposals contained herein. I will keep you posted as more come in.

All the best,

James Lazell, Ph.D.

HOW DO YOU KNOW IT 'S A NEW SPECIES?

by

WENHUA LU

This is perhaps the question we are most frequently asked. It is a hard one to answer because it is often very hard to be sure. Guana's tumbling flower beetles (TFB), family Mordellidae, provide a good example. In 1992 I took on the project of writing a synopsis of the Virgin Islands' TFB, and put in two weeks each year collecting myself for four years. By 1995, after examining all of Mike Ivie's collection at Montana State University, I was convinced that there were seven species of TFB in the Virgin Islands -- six of them present on Guana. My confidence was captured in the video Garden of Eden, where I told the story of dramatic individual variation in a colorful TFB from Guana.

In this pretty group, some have one anterior yellow spot, some have two; some have a white or light yellow head, some have a black or red head; some have black elytra, some have red. I initially had a few specimens and was pretty sure they were just variable individuals. As more specimens accumulated and I had time to examine them back in the lab, I noticed that all the one-spot beetles were males, and all the two-spots with a white head were females. I concluded that it was all one species and new to science.

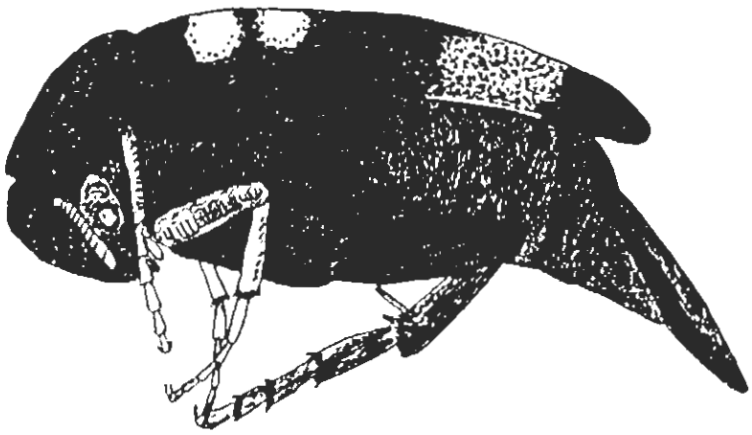
Time flew into 1996. I caught specimens with a red head and red elytra both from Guana and a couple from Sage Mountain. They looked the same as the female with two anterior spots except for their body color, and they were all males. I was puzzled and carefully studied the male genitalia. The news was that there were two kinds of males! The genitalia are totally different. Wow! I thought I had two species new and undescribed from Guana.

Deep in my heart, I thought that was incredible. Back in the old days in the 1930's, an entomologist named Eugene Ray found 14 species on Puerto Rico. Most of them were new species, but two were named by Quedenfeldt in 1860's. Would

Quedenfeldt say anything about my kind of beetles? I was fortunate to get a Smithsonian grant to study further on the matter. I got hold of a copy of Quedenfeldt's paper and could not read it: it is in German! But, being at Smithsonian, you get many experts. There was a German specialist on flies there and I made her translate word by word what Quedenfeldt said in the paper. Oh no! With each word my two "new" species emerged from Quedenfeldt's text. His *leucocephala*, meaning yellow head was one; his *basifulva*, named after the red color on the elytra, was the other. Since his time no one had applied these names to known populations.

To be sure of my discovery, I sent my Quedenfeldt paper all the way to a friend in Germany and put the translation in writing. Then I realized that Quedenfeldt named *leucocephala* based on the female with a yellow head and two anterior spots only. He did not know that its male was sexually dimorphic: never with a yellow head and always with one large anterior yellow spot. So, these two species are not new to science, but the sexual partner of one of the two species is new. So now I am certain that there are eight TFB species in the Virgin Islands and seven of them are present on Guana.

However, a mystery remains. Quedenfeldt named *basifulva* based on the male only, the opposite of *leucocephala*. Who is the sexual partner of *basifulva*?



PROOF

The Termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of the British Virgin Islands: Distribution, Moisture Relations, and Cuticular Hydrocarbons

by

Margaret S. Collins[1], Michael I. Haverty[2], & Barbara L. Thorne[3]

ABSTRACT

A survey of the termites (Isoptera) of 19 islands of the British Virgin Islands (BVI) yielded at least eight species belonging to three families. The Kalotermitidae occurring in the BVI are *Neotermes mona* (Banks), *Incisitermes* species (appear close to *I. snyderi* (Light) and *I. incisus* (Silvestri)), *Procryptotermes corniceps* (Snyder), and *Cryptotermes brevis* (Walker). The only representative of the Rhinotermitidae we collected in the BVI is one or more undetermined species of *Heterotermes* (Froggatt). The three species of Termitidae found on Guana Island are *Nasutitermes acajutlae* (Holmgren), *N. costalis* (Holmgren) and *Parvitermes wolcotti* (Snyder). This report presents brief descriptions of the biology of these termites, along with summaries of the collection sites. Cuticular hydrocarbon composition is correlated with apparent moisture requirements of the termites and availability on the various islands.

INTRODUCTION

The British Virgin Islands (BVI) (Fig. 1) are a complex of more than 50 land masses of various sizes, elevations, and soil types. They are part of the Puerto Rico Bank geological unit, which comprises a broad plateau of land that has been dry or submerged during geological history. The rocks underlying these islands date back to the Cretaceous. Several islands are large enough and possess soils that support Beard's "Evergreen Bushland" (Beard 1945, 1948), as well as littoral and other vegetation associations.

The geographic location and relative size of the several islands of the British Virgin Islands are shown on the map (Fig. 1). All are of volcanic origin except Anegada, a coral and limestone atoll. Tortola, the largest island, has an area of 5444 ha. It supports a protected remnant of

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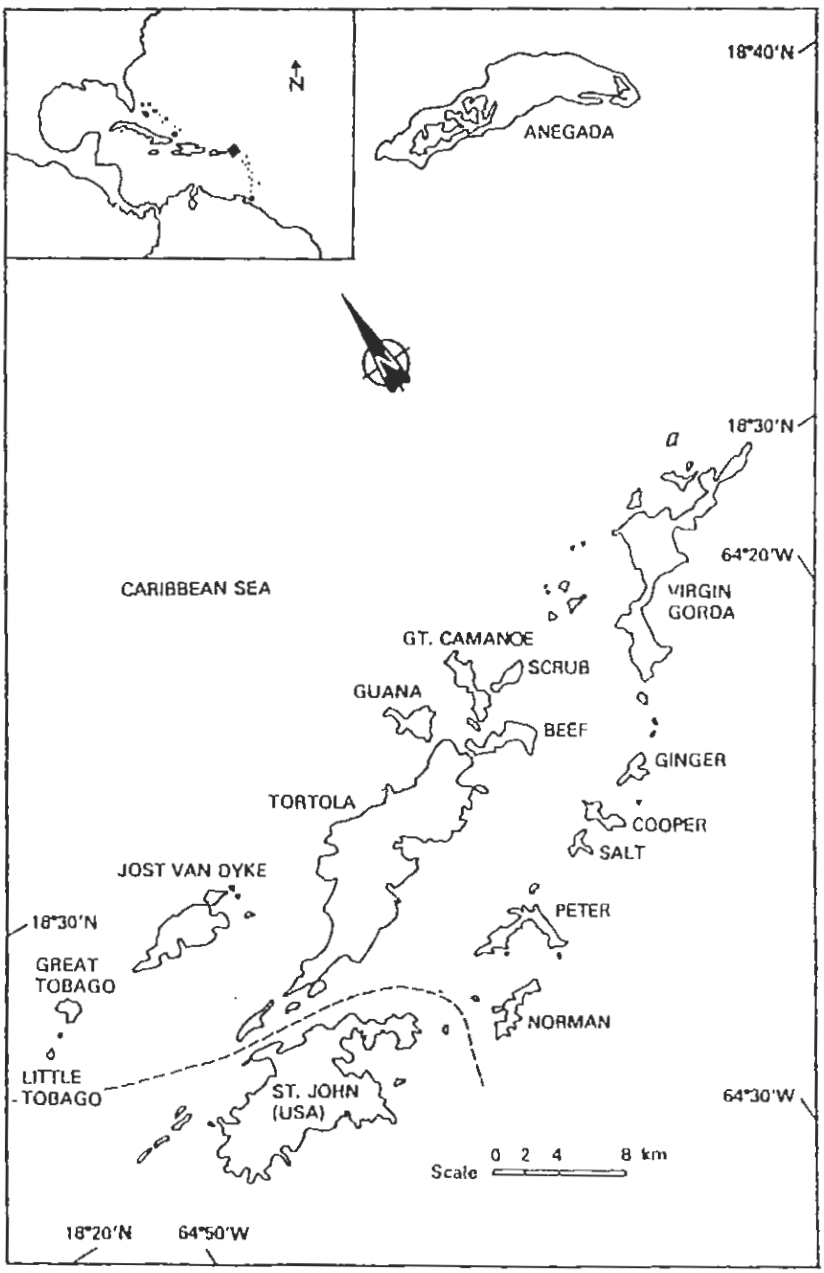

Fig. 1. Diagram showing positions of the British Virgin Islands (from Lazell 1995).

aridulate rain forest (D'arcy 1967) on its highest point, Sage Mountain (522 m). Guana Island is smaller (297 ha.), with a maximum elevation of about 260 m, but has a relatively rich, less-disturbed vegetation cover. A partial listing of typical non-littoral trees and shrubs of Guana Island includes 23 trees, 30 shrubs and 2 palms (G. Proctor, Dept. Botany, University of Puerto Rico, personal communication). The floral and faunal richness of Guana Island may be a result of its origin, size, and history of minimal disturbance (Lazell 1989).

The termite fauna of the West Indies, which included a preliminary, unpublished data in an early version of this report, was reviewed by Scheffrahn *et al.* (1994). In this paper we present a brief report on the biology of each species and revise and update the distribution records of the termite species found in the BVI. A hypothesis is presented regarding the correlation between species distribution, apparent moisture demands, and cuticular hydrocarbon composition of the termites.

METHODS

Collecting periods of 2 to 4 weeks each were spent on Guana Island, with short trips to other islands of the British Virgin Island complex, from 1986 to 1994, most often during the month of October. An attempt was made to sample termite colonies from every suitable habitat that could be reached. Data on flight times were obtained through census of insects attracted to lights in living quarters and trap lights, but such records are limited seasonally. Trees housing carton nests of *Nasutitermes acajutlae* (Holmgren) were sampled in designated areas on Guana Island. Termite samples were transported to the laboratory on Guana Island, where termites were removed and specimens of all available castes were preserved in 85% ethanol. Species diagnoses were made using keys and descriptions, and by comparisons with type and previously identified material. Much work needs to be done to develop usable keys for the Caribbean fauna, and new descriptions are needed for some species. Whenever possible, samples from these same colonies were collected for hydrocarbon analyses (see Haverty *et al.* 1997). Voucher specimens were deposited in the National Museum of Natural History, Smithsonian Institution, Washington D.C.

RESULTS AND DISCUSSION

Collections from 19 islands in the BVI totaled at least eight species in seven genera in three families. The species of termites recorded and the islands on which they were collected, ranked on the basis of moisture availability as determined by vegetation type and density

(Dmi'el *et al.* 1997), are presented in Table 1. These will be considered individually by family.

Kalotermitidae

***Neotermes mona* (Banks).** This is the largest termite in the BVI. This species was once thought to be endemic to Mona Island, but recent collections extended the known range to the Dominican Republic and the Turks and Caicos archipelago, in both natural and structural timber (Scheffrahn *et al.* 1990, 1994). Guana Island was the only island where *N. mona* was collected (Table 1). The two colonies of *N. mona* were collected in dead wood on live trees (one colony from *Morisonia americana* [family Caparaceae]); galleries extended into the live portions of the trees. Mature alates were found on Guana Island during the month of October; flights occurred at night and extended over a period of at least several days. The very large size, clear wings, and large eyes makes this species easy to differentiate from other termites of the area.

***Incisitermes* species.** Members of the genus *Incisitermes* are the most common kalotermitids found in the BVI and were collected on every island except for Carrot Rock (Table 1). Soldiers and alates appear to be close to descriptions and identified specimens of *I. snyderi* (Light) and *I. incisus* (Silvestri). Because of the morphological variation and the uncertainty of the taxonomy of *Incisitermes* in the BVI, we were unable to unambiguously assign specimens to a specific taxon within *Incisitermes* (Haverty *et al.* 1997). Previous species identifications of *Incisitermes* in the BVI (Scheffrahn et. al 1994) should be interpreted with caution because of the uncertainties in diagnosing species within this group (R.H. Scheffrahn, Dept. Entomology, University of Florida, personal communication).

Incisitermes soldiers have head shapes of two distinct types, the "long-headed" and the "short-headed," which develop from different instars of the immature stages. The long-headed soldiers tend to be more deeply pigmented than the short-headed morphs. Small, young colonies have only the short-headed form; older colonies have both morphs. The differences in appearance are so pronounced that they might be regarded as different species if collected separately. Alates are pale yellow or yellow-brown, relatively small night-flyers. Alates were collected during the months of July through November in the BVI.

Incisitermes species live in sound, dead trees of many species, as well as in structural timber in the BVI (see Table 1). *Incisitermes* samples were collected in the mangrove association fringing Beef Island and in trees on the wetter side of Guana Island. *Incisitermes snyderi* is a major pest attacking structures in North and Central America and in the

Table 1. Distribution of termite species in the British Virgin Islands ranked by apparent moisture requirements (termites) and availability (islands).

Island [3] / Termite species [1,2]	N mon	P wol	Het sp	N cos	N aca	P cor	I spp
Tortola			X	X	X		X
Virgin Gorda		X	X		X		X
Greater Jost Van Dyke					X	X	
Beef			X		X		X
Guana	X	X	X	X	X	X	X
Peter			X		X	X	X
Great Camanoe		X			X	X	X
Eustatia		X			X		X
Cooper		X		X	X	X	X
Lesser Jost Van Dyke			X		X		X
Great Tobago					X		X
Scrub					X	X	X
Ginger					X		X
George Dog					X		X
Great Thatch			X		X	X	X
Anegada					X	X	X
Necker			X		X	X	X
Little Tobago					X		X
Carrot Rock					X		

[1]N mon = *Neotermes mona*; P cor = *Procryptotermes corniceps*; I spp = *Incisitermes* species; H sp = *Heterotermes* species; P wol = *Parvitermes wolcotti*; N cos = *Nasutitermes costalis*; N aca = *Nasutitermes acajutlae*. *Cryptotermes brevis* is circumtropical. It is only associated with structures; records of attack from most inhabited Caribbean islands (see Scheffrahn *et al.* 1994).
[2]Decreasing moisture requirements from left to right.
[3]Decreasing moisture availability from top to bottom (Dmi'el *et al.* 1997).

Caribbean, including the Bahamas, Cuba and Puerto Rico (Harris 1961). This species tolerates wide variations in moisture availability in the field, as well as under experimental challenge (Collins 1969).

***Procryptotermes corniceps* (Snyder).** *P. corniceps* is a relatively xerophilic species. Soldiers of *P. corniceps* are distinctive with proportionally long, strongly curved, sharply pointed mandibles. The heads of the soldiers slope steeply in front and have short hornlike projections on the anterolateral region (Krishna 1961). Alates were present in colonies or appeared at lights at night during the months of July through October on Guana Island. *P. corniceps* alates should be redescribed, as the species was named from dealated imagoes and soldiers. In size, alates of *P. corniceps* are very near those of *I.* nr *snyderi*, *C. brevis*, and *I. incisus*, and are somewhat larger than alates of *Heterotermes*; all are of a yellow-brown color, and all fly at night.

P. corniceps was collected on less than half of the islands we visited in the BVI, including some of the driest (Table 1). It is common in old fence posts made from Gumbo Limbo, *Bursera semaruba*, a soft resinous wood not often attacked by other species of termites. Colonies of *P. corniceps* are also frequently found in dead branches on the ground. There are no records of attack of structural timber by this species.

***Cryptotermes brevis* (Walker).** The pragmatic heads of the soldiers, the presence of piles of dry fecal pellets in infested buildings, and the paper-thin outer surface of furniture or structural wood containing colonies of *C. brevis* are diagnostic of this termite. This circumtropical, widely introduced species has been reported only from wood inside buildings. It is not a native termite of the BVI.

C. brevis has been observed in furniture in the lounge of the Guana Island Club. Since dwellings were usually not included in our survey efforts (unless infestations were brought to our attention), we did not encounter this species in any of our collections. Flights occur at night over a long season.

Rhinotermitidae

***Heterotermes* (Froggatt).** Samples of species of this genus from the Caribbean Basin are so variable that Snyder's (1924) query regarding whether the genus comprises a single, highly variable species, a complex of closely related species, or a cluster of evolving species still needs to be addressed. Emerson began re-working the group and left a series of careful, detailed measurements of known species, but had not finished the task at the close of his work. Until useful species limits are diagnosed, members of this group collected in the BVI are referred to as *Heterotermes* sp. (possibly *H. tenuis* (Hagen), *H. convexinotatus* (Snyder), or *H. cardini* (Snyder) or a new species).

Heterotermes sp. was found on eight islands, ranging from the wettest to one of the driest, Little Tobago. Soldiers of this genus are small, with yellow to yellow-brown heads and dark, slender, smooth, sharply pointed mandibles curved only near the tips. The pronotum is narrower than the width of the head capsule, unlike in soldiers of the family Kalotermitidae. Alates are small, light-brown night swarmers; they were observed flying during the month of July on Guana Island.

Termitidae

***Nasutitermes acajutlae* (Holmgren).** *N. acajutlae* is the most obvious and appears to be the most abundant species of termite in the BVI complex, and was collected on every island sampled in this survey (Table 1). This species constructs enormous nests (up to 1.5 m in diameter, up to 3.0 m in height) composed of dark to silvery-brown,

delicate and friable parchment-like outer walls enclosing the variously sized, heavier-walled cells of the carton matrix. Nests are usually spindle-shaped or irregularly rounded. The nests and the nodular food storage bodies contained within are described in more detail by Thorne *et al.* (1996a). *N. acajutlae* was recently resurrected as a species morphologically distinguishable from *N. nigriceps* (Thorne *et al.* 1994, 1996b).

Near White Bay Beach on Guana Island, the most heavily exploited tree species, *Coccoloba uvifera*, had nests, trails, and extensive feeding activity on 79% of 153 trees. *Tabebuia lepidota* had termites on 49.6% of 355 trees. Of the 43 *Pisonia subcordata* 54% had termite galleries, but only three of the trees had nests. In a survey further inland termites were found on 90% of the *C. uvifera* trees, and on 53% of *T. lepidota*. *Bursera semaruba* (Gumbo Limbo or "Terpentine Tree") showed no feeding tunnels and rarely had either nests or runways leading to foraging sites on stems or branches. This may be related to the similarity between some components of the sap of the living tree and the defense secretion of the nasute soldiers (as suggested in other species by Moore (1956); Vrkoc et al. (1978); Valterova et al. (1984)).

Mature nests and individuals of *N. acajutlae* are larger than those of the other carton-nest building nasute of the Guana Island, *N. costalis*. Soldiers of *N. acajutlae* have reddish to dark brown heads; alates are quite large, and chestnut brown in color (Thorne *et al.* 1994). Flights were observed to occur during evening hours in October.

***Nasutitermes costalis* (Holmgren).** *N. costalis* is a carton nest-building nasute that is far less common than *N. acajutlae* and is found primarily in the wetter areas of the BVI. Tortola and Guana Island are the only islands where we collected colonies of this arboreal species (Table 1). Mature nests of this species are smaller, often darker in color, and more friable internally than those of *N. acajutlae*.

Soldiers of *N. costalis* are smaller and have reduced pilosity on the head capsule when compared to *N. acajutlae*. The alates are also smaller and darker, with dark brown to black bodies and charcoal wings. Flights occur during the day and were observed during the month of October on Tortola.

***Parvitermes wolcotti* (Snyder).** *P. wolcotti* is a small nasute that lives on dead wood in or on the ground in areas with fairly dense tree cover. Main nests so far observed in the BVI were under stones in the comparatively moist Pinguin Ghut area of Guana Island. Foraging groups have been taken on three other islands in zones of fairly high moisture. *P. wolcotti* alates have very large, protruding compound eyes,

and large ocelli mounted on raised projections.

MOISTURE RELATIONSHIPS AND TERMITE DISTRIBUTIONS

The distribution pattern shown by termite species collected in the BVI during this study (Table 1) reflects the importance of moisture which, with temperature, directly affects termite distribution (Collins 1991). However, factors such as deforestation; agricultural, residential, and industrial development; reduction of mangroves; overgrazing by sheep and goats; and restrictions of access by collectors all determine whether or not a termite species is collected on a given island. Therefore, the history of disturbance on an island, along with accidents of sampling, may influence diversity records.

All of the species of termites collected from the British Virgin Islands can be found on Guana Island (Table 1). Despite its small size, Guana Island hosts a diversity of habitats that comprise the range of habitats of the BVI (mangroves, dry lowland shrub and cactus, dry forest, and mesic ravine forests) found within the rest of the island complex, except for an aridulate rain forest. The great majority of our time in the field was spent on Guana Island. This perspective has helped to shape our views on specific termite habitats and is, without doubt, the reason we have collected all species on Guana Island.

Species of termites are known to differ in their moisture requirements (Collins 1969). The BVI fauna includes species at both ends of the moisture-dependence spectrum, *N. mona* and *C. brevis*. On Guana Island *N. mona* appears to require a high and constant moisture supply; our one colony was found in wet wood in a live tree. In contrast, *C. brevis*, the "furniture termite," is capable of living without access to free water and is intolerant of exposure to high moisture (Collins 1969; Williams 1977; Steward 1982). The ability to tolerate extremely dry conditions appears to have evolved independently in several groups of Kalotermitidae, and depends on rectal water retention and on restriction of water loss by cuticular lipids (Collins 1991).

Of the species present on this island complex, *Nasutitermes acajutlae* seems to be the least moisture sensitive of the Termitidae (Table 1); this is reflected in collections of this species on even the most arid of the islands (Dmi'el *et al.* 1997). A similar pattern is shown by the closely related species, *N. nigriceps*, found elsewhere in the Caribbean, Central, and South America (Thorne *et al.* 1994). *N. costalis* occurs on many of the islands in the Greater and Lesser Antilles, from Cuba south to Trinidad and Tobago (Snyder 1949; Araujo 1977). The relative scarcity of *N. costalis* in the BVI may be related to the lower moisture availability on most of the islands of the complex. Krecek (1970) found that *N.*

costalis distribution patterns and nest composition on Cuba indicated a higher moisture demand than that shown by the other common nasute, *N. rippertii* (Rambur).

Incisitermes snyderi has been shown to have great flexibility in tolerance of different moisture availability, surviving well in both moist and arid environments (Collins 1969). The apparent scarcity of the dry-adapted *Procryptotermes corniceps* on islands where *Incisitermes* has been collected may reflect sampling accident. *Heterotermes* sp. is common in buildings through the Caribbean, and in natural habitats with suitable soil types. The subterranean habit probably permits access to more constant water sources and thereby increases the diversity of habitats occupied by the species. *P. wolcotti* and *N. costalis* appear to have low tolerance to desiccation; these two species are found either on the "wettest" islands or the more mesic portions of other islands of the BVI.

Epicuticular lipids provide a primary barrier to water loss in terrestrial arthropods (Edney 1967; Hadley 1980, 1985). The physical properties of these lipids are involved in determining rates of water loss from the organism. Studies of water loss rates show that specific temperatures, characteristic of the taxon under study, determine the point at which transpiration rate increases rapidly (Collins 1991). Such "critical temperature" responses have been postulated to result from lipid phase transition in the surface layer (Beament 1961, 1964; Wigglesworth 1972). When extracts of cuticular hydrocarbons from the BVI termite species were analyzed and the percentage of components determined (Haverty *et al.* 1997), the desiccation-tolerant species (*C. brevis*, *Incisitermes*, *P. corniceps*, and *N. acajutlae*) all showed a preponderance of high-melting point (late-eluting) components that were absent from profiles of species found in wetter areas or in living trees (Table 2). The presence of large amounts of late-eluting components in a cuticular hydrocarbon mixture suggest a waterproofing layer of higher stability at normal environmental temperatures than would be characteristic of a mixture of lower-melting point hydrocarbons. It is of interest that, for the BVI termite species, the presence of very late-eluting components in the hydrocarbon mixture is generally correlated with habitat (Haverty *et al.* 1997).

Parvitermes wolcotti is the one exception to this pattern. This species is comprised of very small, fast-moving individuals. The cuticular hydrocarbons of this species consists of two groupings: early-eluting components and late-eluting components much like *N. acajutlae* (Haverty et al. 1997). More must be learned about the ecology and evolutionary biology of this species before these patterns can be interpreted.

Table 2. Predominant cuticular hydrocarbons from pseudergates, larvae and nymphs, too, of workers of 8 termite taxa from the British Virgin Islands.[1]

	Termite species[2]							
Hydrocarbon	N mon	P wol	Het sp	N cos	N aca	P cor	I spp	C bre
2-MeC24	++					+++	+++	+++
C25:1					+	++	+++	
C25	+++			++	+++	+++	+++	+++
13-; 11-; 9-MeC25	+++				tr		+	+
2-MeC25	+++					+++	+++	++
3-MeC25	+++					+++	+++	+++
C26	+++		tr		tr	++	++	++
13-; 12-MeC26	+++							
2-MeC26	++		+			+++	++	+
C27:1				tr	+		+++	
C27	+++	+++	+++	++	++	+++	+++	+++
13-; 11-; 9-; 7-MeC27	+++		+++	+	tr	tr	tr	tr
11,15-DimeC27	++		+++	++				
9,17-DimeC27			+++					
14-; 13-; 12-; 9-; 7-MeC28	+		+++	+			tr	
2-MeC28			+++	+			tr	
15-; 13-; 11-; 9-; 7-; 5-MeC29	tr	+	+++	+++			+	
9,X-DimeC28			+++					
13,17-; 11,15-DimeC29				+++				
9,19-; 9,17-DimeC29			+++				+	
7,21-DimeC29			+++					
2-MeC29		++						tr
11,15-; 12,16-; 13,17-DimeC30					+++			

9-MeC2[illegible]			++					
11,15-; 12,16-; 13,17-DimeC30				+++				
13,17-DimeC31				+++				
15-; 13-; 11-; 9-MeC31	tr		+	+++				
C37:2						++		+++
C39:2					+	+++	+	+++
C39:1			tr		+++	++	tr	++
C40:1					+++			
C41:5					+++			
C41:4					+++			
C41:3		+++				+++	+	+++
C41:2					++	+++	++	+++
C41:1					+++	+++	+	++
C43:4		+++			+++	+		
C43:3		+++				++	+++	++
C43:2					+	++	+++	++
C43:1					+++		++	++
C43:54		+++					+	
C45:3		+++					++	++
C45:1					++		+++	

[1]Relative proprtions of the total hydrocarbon mixture for each species. +++ = > 3.0 %; ++ = 1.0 to 3.0 %; + = 0.3 to 0.99 %; and tr = < 0.3 %; blank = not detected.
[2]N mon = *Neotermes mona*; C bre = *Cryptotermes brevis*; P cr = *Procryptotermes corniceps*; I spp = *Incisitermes* species; H sp = *Heterotermes* species; P wi = *Parvitermes wilcti*; N cs = *Nasutitermes costalis*; N aca = *Nasutitermes acajutlae*.

Our survey of the termite fauna of the British Virgin Islands has provided baseline information on the biodiversity of the region and the data to evaluate the association between cuticular hydroearbons and the arid and mesic termite habitats.

ACKNOWLEDGMENTS

We extend sincere thanks to Dr. Henry and Gloria Jarecki and the staff of the Guana Island Club for their support and hospitality during the course of this project. We thank R.H. Scheffrahn for constructive comments on an earlier draft of this manuscript, and J. Lazell for support and discussion throughout the project. L.M. Hernandez and J. Krecek assisted in the collection of several species. L.J. Nelson characterized the cuticular hydrocarbons for each species. This research was funded, in part, by a series of grants to the authors by The Conservation Agency through grants from the Falconwood Foundation and a cooperative agreement between the Pacific Southwest Research Station and the University of Maryland.

TRIBUTE

Dr. Margaret S. Collins (1922-1996) passed away while on a Caribbean island, studying the termites that captured her interest so completely. Well known as an adventurous tropical field biologist, Dr. Collins energetically devoted her career to work on termites in a variety of locations in the Neotropics and North America. It was our privilege to have this exceptional person as our friend and collaborator. She lived her life with enthusiasm, integrity, and a rich sense of humor, and she made many substantive contributions to the field of termite biology.

- MIH and BLT

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BIOTROPICA 28(4b): 781–785 1996

Associations Between Termites and Bromeliads in Two Dry Tropical Habitats[1]

Key words: British Virgin Islands; Bromeliaceae; fire protection; Guana Island; Isoptera; Minas Gerais, Brazil; Nasutitermes; *symbiosis;* Tillandsia.

THE ECOLOGICALLY AND NUMERICALLY PROMINENT TERMITE *Nasutitermes acajutlae* (Holmgren) occurs through the Caribbean from Puerto Rico south to Trinidad, possibly extending into South America (Thorne *et al.* 1994). This species builds arboreal nests that are among the largest known for any termite, measuring up to 2 m in height and 1 m in diameter (Thorne *et al.* 1994). Like other *Nasutitermes,* these termites build extensive networks of covered tunnels connecting the nest to distant foraging sites in the tree crown and ground below. Workers and soldiers, numbering well over a million individuals in mature colonies, travel within these "carton" tunnels which consist of partially digested wood, fecal material, soil, and water (Light 1933). We discovered an association between *N. acajutlae* termites and the bromeliad *Tillandsia utriculata* on Guana Island, British Virgin Islands (B.V.I.), during October 1994. *T. utriculata* is the largest of the local tank-forming or phytotelm bromeliads, that by definition impound water and litter as major sources of moisture and nutrients among inflated, tightly overlapping leaf bases.

Guana Island, a wildlife sanctuary which lies directly north of the eastern end of the island of Tortola, B.V.I., covers about 340 ha and rises to about 246 m at maximum elevation. Guana Island is volcanic and supports a rich diversity of flora and fauna due to habitat variation and a history of minimal disturbance (Lazell 1989). Dry lowland scrub forest predominates, while abundant cacti characterize scattered, drier sites. Wet areas are confined to the few ravines on the island. *T. utriculata* occurs abundantly in some of the drier woodlands, but they were not surveyed comprehensively.

[1] Received 5 April 1995; revision accepted 15 September 1995.

TABLE 1. *Transect survey of the association between active trails of the termite* Nasutitermes acajutlae *and the bromeliad* Tillandsia utriculata *in four locations on Guana Island, British Virgin Islands. Only trees (including cacti) that contained both termite trails and at least one bromeliad were included in the study.*

Location transect on Guana Island	Number of trees with termite trails intersecting the bromeliad	Number of trees with termite trails not intersecting the bromeliad
"Garden of Eden" nr. Guana Island Club	11	1
Trail to Longman's Point	75	2
White Bay Beach	17	0
Wei Ping Liao Trail	12	0
Total	115 (97.4%)	3 (2.6%)

The association between the termites and bromeliads on Guana Island was particularly easy to document because *N. acajutlae* builds distinctive and conspicuous trails, and *T. utriculata* often grows in relatively low canopies. We inspected every tree and large cactus that we could readily see along four transects in four locations on the island. Only trees and cacti that had both active termite foraging tunnels and at least one *T. utriculata* plant were examined.

Termite tunnels intersected a bromeliad in 97.4 percent of the 115 possibilities (Table 1; Fig. 1). Usually, *N. acajutlae* tunnels went to the root system or above to the water-filled leaf axils. Often tunnels continued into the shoots to cover impounded water. Some hung suspended for short distances (4–12 cm) between adjacent bark and the plant. A few tunnels led only to the moist area where the bromeliad rooted, but usually at least one leaf axil was accessed.

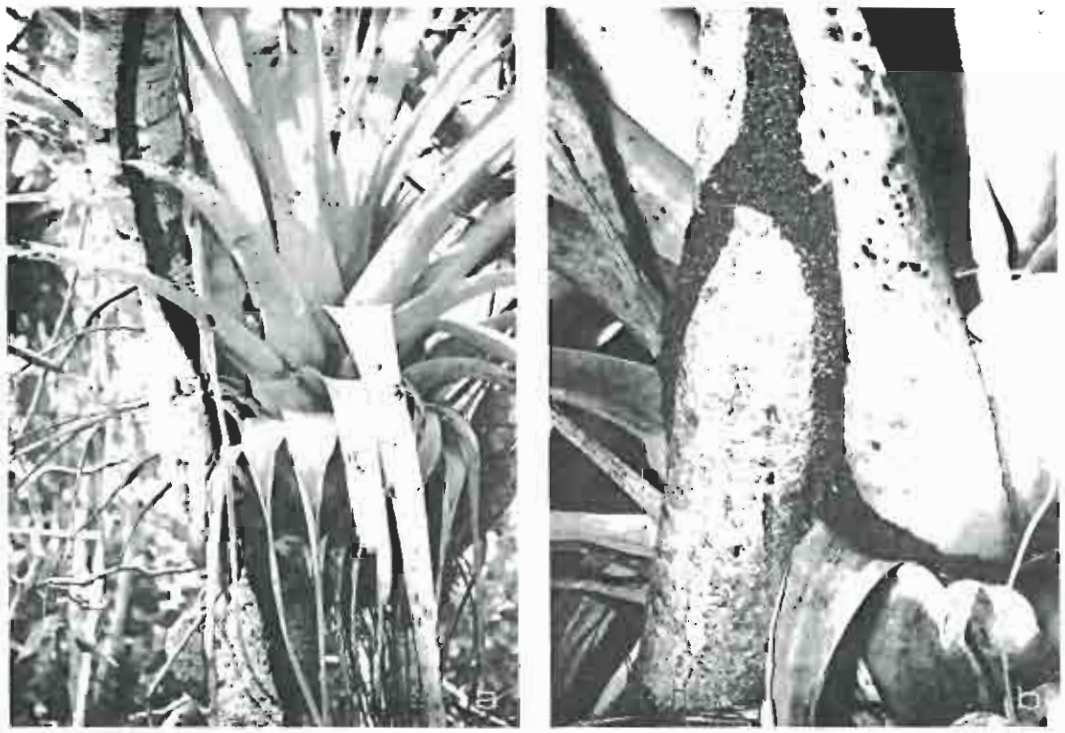

FIGURE 1. Photographs of tunnels built by the termite *Nasutitermes acajutlae* intersecting leaf base chambers of the bromeliad *Tillandsia utriculata* on Guana Island, British Virgin Islands. a. *N. acajutlae* trail built up to the moist root cluster and leaf base reservoirs of *T. utriculata*. b. *N. acajutlae* trail built over leaf bases of a *T. utriculata* plant.

Each tree was counted only once in a transect, but many trees hosted more than one *T. utriculata* plant, and termite trails intersected up to eight bromeliads on a single tree. We were unable to determine the extent of a complete network, *i.e.* how many bromeliads were tapped by a given *N. acajutlae* colony, in part because *N. acajutlae* foragers also travel within decayed wood. Quite likely, termites inconspicuously tap bromeliads from inside supporting trunks and branches. *N. acajutlae* may also tap *T. utriculata* rooted on the ground. Hence, our data probably present a conservative measure of the termite/bromeliad association.

Although diverse invertebrates occupy bromeliad tanks (*e.g.*, Picado 1911, Pittendrigh 1948, Laessle 1961, Fish 1983, Benzing 1990, Nadkarni & Longino 1990), this is the first report of an association with termites. Most likely the termites are visiting *T. utriculata* to obtain water, a scarce commodity given the xerophytic vegetation and porous soils of Guana Island. No formal weather records exist, but longtime residents and scientific researchers agree that the two years prior to our study were uncharacteristically dry in the Virgin Islands. Drought was particularly acute where aridity generally prevails as on Guana Island. Our related studies of termites on Guana Island (1989–1994), suggest diminished populations of *Nasutitermes* and other termites caused by abnormally low rainfall in 1993 and 1994, but more extensive records are needed to assess the magnitude and rarity of this presumed stress.

Organic matter suspended in the water impounded by *Tillandsia* shoots is a potential source of termite nutrients (Huxley 1980; Benzing 1981, 1990). However, preliminary bioassays ($N = 4$) using disks of saturated Whatman #1 filter paper and several hundred termite foragers indicated no preferences between bromeliad extracts and tap water. We do not know if termites feed on tank debris as the water level recedes.

Although termites probably visit *T. utriculata* primarily to exploit moisture, effects on plants more likely vary with local circumstances. Absorbant termite carton tunnels wick moisture away from shoots. Up to 35-cm lengths of termite tunnel were saturated with moisture siphoned from leaf axils or areas of seepage around roots. *Tillandsia utriculata* tolerates considerable drought, but any tank bromeliad fails if leaf axils dry out for extended intervals (Madison 1977; Benzing 1981, 1990). Conversely, overhanging portions of termite carton should reduce losses from a plant's supply of moisture.

Termites are ill-suited to match certain ants as plant mutualists. Termites typically remain confined to tunnels and are therefore unavailable, even if disposed, to deter plant predators. Some ants deposit nutritive materials in bromeliad shoots, thus contributing to plant welfare (reviewed in Huxley 1980, Benzing 1990). An occasional termite may die or drown in a leaf axil, but probably not often enough to significantly promote plant nutrition. Termite tunnel carton that ends up in the tanks is also unlikely to constitute much of a nutritional supplement for the bromeliad.

The termite diet precludes bromeliad seed dispersal, a phenomenon known from several ant/epiphyte relationships (reviewed in Huxley 1980, Benzing 1990, Yu 1994). Only once did we observe a juvenile *T. utriculata* rooted in a termite tunnel or a bromeliad growing adjacent to a termite nest. Furthermore, a number of the termite trails turned toward a bromeliad, suggesting that the plant had arrived first and had been subsequently located by exploring termites. Thus, the relationship between termites and plant probably pivots on moisture rather than predator deterence, nutrition, or seed dispersal. The dynamic may shift according to circumstance from weakly mutualistic through commensalistic to parasitic if plants become water-stressed.

A heavy rain in October 1994, the first following a prolonged drought, provided an opportunity to observe termite trail construction. As is typical for arid-land *Nasutitermes*, trail building escalated because workers need moisture to masticate and manipulate materials used for tunnel construction. Trails were rapidly laid down on four trees which supported bromeliads that *N. acajutlae* had only recently discovered. In each case, the termites first lay a trail of fecal deposits and secretions on the tree bark, typical of their normal building behavior (Jones 1980). Tunnel construction then began at the junction with the bromeliad, proceeding down the tree. By working from the bromeliad down instead of from the base of the tree up, the termites had ample moisture for their work.

Do termites use trees with bromeliads for *both* food and water, or are they exploring some supports only to obtain moisture from the attached epiphytes? Host trees we examined were generally mature with apparent or probable zones of dead wood. In addition to the tunnels leading to the bromeliad, usually one or more of the trails led to a feeding site through a knothole or dead branch. However, young trees with no dead wood would, because of their age, also be the trees least likely to support

epiphytes. If one found a case in which the termites appeared to be occupying the tree only for access to the bromeliad, the carton tunnels should be scraped away to confirm presence or absence of any covered access holes into the tree wood.

Although *N. acajutlae* appears to be remarkably drought tolerant, probably more so than other *Nasutitermes* species in the Caribbean, bromeliads may at least occasionally provide essential moisture. *N. acajutlae* workers possess comparatively large water sacks, sufficient to store enough water for prolonged dry weather (M. S. Collins, pers. comm.). *N. acajutlae* is clearly not a specialist on trees with bromeliads, but linking to one or more tank reservoirs may enable these termites to thrive or survive in an otherwise inhospitable season or habitat. To further understand the ecological dynamics of this association, we plan to seek comparative data on intersections between *N. acajutlae* foraging trails and *T. utriculata* plants on wetter islands in the Virgin Islands cluster, and on Guana Island in times of heavier rainfall.

Preliminary observations in the rocky, semiarid "campos rupestres" of Minas Gerais state in southeastern Brazil indicate that associations between termites and water-impounding bromeliads occur beyond Guana Island. A brief foray into the field near the city of Diamantina revealed covered termite trails leading from the soil up along the precipitous faces of large rocks to the bases of several large, lithophytic and water-impounding *Aechmea phanerophlebia*. Unlike *Tillandsia utriculata*, *Achmea phanerophlebia* produces an urn-shaped shoot that intercepts no litter, but often houses ant colonies. In all other respects, the relationship appeared to be equivalent to that observed on the Caribbean island.

Several more locally abundant terrestrial Bromeliaceae representing *Dyckia* and *Encholirium* also attracted unidentified termites at the Brazilian site, as did some companion flora (*e.g.*, Cyperaceae, Vellosiaceae) of similar low stature. Virtually every mature plant contacted mounds of termite carton. Inspection indicated no damage from the insects. Root systems located in soil rather than in adjacent termite construction indicated that termites build around established plants as in the case of *Tillandsia utriculata* in the British Virgin Islands.

Drought may render bromeliad phytotelmata an important termite resource in the high rocky grasslands of southern Brazil as on Guana Island. A broader variety of terrestrial herbs, including Bromeliaceae, could provide similar assistance, not as liquid water but as vapor to humidify termite galleries. A variety of benefits may accrue in turn to the flora. Abundant, charred vegetation and extraordinary insulation on endemic flora (thick bark, persistent leaf bases on herbs) reflect frequent natural fires in the campos rupestres. Recent fires had seriously burned some of the few exposed bromeliads, but none of those plants embedded in termite carton (similar findings elsewhere noted by Morison *et al.* 1948, Harris 1961). The survivorship and fecundity of bromeliads among other low-growing vegetation would also be improved if these insects promote plant nutrition or deter herbivores. Additionally, spiny-leafed *Dyckia* and *Encholirium* may discourage large, local ant-eaters seeking termites as food.

Termites constitute prominent components in diverse ecosystems, particularly in the tropics, where they substantially enhance nutrient cycles. Observations reported here indicate that termite colonies in some dry habitats may establish long-term relationships with bromeliads to access water. Consequences probably vary for the plants. Some of these relationships seem pervasive enough to warrant further inquiry.

Sincere thanks to H. and G. Jarecki and the staff of The Guana Island Club for their support and hospitality during the course of this project. We gratefully acknowledge constructive input and productive discussions with Drs. M. S. Collins, J. D. Lazell, and N. M. Nadkarni. This research was funded by a grant from The Conservation Agency, through a grant from the Falconwood Foundation, and by a cooperative agreement between the University of Maryland and the Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture.

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Reprinted from *Florida Entomologist*, Volume 79, Number (1), March, 1996

ARCHITECTURE AND NUTRIENT ANALYSIS OF ARBOREAL CARTON NESTS OF TWO NEOTROPICAL *NASUTITERMES* SPECIES (ISOPTERA: TERMITIDAE), WITH NOTES ON EMBEDDED NODULES

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ABSTRACT

Nest architecture of the arboreal Neotropical termites *Nasutitermes acajutlae* (Holmgren) and *N. nigriceps* (Haldeman) is described, with special reference to carton inclusions or nodules found within the normal gallery matrix of some nests. Nutrient analyses of these nodules show that they have high cellulose and low cutin concentrations in comparison to normal nest carton. These data support the hypothesis that the nodule inclusions serve as a form of facultative food storage in some nests of these termite species. These cases appear to represent a rare situation in which food is not stockpiled or cultured by termites, but rather some partially processed, masticated food is incorporated into the nest matrix for future consumption.

Key Words: Termites, Nasutitermitinae, inclusions, food storage.

RESUMEN

Se describe la arquitectura del nido de las termitas neotropicales *Nasutitermes acajutlae* (Holmgren) y *N. nigriceps* (Haldeman), con referencia especial a inclusiones de cartón o nódulos encontrados dentro de la matriz de la galería de algunos nidos. El análisis de nutrientes de los nódulos muestra que estos tienen concentraciones altas de celulosa y bajas de cutina, en comparación con el cartón normal de los nidos. Los datos sostienen la hipótesis de que las inclusiones de los nódulos sirven como una forma facultativa de almacenamiento de alimento en algunos nidos de termitas de esas especies. Estos casos parecen representar una rara situación en la cual el alimento no es almacenado en pilas o cultivado por las termitas, sino masticado y parcialmente procesado e incorporado a la matriz del nido para consumo futuro.

The tropicopolitan termite genus *Nasutitermes* (Termitidae; Nasutitermitinae) is the most speciose of all isopteran genera, containing approximately 75 described species from the Neotropics alone (Araujo 1977). Unlike most termites, many species of *Nasutitermes* build arboreal carton nests composed of wood and salivary and fecal flu-

ids (Light 1933), and occasionally other materials such as sand (Thorne & Haverty, pers. obs.). Most other nest-building termites build mounds on the ground (e.g., Emerson 1938), but nesting in trees has enabled species of *Nasutitermes* and several other genera to colonize and exploit a new habitat.

Nasutitermes nigriceps (Haldeman) is a geographically widespread termite, ranging at least from Panama north throughout the lowland forests of Central America into Mexico. It is also found on Jamaica and on Grand Cayman Island (Araujo 1977, Thorne et al. 1994). *N. acajutlae* (Holmgren), which is morphologically very similar to *N. nigriceps*, is found on Puerto Rico, the US and British Virgin Islands (BVI), Trinidad, and Guyana (Emerson 1925, Araujo 1977, Thorne et al. 1994). There are isolated reports of members of the *N. nigriceps* "complex" from other parts of South America, but a comprehensive taxonomic analysis of specimens is needed to verify species identity of the South American fauna.

Despite the abundance of *Nasutitermes* arboreal nests, the chemical composition of the carton material has not been examined in detail in any species (but see Becker & Seifert 1962 for data on ash and lignin content). Knowledge of the composition of the nest is fundamental in determining origin of nesting materials, cost of construction, variation among colonies and species, and ability of the termites to allocate components of their diet for nest construction.

A distinctive feature of some *N. acajutlae* and *N. nigriceps* nests is the presence of rounded carton inclusions or "nodules" within the typical gallery matrix (Hubbard 1877 pp. 268, 270, Andrews 1911 pp. 200-202, Emerson 1938 p. 264, Wolcott, cited in Martorell 1945 p. 361). These nodules appear to be of a similar carton construction as the rest of the nest, but they are a lighter brown color, are formed in dense concentric sheaths (Fig. 1), and they may possibly serve as a form of food storage (Hubbard 1877; Andrews 1911). Kemner (1929) interprets the presence of carton nodules in the Javan termite *Microcerotermes depokensis* Kemner as food storage structures. Noirot (1959) reported compact masses of wood fragments in the central cavity of nests of *Globitermes sulphureus* (Haviland). Some termite genera do store food as dried vegetative elements in specialized portions of their nests ("attics") [e.g., Hodotermitinae (*Hodotermes*, *Microhodotermes*, *Anacanthotermes*); Rhinotermitidae (*Psammotermes*); Termitidae: Amitermitinae (certain *Amitermes* and *Drepanotermes*), Nasutitermitinae (certain species of *Tumulitermes*, *Nasutitermes* and *Trinervitermes*)] (Noirot 1970, Bouillon 1970). The "fungus growing" termites (certain Macrotermitinae) culture fungus within the nest as a supplemental food source. Interestingly, some fungus growing termites store vegetative materials within the nest before they are included in the fungus garden (*Pseudacanthotermes*, *Acanthotermes*, some *Macrotermes*) (Grasse & Noirot 1951). If the *Nasutitermes* nodules described in this paper are indeed food reserves, they are not simply stored food but rather elements which have already been masticated and partially processed by the termites, then positioned within the nest matrix for future consumption.

In this paper we describe the architecture of *N. acajutlae* and *N. nigriceps* nests from sites in Panama and the BVI. Observations of the nodule inclusions are reported. Nutrient analyses of two nests without nodules and comparative chemical analyses of nodule material versus the surrounding "normal" carton matrix of two nests with nodules are presented and reported.

Materials and Methods

Eight *N. nigriceps* nests were collected within 5 km of the Panama Canal in 1980 and 1981; only one of these contained the distinctive nodules within the carton next matrix. This arboreal nest was collected from the Gigante East Peninsula near Barro

Colorado Island on 7 April 1981. The entire nest was pried from the host tree, placed within a plastic bag, and taken to the laboratory of the Smithsonian Tropical Research Institute on Barro Colorado Island. The nest was dissected by sequential shaving after being refrigerated for 24 hours to inactivate the termites (technique described in Thorne 1984).

Nest carton from four colonies (one *N. nigriceps* nest collected near Barro Colorado Island, Panama in 1981; three *N. acajutlae* nests collected in 1988 and 1989 on Guana Island, BVI) was analyzed in 1989-1990. Two of the nests (the *N. nigriceps* nest from Panama and a 1988 *N. acajutlae* nest from the BVI) contained nodules. Chemical composition of both the nodules and a sample of the more typical dark carton material was determined from those two nests, and samples of typical carton matrix from parts of two additional Guana Island *N. acajutlae* nests (which did not contain nodules) were also analyzed. Type of nest material examined is presented in Table 1.

Materials and Methods for Nutritional Analyses of Nest Samples

In the laboratory, samples were dried at 60°C to constant mass (approximately 24 h). Dried samples were ground to pass through a 1 mm screen in a Wiley mill. A portion of each sample was dried at 105°C to determine percent dry matter and then placed in a muffle furnace for 3 h at 500°C to determine percent organic matter and ash (an estimate of total mineral content). In vitro organic matter digestibility, or percent fermentable substrate, was determined by the Tilley & Terry (1963) method as modified by Moore & Mott (1974). This analysis consists of a 48 h incubation under CO_2 at 39°C with an inoculant of steer rumen fluid followed by a 48 h acid-pepsin treatment to remove undigested microbes. The percent of organic matter that disappears during the 96 h is the in vitro organic matter digestibility.

Percentage of neutral detergent fiber (NDF: cellulose, hemicellulose, lignin and cutin) was measured by the Van Soest technique (Goering & Van Soest 1970) with decalin and sodium sulfite omitted (Golding et al. 1985). Analyses for percentages of acid detergent fiber (ADF: cellulose, lignin and cutin), potassium permanganate lignin, and cutin followed Goering & Van Soest (1970). Percent hemicellulose is estimated by subtracting ADF from NDF. Lipids were extracted with ethyl ether in a Goldfisch apparatus for 8 h. Percent concentrations of total (Kjeldahl) nitrogen and phosphorus were measured with a block digester (Gallaher *et al.* 1975) and an automated Technicon analyzer (Hambleton 1977). Energy content of food and feces was determined in a bomb calorimeter following standard procedure (Parr Instrument Co. 1960).

One sample was analyzed from each source of nest material. In each analysis, two subsamples were evaluated. Values for replicates of each sample were accepted within 1% relative error. Relative error is calculated as $(a-b)/(a+b)$ where a and b are replicate values. Rarely, the values obtained for the duplicates were not within 1 relative percentage, in which case a third subsample was analyzed. Table 1 reports the mean of the analyzed values for each sample.

Results

Nest Architecture

Nests built by *N. acajutlae* and *N. nigriceps* can be among the largest of any arboreal nesting *Nasutitermes*. Maximum dimensions of an ellipsoidal nest can approach 2 m in height and 1 m in girth (Thorne et al. 1994). The exterior of these nests is typically medium to greyish brown in color and irregularly mottled, generally with

rather shallow bumps, unlike the dark nests with small bumps characteristic of the exterior of *N. corniger* (Motschulsky) and *N. costalis* (Holmgren) nests or the lighter brown, smooth shell typical of *N. ephratae* (Holmgren) (Thorne 1980; Haverty et al. 1990). Young nests may be difficult to identify to species, but differences in exterior appearance make it possible to visually discriminate most mature nests of *N. acajutlae* and *N. nigriceps* from those of *N. corniger*, *N. costalis*, or *N. ephratae*. The outer carton shell of nests of all of these *Nasutitermes* species has small pinpoint holes, visible if a piece is held up to a light. These holes presumably function in gas exchange.

The intercalated matrix of galleries within mature nests of *N. acajutlae* and *N. nigriceps* tends to be larger (chamber diam up to 2.1 cm) and with thicker carton walls (up to 0.6 cm near the exterior of a nest; exceeding 1.7 cm near the interior of the nest) than in nests of arboreal *Nasutitermes* found sympatrically with one or both of these species (*N. columbicus*, *N. corniger*, *N. costalis*, *N. ephratae*). The royal "cell" within the nest is often positioned near the central longitudinal axis of the nest, and frequently located in or near a branch fork or knothole of the host tree. In small to medium sized nests (< 60 cm diam) the royal cell is a distinctly thicker sphere or ellipsoid of layered carton (generally up to 12-15 cm in diam) surrounding the royal chamber. In nests exceeding 1 m on an axis the royal chamber can be embedded in the dense carton center of the nest, with the royal cell becoming indistinct from the remainder of the central, reinforced portion of the nest. We have not found distinguishing characters to differentiate nest architecture of *N. acajutlae* from *N. nigriceps*.

The carton-covered foraging trails built by large *N. acajutlae* and *N. nigriceps* colonies are wider and less regular than in *N. corniger*, *N. costalis*, or *N. ephratae*. Termites occupying small to medium sized *N. acajutlae* and *N. nigriceps* nests frequently build simple, linear trails 0.9-1.5 cm wide, thus they are indistinct from trails of *N. corniger*, *N. costalis*, or *N. ephratae*. However, trails leaving large nests of *N. acajutlae* and *N. nigriceps* are often broad (up to 14 cm in width) and deep (up to 8 cm from the tree to ceiling of the gallery). Trails from large nests are often highly irregular along the edges. Occasionally a "floor" is built as well so that the trail becomes a tube that can, for a limited distance, be separate from the tree or branch. As is typical for many arboreal *Nasutitermes*, tunnels built on the exterior of tree branches are frequently on the underside of the branch. We hypothesize that this minimizes disturbance by hard rainfall or by creatures traveling along the tops of branches. Building galleries in the "shade" of branches would also minimize desiccation from direct sunlight. A further advantage would be that foraging tunnels built on the undersides of branches would receive maximum moisture from rain running off the branch. This would be beneficial for *N. acajutlae* or *N. nigriceps* since they often live in relatively dry habitats (Thorne et al. 1994).

Description of Nodules and Nest Population

We describe nodule inclusions in three nests: one *N. nigriceps* nest dissected in April, 1981 in Panama, one *N. acajutlae* nest dissected in July, 1988 on Guana Island, BVI, and a nest dissected on the island of Tortola, BVI in October, 1994 (nodules from the latter nest were not analyzed for nutritional content).

Photographs of the interior of the *N. nigriceps* nest collected in Panama are shown in Fig. 1. The nest was generally spherical, about 46 cm in diam, which placed it in a medium size category for conspecific nests in that area. Twenty nodule formations, 14 of which measured 3.0-4.8 cm in diam, but some as small as 1 cm diam, were removed from the nest. All nodules were positioned within 4-10 cm of the nest exterior. The nodules were of a uniform light brown color in contrast to the dark brown surrounding

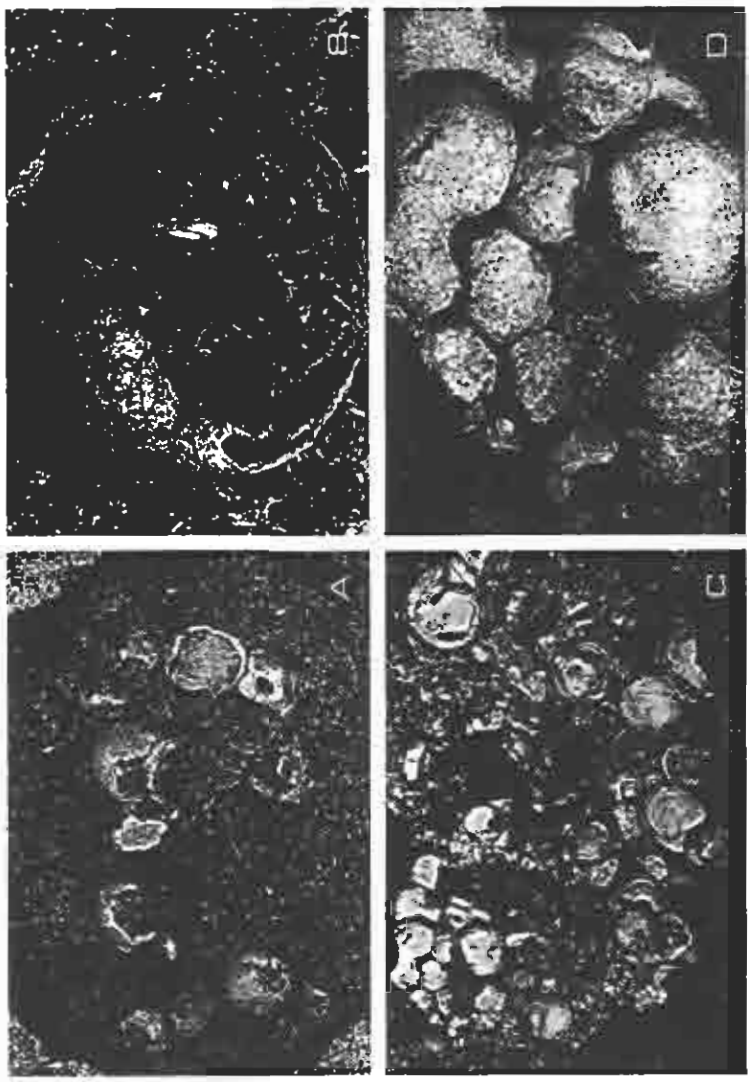

Figure 1. Photographs of nodules embedded within normal nest carton matrix in the *Nasutitermes nigriceps* nest collected in Panama in April 1981 (A,B) and in an *N. acajutlae* nest collected on Tortola, BVI in October 1994 (C,D).

nest matrix. Nodule shape was generally spherical although some had distortions or were irregular ellipses. The nest contained an active population of soldiers and workers, as well as a conspicuous brood of wingbud nymphs in the penultimate and ultimate instars. Many of the nymphs occupied the interiors of the nodule spheres. No reproductives, eggs or immatures were found within the nest.

The *N. acajutlae* nest on Guana Island was irregularly ellipsoidal, measuring approximately 1 m in height with a maximum diam of 75 cm. The nest contained a primary queen, developing nymphs of a variety of instars, many eggs and white larvae, and a large population of workers and soldiers. No primary king was retrieved but that is typical during field dissections because mature kings are small enough to retreat quickly and evade capture. The light-colored nodules were located in a zone surrounding the hard, inner core of the nest, all positioned at least 2 cm from the exterior nest wall. Many of the nodules were scalloped, possibly indicating consumption by termites. As with the *N. nigriceps* nest, immature termites occupied the interior of hollowed-out nodules.

An *N. acajutlae* nest collected on the island of Tortola, BVI was brought to us in several pieces during a field trip in October 1994. This large nest, estimated to have been just over a meter in height and about 80 cm in diam, contained eggs, white immature, soldiers, and workers. There were relatively few brachypterous nymphs, but numerous mature alates were present. The primary queen and king were not recovered, but the presence of egg caches suggests that the reproductives were present in the intact nest or in the portion of the host tree surrounded by the nest. This nest had clusters of nodules positioned within the inner perimeter of the nest (Fig.1 C, D) Again, the outer 2 cm or more of nest material was dark, more typical carton matrix with no nodules. Because the nest arrived in pieces, it was impossible to tell whether nodules were built in the center core of the nest.

We did not do nutrient analyses of nodule material from the Tortola nest, but we measured each of the 75 nodules that were retrieved. The distribution of nodule sizes recovered from this nest is shown in Fig. 2. Some of the irregularly spherical nodules in this nest were solid, dense material; most were hollowed to some extent as seen in the Panama and Guana Island nests. Hollowed nodules contained large numbers of immatures. Eight of the nodules from this nest were bilobed, as if two units had been constructed or fused together.

The nodules from all three of these nests were generally similar in size, shape, color, and position within the nest matrix. In each case immatures occupied excavated nodules.

Chemical Analyses of Nest and Nodule Material

The most striking aspect of the nutrient composition of nest materials (Table 1) is the consistency among these nests. The only apparent differences are the higher cellulose and lower cutin concentrations in nodule samples than in carton samples and the higher in vitro organic matter digestibility values of the samples from Panama. No statistical analyses were performed because only two samples were available in each category. Hemicellulose was absent or present in only trace amounts in the samples, so was not included in Table 1. Nutrient composition of the nest material apparently does not change with age. The composition of recently constructed normal carton was very similar to that of old carton material from the North Bay and White Bay Beach nests on Guana Island. The high organic matter content indicates that little, if any, soil or sand is incorporated into these samples of nest or nodule material.

TABLE 1. NUTRIENT COMPOSITION OF NODULE AND NORMAL NEST CARTON SAMPLES[1]

	Organic Matter	Cellulose	Lignin	Cutin	Lipids	Nitrogen	Phosphorus	In Vitro Organic Matter Digestibility	Energy
Panama-1981									
Nodule	96.0	30.9	35.8	5.1	1.6	0.66	0.06	15.0	19.6
Normal nest carton	94.5	16.6	31.9	30.9	0.8	0.73	0.06	10.2	20.7
BVI-1988									
Nodule	95.2	29.9	37.5	9.0	0.8	0.75	0.04	5.6	19.8
Normal nest carton	94.5	16.9	40.9	17.7	0.8	1.03	0.08	6.2	20.7
BVI-1989 North Bay Beach									
Interior, dense carton	93.4	14.6	36.3	34.4	0.9	0.96	0.09	4.0	20.5
Exterior, thin carton	92.9	18.1	32.2	34.8	1.0	1.01	0.07	3.1	20.2
BVI-1989 White Bay Beach									
New, thin carton	91.1	18.7	36.5	27.4	1.2	0.70	0.05	4.2	19.5
Fresh, very thin carton	91.7	19.7	35.2	30.8	1.0	0.67	0.05	3.0	19.5
Dry nest carton	88.3	15.3	35.1	30.6	1.4	0.80	0.08	2.5	19.6

[1]All values are based on one sample, each analyzed in duplicate. If the values obtained for the duplicates were not within 1 relative percentage, a third replicate was analyzed. Means of the two or, rarely, three values are listed. All values are presented as percent dry matter except in vitro organic matter digestibility is expressed as percent organic matter and energy is expressed a kJ/g dry matter. Panama samples are from an *Nasutitermes nigriceps* colony; BVI (British Virgin Islands) samples are from *N. acajutlae* colonies

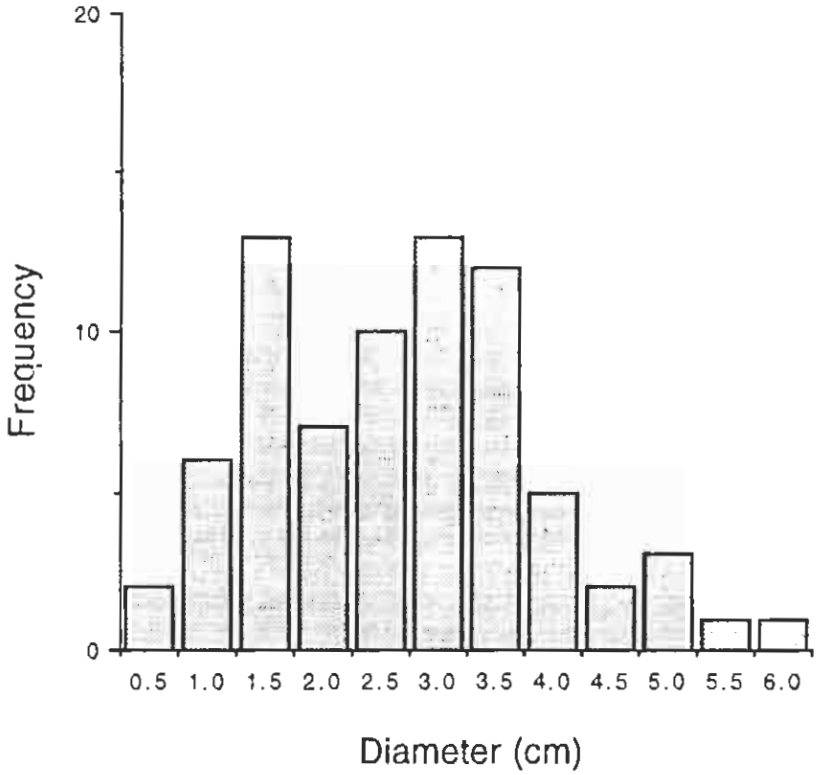

Figure 2. Size frequency distribution of nodules removed from the *Nasutitermes acajutlae* nest collected on the island of Tortola, BVI in October 1994 (N = 75; x = 2.6 ± 1.2 cm).

Discussion

Nasutitermes acajutlae and *N. nigriceps* are exceptional among termites in building distinctive inclusions or nodules within the normal carton matrix of their nests. The only other termite reported to build similar structures is the Javan termite *Microcerotermes depokensis* (Kemner 1929).

Two contrasts between the composition of nodules versus normal nest carton analyzed in this study may be biologically significant. First, the nodule samples have lower cutin and higher cellulose percentages than do samples of the surrounding, dark carton matrix. Cutin degradation is not possible for most organisms except some specialized fungi; digestion of cutin by termites is unknown (Breznak, pers. comm.). The differences in cutin and cellulose percentage may indicate that the termites are constructing the nodules from materials with greater digestibility. The relatively high cutin percentages in typical carton probably enhances water-proofing and construction strength. It is unlikely that the difference in cutin abundance is due to transfer of waxes from the insect exocuticle to the nest walls. The percentage of cutin in fresh, newly constructed carton (having minimal opportunity for contact transfer of waxes from passing insects) does not differ markedly from that of old, dense, interior carton

(see samples from North Bay and White Bay Beach nests, Guana Island, BVI in Table 1).

A second distinction is that both the nodule sample and the normal gallery within the nodule sample from Panama have higher in vitro digestibility than do any of the BVI samples. This may reflect species differences in carton processing, or a difference in diet among the two populations (*N. acajutlae* sampled from Guana Island were feeding substantially on sea grape, *Coccoloba uvifera*, the diet of the *N. nigriceps* from Panama is unknown). Clearly, further sampling and geographic variation within each species must be examined before differences of this type can be further evaluated.

Hubbard (1877) and Andrews (1911) hypothesized that these *Nasutitermes* nodules serve as food storage. Kemner (1929) came to a similar conclusion in the case of *Microcerotermes depokensis*. The food storage hypothesis is supported by the higher cellulose content of nodules in comparison to surrounding nest carton in both *N. acajutlae* and *N. nigriceps*. It is difficult to know the conditions under which the nodules might be naturally consumed in a nest, but 0.3 g portions of both *N. acajutlae* and *N. nigriceps* nodules offered to 100 workers of *N. acajutlae*, *N. nigriceps*, *N. costalis*, and *Zootermopsis nevadensis* (Hagen) were consumed in the laboratory within 24 h, and consumed by 100 *Reticulitermes flavipes* (Kollar) workers within 48 hr (N=3 per species). These species did not consume the normal carton of either *N. acajutlae* or *N. nigriceps* nests.

Termite nest material can be used as nutritional food reserves in some species. Hegh (1922) commented that termites in mature colonies of *Microcerotermes fuscotibialis* (Sjostedt) eat the internal walls of their nests during times of food stress. Noirot (1970) reported that central walls of *Cephalotermes rectangularis* (Sjostedt) nests can be used to culture the termites in the laboratory.

The function of nodules and circumstances under which they are constructed are difficult to identify because they are found so rarely. In both Panama and the BVI, examination of nests of approximately the same size, in the same local area, at the same season never revealed another live colony with nodules. Because young were found within the nodules of both *N. acajutlae* (white immatures instars 1-3) and *N. nigriceps* (developing alate nymphs) the nodule food reserves may be sequestered for juveniles. Comparable nests with immatures, however, did not have nodules. Nodule construction may be influenced by individual colony health, age, microhabitat, food resources, caste proportions, or population size. Even among colonies producing nodules, they may be ephemeral within a nest. Nodules may only be present seasonally, stockpiled as food reserves and then consumed during times of high demand (as when alate brood is present), when food is scarce, or when travel from the nest is physiologically expensive (as in a drought). It is notable that the only two *Nasutitermes* species known to construct these nest inclusions are the closely related species *N. acajutlae* and *N. nigriceps*, both of which can occupy dry and thus potentially stressful environments (Thorne *et al.* 1994). The facultative ability to store food in nodules, combined with an exceptional desiccation tolerance of individuals, may contribute to the survival of these two *Nasutitermes* species in arid or otherwise marginal habitats not colonized by other members of the genus.

ACKNOWLEDGMENTS

We extend sincere thanks to Dr. and Mrs. Henry Jarecki and the staff of The Guana Island Club for their support and hospitality during the course of this project. John A. Breznak, Michael I. Haverty, and James D. Lazell provided constructive input and productive discussions. Field assistance was contributed by James Egelhoff,

Thomas Jarecki, and Ralph Rusher. Portions of this research were funded by The Conservation Agency through a grant from the Falconwood Foundation, The Smithsonian Tropical Research Institute, NSF grant DEB-8607407 to BLT, and by a cooperative agreement between the University of Maryland and the Pacific Southwest Research Station, U.S. Department of Agriculture.

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Journal of Chemical Ecology, Vol. 23, No. 4, 1997

CUTICULAR HYDROCARBONS OF TERMITES OF THE BRITISH VIRGIN ISLANDS (ISOPTERA: KALOTERMITIDAE, RHINOTERMITIDAE, TERMITIDAE)

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(Received June 3, 1996; accepted November 22, 1996)

Abstract—A survey of the termites (Isoptera) of 17 islands of the British Virgin Island (BVI) complex yielded eight taxa belonging to three families. The Kalotermitidae include *Neotermes mona* (Banks), *Cryptotermes brevis* (Walker), *Procryptotermes corniceps* (Snyder), and an undetermined species of *Incisitermes*, likely *Incisitermes* nr *snyderi* (Light) or *I. incisus* (Silvestri). The only rhinotermitid collected is an undetermined species of *Heterotermes* (Froggatt). *Parvitermes wolcotti* (Snyder), *Nasutitermes costalis* (Holmgren), and *N. acajutlae* (Holmgren) comprise the Termitidae. Cuticular hydrocarbon mixtures were characterized for each of the taxa. Blends of abundant hydrocarbons are species-specific and can be used to identify a given taxon without the diagnostic castes, soldiers, or imagoes, although the species of *Incisitermes* were not separable on the basis of cuticular hydrocarbons.

Key Words—Cuticular hydrocarbons, chemotaxonomy, Isoptera, tropical termites, gas chromatography, mass spectrometry, Virgin Island, Caribbean termites, olefins, methylalkanes.

INTRODUCTION

The termite fauna of the West Indies was summarized first by Banks (1919), who described termites collected from the larger islands, except Puerto Rico

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0098-0331/97/0400-0927$12.50/0

and other islands of the Puerto Rico Bank. Individual collections in the West Indies placed in the US National Museum were included in Snyder's compilation (Snyder, 1956). Scheffrahn et al. (1994) summarized the literature and unpublished records of the termites of the West Indies. From this survey it is clear that, until recently, little effort has been devoted to collecting the fauna of the Virgin Island complex, especially the British Virgin Islands (BVI).

The BVI are a complex of more than 50 land masses that are part of the Puerto Rico Bank. The BVI were apparently not intensively collected until M. S. Collins began systematic work in 1986 (Collins et al., 1997). In this paper we expand this work to include documentation of the cuticular hydrocarbon mixture of all termite taxa collected from the BVI. Characterization of the cuticular hydrocarbons of each taxon supports the species specificity of hydrocarbon mixtures for this region.

METHODS AND MATERIALS

Collection of Termites. Collecting periods of two to four weeks each were spent on Guana Island from 1986 to 1994, most often during the month of October. During those stays, short trips were made to other islands of the BVI complex. We attempted to sample termite colonies from every habitat that could be reached. Termite samples were bagged and brought to the laboratory on Guana Island where the termites were separated from soil, nest, and wood debris.

Samples of workers, soldiers, larvae, pseudergates, nymphs, or alates were placed in separate dishes or vials and dried. The number of termites in a sample varied by species; 15–20 nymphs or pseudergates of kalotermitids or up to 200 workers of the nasutes were dried. These samples were placed over a single incandescent light. Initially samples were dried in whatever vessel was available over whatever lamp was available in the guest cottages on Guana Island. From 1991 to 1994, we dried termite samples in 20-ml scintillation vials over a single 75-W, reflecting incandescent light. Vials were moved periodically in an attempt to make drying uniform (Haverty et al., 1996).

The amount of time required to completely dry termites varied slightly as a function of the number and size of the termites in the sample and the position of the vials over the bulb. With some of the kalotermitid species, drying was accelerated by decapitating termites. Internal hydrocarbons do not appear to affect characterization of cuticular hydrocarbons (Haverty et al., 1996). Once the termites were completely dry, specimens were placed in a vial that was tightly capped. Dried samples were returned to our laboratory in California for extraction and characterization of cuticular hydrocarbons. Concurrently, fresh (i.e., not dried) voucher samples from each collection were preserved in 85%

ethanol and deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Species diagnoses were made primarily by M. S. Collins using keys, original references and descriptions, and by comparison with type and previously identified material. Much work needs to be done to develop usable keys for the Caribbean fauna, and new descriptions are needed for some species.

Extraction Procedure and Characterization of Cuticular Hydrocarbons. In this study cuticular lipids were extracted by immersing termites, as a group, in 10 ml of *n*-hexane for 10 min. After extraction, hydrocarbons were separated from other components by pipetting the extract through 4 cm of activated BioSil-A in Pasteur pipet mini-columns. An additional 5 ml of clean hexane was dripped through the BioSil-A. The resulting hydrocarbon extracts were evaporated to dryness under a stream of nitrogen and redissolved in 60 μl of *n*-hexane for gas chromatography—mass spectrometry (GC-MS) analyses. A 3-μl aliquot was injected into the GC-MS.

GS-MS analyses were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a HP 5970B Mass Selective Detector interfaced with a computer and HP Chemstation data analysis software (HP59974J Rev. 3.1.2). The GC-MS was equipped with an HP-1, fused silica capillary column (25 m × 0.2 mm ID) and operated in split mode (with a split ratio of 8 : 1). Each mixture was analyzed by a temperature program from 200°C to 320°C at 3°C/min with a final hold of 11 or 16 min. Electron impact (EI) mass spectra were obtained at 70 eV.

n-Alkanes were identified by their mass spectra. Mass spectra of methylalkanes were interpreted as described by Blomquist et al. (1987) to identify methyl branch locations. Mass spectra of di- and trimethylalkanes were interpreted as described in Page et al. (1990) and Pomonis et al. (1980). Alkenes were identified by their mass spectra and/or retention times relative to *n*-alkanes. A typical alkene mass spectrum shows a molecular ion and a series of fragments at 14-mass-unit intervals (69, 83, 97), similar to those displayed by *n*-alkanes, less 2 mass units. Interpretation of the mass spectra of dienes and polyunsaturated hydrocarbons was extrapolated from this pattern, i.e., for each double bond, the molecular ion is decreased by 2 mass units.

Integration of the total ion chromatogram was performed using the HP Chemstation data analysis software. GC-MS peak areas were converted to percentage of the total hydrocarbon fraction. Summary statistics for percentages of each hydrocarbon for each taxon or geographic location of a taxon were computed using SAS (1990) to make comparisons.

In the text and tables, we use shorthand nomenclature to identify individual hydrocarbons or mixtures of hydrocarbons. This shorthand uses a descriptor for the location of methyl groups (X-Me), the total number of carbons (C_{XX}) in the hydrocarbon component excluding the methyl branch(es), and the number of

double bonds following a colon ($C_{XX:Y}$). Thus, pentacosane becomes *n*-C_{25}; 3-methylpentacosane becomes 3-MeC_{25}; 3,13-dimethylpentacosane becomes 3,13-DimeC_{25}; and pentacosadiene becomes $C_{25:2}$. Hydrocarbons are presented in the tables for each taxon in the order of elution on our GC-MS system.

RESULTS AND DISCUSSION

In their survey of the termites of the West Indies, Scheffrahn et al. (1994) listed a total of nine species in seven genera and three families found in the BVI. We characterized cuticular hydrocarbons for all of these species, although we were unable to differentiate *Incisitermes incisus* (Silvestri) and *I. snyderi* (Light) (or *I.* nr *snydreri*). Most of the specimens used for these analyses were collected on Guana Island, and incidentally from many other islands in the BVI complex (Table 1). Whenever possible, we used collections from locations in addition to Guana Island to include interisland variation. We summarize the relative proportions of each hydrocarbon for eight taxa; hydrocarbons are presented in order of elution within a hydrocarbon class (Table 2). Hydrocarbon mixtures for pseudergates or workers are discussed for each taxon within the three families. Comparison of castes within a taxon or island-to-island variation are presented separately for select taxa.

TABLE 1. SPECIES OF TERMITES COLLECTED FROM VARIOUS LOCATIONS IN BRITISH VIRGIN ISLANDS FOR CHARACTERIZATION OF CUTICULAR HYDROCARBONS

Species	Collection sites
Neotermes inona	Guana
Cryptotermes brevis	Oahu, Hawaii[a]
Procryptotermes corniceps	Guana, Lesser Jost Van Dyke, Great Camino, Great Thatch
Incisitermes species	Guana, Lesser Jost Van Dyke, Greater Jost Van Dyke, Eustatia, Scrub, Anegada
Heterotermes spp.	Guana, Tortola, Great Thatch
Parvitermes wolcotti	Peter
Nasutitermes costalis	Guana, Tortola
Nasutitermes acajutloe	Guana, Great Camino, Scrub, Eustatia, Virgin Gorda, Lesser Jost Van Dyke, Greater Jost Van Dyke, Great Thatch, Cooper, Necker, Tortola

[a] *Cryptotermes brevis* occurs only in structures. We were not able to collect a sample from a building or furniture. We received these from a colleague in Hawaii, where this species is quite common.

TABLE 2. RELATIVE QUANTITIES OF CUTICULAR HYDROCARBONS FROM PSEUDERGATES (LARVAE AND NYMPHS) OR WORKERS OF 8 TERMITE TAXA FROM BRITISH VIRGIN ISLANDS[a]

Hydrocarbon	Termite species[b]							
	N mon	C bre	P cor	I spp'	Het sp	P wol	N cos	N aca
n-Alkanes								
C_{23}	0	tr	0	0/+	0	0	0	+
C_{24}	tr	0	tr	tr/+	0	0	0	tr
C_{25}	+++	+++	+++	+++	0	0	++	+++
C_{26}	+++	++	++	++	tr	0	0	tr
C_{27}	+++	+++	+++	+++	+++	+++	++	++
C_{28}	tr	+	tr	0/+	++	++	+	tr
C_{29}	+	++	+	+	++	++	++	+
C_{30}	0	0	0	0	tr	0	0	0
C_{31}	0	tr	0	0	tr	0	0	tr
Internally branched methylalkanes								
12-; 11-; 10-MeC_{24}	+	0	0	0	0	0	0	0
13-; 11-MeC_{25}	+++	+	0	+	0	0	0	tr
13-; 12-MeC_{26}	+++	0	0	0	0	0	0	0
13-; 11-; 9-; 7-MeC_{27}	+++	tr	tr	0/tr	+++	0	+	tr
14-; 13-; 12-; 9-; 7-MeC_{28}	+	0	0	0/tr	+++	0	+	0
15-; 13-; 11-; 9-; 7-; 5-MeC_{29}	tr	0	0	tr/+	+++	+	+++	0
15-; 14-; 12-; 11-; 10-; 9-MeC_{30}	0	0	0	0	+	0	+	0
15-; 13-; 11-; 9-MeC_{31}	tr	0	0	0	+	0	+++	0
14-MeC_{32}	0	0	0	0	0	0	+	0
13-MeC_{33}	tr	0	0	0	0	0	+	0
15-; 13-MeC_{35}	tr	0	0	0/tr	0	0	0	0
12-MeC_{36}	tr	0	0	0	0	0	0	0

TABLE 2. Continued

Hydrocarbon	Termite species[b]							
	N mon	C bre	P cor	I spp[c]	Het sp	P wol	N cos	N aca
Internally branched methylalkanes Continued								
17-; 15-; 13-MeC_{37}	tr	tr	0	0/tr	tr	0	0	0
12-MeC_{38}	tr	0	0	0	0	0	0	0
15-; 13-MeC_{39}	+	tr	+	0/+	0	0	0	tr
12-MeC_{40}	+	0	0	0	0	0	0	0
15-; 13-C_{41}	++	tr	+	0	0	0	0	tr
12-MeC_{42}	tr	0	0	0	0	0	0	0
13-MeC_{43}	+	0	0	0	0	0	0	0
Terminally branched methylalkanes								
2-MeC_{23}	0	+	0	0/tr	0	0	0	0
3-MeC_{23}	0	++	0	0	0	0	0	0
2-MeC_{24}	++	+++	+++	0/+++	0	0	0	0
3-MeC_{24}	tr	+	tr	0	0	0	0	0
2-MeC_{25}	+++	++	+++	+++	0	0	0	0
3-MeC_{25}	+++	+++	+++	+++	0	0	0	0
2-MeC_{26}	++	+	+++	+/++	+	0	0	0
3-MeC_{26}	+	tr	tr	0/tr	tr	0	0	0
2-MeC_{27}	0	tr	+	tr/+	++	++	0	tr
3-MeC_{27}	+	+	++	0/+	++	+	tr	0
2-MeC_{28}	0	0	tr	0	+	+++	0	0
2-MeC_{29}	0	tr	0	0	0	++	0	0
3-MeC_{29}	0	0	0	0	0	++	tr	0
Dimethylalkanes								
3,X-$DimeC_{25}$	++	tr	0	0	0	0	0	0
11,15-$DimeC_{27}$	++	0	0	0	+++	0	++	0
9,17-$DimeC_{27}$	0	0	0	0	+++	0	0	0

5,15-DimeC$_{27}$	tr	0	0	0	0	0	0	0
3,13-DimeC$_{27}$	tr	0	0	0	0	0	0	0
11,15-; 12,16-DimeC$_{28}$	tr	0	0	0	0	0	++	0
9,X-DimeC$_{28}$	0	0	0	0	+++	0	0	0
13,17-; 11,15-DimeC$_{29}$	0	0	0	0	0	0	+++	0
9,19-; 9,17-DimeC$_{29}$	0	0	0	0/+	+++	0	0	0
7,21-DimeC$_{29}$	0	0	0	0	+++	0	0	0
5,X-DimeC$_{29}$	tr	0	0	0	tr	++	0	0
3,21-DimeC$_{29}$	0	0	0	0	tr	0	0	0
11,15-; 12,16-; 13,17-DimeC$_{30}$	0	0	0	0	0	0	+++	0
9,X-DimeC$_{30}$	0	0	0	0	++	0	0	0
13,17-DimeC$_{31}$	0	0	0	0	0	0	+++	0
9,21-DimeC$_{31}$	0	0	0	0	++	0	0	0
3,X-DimeC$_{31}$	tr	tr	0	0	0	0	0	0
14,18-DimeC$_{32}$	0	0	0	0	0	0	+	0
11,21-; 13,21-; 15,19-DimeC$_{33}$	0	0	0	0/tr	0	0	+	0
13,X-; 11,23-DimeC$_{35}$	tr	0	0	0/+	0	0	0	0
12,24-DimeC$_{36}$	0	0	0	0/tr	0	0	0	0
17,X-; 15,X-; 13,X-DimeC$_{37}$	+	0	0	0/+	0	0	0	0
12,16-DimeC$_{38}$	tr	0	0	0	0	0	0	0
11,15-DimeC$_{39}$	++	0	0	0	0	0	0	0
12,16-DimeC$_{40}$	tr	0	0	0	0	0	0	0
13,17-DimeC$_{41}$	++	0	0	0	0	0	0	0
12,16-DimeC$_{42}$	tr	0	0	0	0	0	0	0
13,17-DimeC$_{43}$	+	0	0	0	0	0	0	0
Trimethylalkanes								
9,13,17-TrimeC$_{27}$	0	0	0	0	0	0	+	0
11,15,19-TriMeC$_{29}$	0	0	0	0	0	0	+	0
7,13,21-TrimeC$_{29}$	0	0	0	0	tr	0	0	0
5,11,21-TrimeC$_{29}$	0	0	0	0	tr	0	0	0
3,11,21-TrimeC$_{29}$	0	0	0	0	tr	0	0	0
13,17,21-; 11,15,21-TrimeC$_{33}$	0	0	0	0/tr	0	0	0	0
13,17,23-; 11,15,19-TrimeC$_{35}$	0	0	0	0/tr	0	0	0	0
13,17,21-TrimeC$_{37}$	0	0	0	0/tr	0	0	0	0

TABLE 2. Continued

Hydrocarbon	Termite species[b]							
	N mon	C bre	P cor	I spp[c]	Het sp	P wol	N cos	N aca
Olefins								
$C_{23:1}$	0	0	0	0	0	0	0	+
$C_{24:1}$	0	0	0	tr	0	0	0	0
$C_{25:1}$	0	0	+ +	+ + +	0	0	0	+
$C_{25:2}$	0	0	0	+	0	0	0	0
$C_{26:1}$	0	0	0	tr	0	0	0	0
$C_{27:1}$	0	0	0	+ + +	0	0	tr	+
$C_{27:2}$	0	0	0	tr	0	0	0	0
$C_{27:3}$	0	0	0	0/tr	0	0	0	0
$C_{29:1}$	0	tr	0	0/tr	0	0	0	0
$C_{31:2}$	0	0	0	0/+	0	0	0	0
$C_{31:1}$	0	tr	0	0/+	0	0	0	0
$C_{33:2}$	0	tr	0	0/tr	0	0	0	0
$C_{33:1}$	0	tr	0	0/tr	0	0	0	0
$C_{35:2}$	0	+ +	tr	0	0	0	0	0
$C_{35:1}$	0	tr	0	0	0	0	0	0
$C_{36:2}$	0	+	0	0	0	0	0	0
$C_{37:3}$	0	+	+	0	0	0	0	0
$C_{37:2}$	0	+ + +	+ +	0	0	0	0	0
$C_{37:1}$	0	+ +	tr	0	+	0	0	+
$C_{38:3}$	0	0	tr	0	0	0	0	0
$C_{38:2}$	0	+ +	+	0	0	0	0	0
$C_{38:1}$	0	0	0	0	0	0	0	+
$C_{39:5}$	0	0	0	0	0	0	0	+
$C_{39:4}$	0	0	0	0	0	0	0	+ +
$C_{39:3}$	0	+ +	+ +	0/tr	0	0	0	0

$C_{39:2}$	0	+ + +	+ + +	0/+	0	0	0	+
$C_{39:1}$	0	+ +	+ +	0/tr	tr	0	0	+ + +
$C_{40:3}$	0	0	+	0	0	0	0	0
$C_{40:3}$	0	+	0	0	0	0	0	0
$C_{40:2}$	0	+ +	+ +	0	0	0	0	0
$C_{40:1}$	0	0	0	0	0	0	0	+ + +
$C_{41:5}$	0	0	0	0	0	0	0	+ + +
$C_{41:4}$	0	0	0	0	0	0	0	+ + +
$C_{41:3}$	0	+ + +	+ + +	0/+	0	+ + +	0	0
$C_{41:2}$	0	+ + +	+ + +	0/+ +	0	0	0	+ +
$C_{41:1}$	0	+ +	+ + +	0/+	0	0	0	+ + +
$C_{42:3}$	0	+	+ +	0	0	0	0	0
$C_{42:2}$	0	+	+	0/tr	0	0	0	0
$C_{42:1}$	0	0	tr	0	0	0	0	+ +
$C_{43:6}$	0	0	0	0	0	0	0	tr
$C_{43:5}$	0	0	0	0	0	0	0	tr
$C_{43:4}$	0	0	+	0	0	+ + +	0	+ + +
$C_{43:3}$	0	+ +	+ +	0/+ + +	0	+ + +	0	0
$C_{43:2}$	0	+ +	+ +	+ + +	0	0	0	+
$C_{43:1}$	0	+ +	0	0/+ +	0	0	0	+ + +
$C_{44:2}$	0	0	0	0/tr	0	0	0	0
$C_{45:4}$	0	0	0	0/+	0	+ + +	0	0
$C_{45:3}$	0	+ +	0	0/+ +	0	+ + +	0	0
$C_{45:2}$	0	+ +	0	0/+ +	0	0	0	0
$C_{45:1}$	0	0	0	0/+ + +	0	0	0	+ +

[a] Relative proportions of the total hydrocarbon mixture for each species. + + + = >3.0%; + + = 1.0–3.0%; + = 0.3–0.99%; and tr = <0.3%; 0 = not detected.

[b] N mon = *Neotermes mona*; C bre = *Cryptotermes brevis*; P cor = *Procryptotermes corniceps*; I spp = *Incisitermes* species; H sp = *Heterotermes* species; P wol = *Parvitermes wolcotti*; N cos = *Nasutitermes costalis*; N aca = *Nasutitermes acajutlae*.

[c] *Incisitermes* spp. displayed a wide range of hydrocarbon mixtures. For example, 0/+ + + would denote the range from absent to above 3%.

Kalotermitidae

We characterized the cuticular hydrocarbons of Kalotermitidae identified as *Neotermes mona* (Banks), *Cryptotermes brevis* (Walker), *Procryptotermes corniceps* (Snyder), and *Incisitermes* spp. Species of the family Kalotermitidae, although commonly known as drywood termites, differ widely in their moisture requirements. The fauna of the BVI includes species at both ends of the moisture-dependence spectrum. In the BVI we feel that *N. mona* is dependent upon a high constant environmental moisture supply, usually obtained by inhabiting living trees, whereas *C. brevis*, the "furniture termite," is capable of living without access to free water and is unable to thrive when exposed to sustained presence of free water (Collins, 1969; Williams, 1977).

Neotermes mona (Banks). This is the largest termite of the area. We found it on the relatively moist, north slope of Guana Island. The bulk of the colony developed excavations in living, as well as dead, wood. This species was once thought to be endemic to Mona Island, but its range was recently extended west through the Dominican Republic to the Turks and Caicos archipelago (Scheffrahn et al., 1990; Jones et al., 1995).

The cuticular hydrocarbon mixture of *N. mona* reflected a general pattern seen in most of the termite species examined thus far in the West Indies. Cuticular hydrocarbons occurred in two distinct groups: early-eluting compounds (24–29 carbons in the parent chain) and late-eluting compounds (37–43 carbons in the parent chain) (Table 3; Figure 1). In *N.* mona the early-eluting compounds predominate, representing over 90% of the total hydrocarbon in nymphs and pseudergates (Figure 1). The hydrocarbon mixtures of pseudergates and nymphs were very similar to the one alate sample (Table 3).

n-Alkanes present were *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{27}, *n*-C_{28}, and *n*-C_{29}. The most abundant were *n*-C_{25} and *n*-C_{27}, comprising about 13% and 12% of the total hydrocarbons, respectively. Slightly lower amounts were seen in the alate sample. The other *n*-alkanes accounted for about 5% of the total hydrocarbons.

We identified isomeric mixtures of internally branched monomethylalkanes with parent chains ranging from C_{24} to C_{43}, except for C_{30}, C_{32}, and C_{34}. Positions of methyl branches ranged from C-10 to C-15. Internally branched monomethylalkanes were the most abundant class of hydrocarbons produced by *N. mona*, representing about 42% of the total hydrocarbon. One isomeric mixture, 13-; 11-MeC_{25}, accounted for 22–26% of the total hydrocarbon (Table 3; Figure 1).

2- and 3-Methylalkanes were identified for C_{24} to C_{29}. These terminally branched monomethylalkanes comprised approximately 17–20% of the total hydrocarbon. Internally branched dimethylalkanes constituted < 7% of the total cuticular hydrocarbon fraction of *N. mona*. There was only one type of internally branched dimethylalkane, those with three methylene groups separating the

TABLE 3. RELATIVE QUANTITIES (MEAN, STANDARD DEVIATION, AND RANGE) OF CUTICULAR HYDROCARBONS OF PORTIONS OF COLONIES OF *Neotermes mona* (BANKS) FROM BRITISH VIRGIN ISLANDS[a]

Hydrocarbon	Larvae, nymphs, and pseudergates		Alates (mean)
	Mean ± SD	Range	
n-C_{24}	0.20 ± 0.17	0–0.30	0.24
12-; 11-; 10-MeC_{24}[b]	0.92 ± 0.31	0.68–1.27	1.18
2-MeC_{24}	1.74 ± 0.92	1.19–2.80	1.74
3-MeC_{24}	0.06 ± 0.10	0–0.17	0
n-C_{25}	13.00 ± 4.14	8.72–16.98	11.45
13-; 11-MeC_{25}[b]	21.39 ± 5.32	18.06–27.52	25.85
2-MeC_{25}	7.25 ± 0.66	6.79–8.01	7.58
3-MeC_{25}	4.78 ± 1.92	3.34–6.96	4.89
n-C_{26}	4.04 ± 1.41	2.41–4.97	3.00
3,13-$DimeC_{25}$	1.19 ± 0.35	0.82–1.51	1.38
13-; 12-MeC_{26}[b]	4.33 ± 1.06	3.11–4.95	5.17
2-MeC_{26}	2.34 ± 0.42	1.90–2.73	1.71
$C_{27:1}$ + 3-MeC_{26}[c]	0.47 ± 0.46	0–0.91	0.97
n-C_{27}	11.94 ± 6.20	7.65–19.06	9.55
13-; 11-MeC_{27}[b]	9.48 ± 2.95	7.72–12.89	11.51
11,15-$DimeC_{27}$; 2-MeC_{27}[d]	4.60 ± 1.31	3.40–6.00	4.79
3-MeC_{27}	0.35 ± 0.34	0–0.69	0.71
5,15-$DimeC_{27}$	0.06 ± 0.11	0–0.19	0
n-C_{28}	0.09 ± 0.15	0–0.27	0
3,13-$DimeC_{27}$	0.07 ± 0.12	0–0.21	0
12-MeC_{28}	0.56 ± 0.56	0–1.13	0.81
12,16-; 11,15-$DimeC_{28}$[e]	0.11 ± 0.19	0–0.33	0
n-C_{29}	0.66 ± 0.65	0–1.31	0.84
13-; 11-MeC_{29}[b]	0.25 ± 0.43	0–0.75	0.44
13,17-$DimeC_{29}$	0.10 ± 0.17	0–0.30	0
3-MeC_{29}	0.07 ± 0.12	0–0.21	0
5,17-$DimeC_{29}$	0.04 ± 0.08	0–0.13	0
15-; 13-MeC_{31}[b]	0.05 ± 0.08	0–0.14	0
3,7-$DimeC_{31}$	0.03 ± 0.06	0–0.10	0
13-MeC_{33}	0.06 ± 0.11	0–0.18	0
13-MeC_{35}	0.07 ± 0.13	0–0.22	0
13,17-$DimeC_{33}$	0.07 ± 0.13	0–0.22	0
12-MeC_{36}	0.06 ± 0.11	0–0.19	0
13-MeC_{37}	0.81 ± 0.40	0.53–1.26	0.57
13,17-; 11,15-$DimeC_{37}$[e]	0.38 ± 0.66	0–1.15	0
12-MeC_{38}	0.28 ± 0.30	0–0.59	0.20
12,16-$DimeC_{38}$	0.12 ± 0.20	0–0.35	0
13-MeC_{39}	0.94 ± 0.30	0.59–1.12	0.53
11,15-$DimeC_{39}$	1.51 ± 0.41	1.26–1.98	0.76

TABLE 3. Continued

Hydrocarbon	Larvae, nymphs, and pseudergates		Alates (mean)
	Mean ± SD	Range	
12-MeC$_{40}$	0.53 ± 0.06	0.46–0.57	0.30
12,16-DimeC$_{40}$	0.22 ± 0.39	0–0.67	0.20
13-MeC$_{41}$	1.25 ± 0.62	0.59–1.81	0.77
13,17-DimeC$_{41}$	1.60 ± 0.80	0.84–2.44	1.10
12-MeC$_{42}$	0.07 ± 0.12	0–0.21	0
12,16-DimeC$_{42}$	0.11 ± 0.19	0–0.33	0
13-MeC$_{43}$	0.77 ± 0.21	0.53–0.89	0.72
13,17-DimeC$_{43}$	0.98 ± 1.41	0–2.23	1.04

[a] The data from this table are derived from separate samples taken in 1991 and 1992 from the same colony on Guana Island. Samples include combinations of large larvae, nymphs, and/or pseudergates. The single alate sample was extracted in hexane before drying. No soldiers are included in the hydrocarbon samples.
[b] An isomeric mixture. These monomethylalkanes coelute.
[c] This alkene and monomethylalkane coelute.
[d] This monomethylalkane and two isomers of this dimethylalkane coelute.
[e] An isomeric mixture. Two or more isomers of these dimethylalkanes coelute. Distinct, separate isomers can be distinguished from mass spectra.

methyl branches. Terminally branched dimethylalkanes were not abundant (< 1.4% of the total hydrocarbon) and were trivial except for 3,13-DimeC$_{25}$. No trimethylalkanes were found. Only one alkene, C$_{27:1}$, was found in *N. mona*.

Cryptotermes brevis (Walker). The phragmatic heads of the soldiers, the presence of piles of dry fecal pellets in infested buildings, and the paper-thin outer surface of furniture or wood containing large colonies of *C. brevis* are characteristic of this termite. This species has never been reported in the BVI from habitats other than structural timber, furniture, and objects of art not exposed to moisture. This habitat limited our ability to collect samples in the BVI. Because *C. brevis* is very common in Hawaii, we were able to obtain a sample from Oahu, Hawaii, so that we could present the cuticular hydrocarbon mixture of this now circumtropical species.

C. brevis clearly reflects the general pattern of hydrocarbon mixtures of drywood termites of the West Indies. In this species hydrocarbons occurred in two groups: the early-eluting compounds consisted almost exclusively of *n*-alkanes and terminally branched monomethyl alkanes, and late-eluting compounds were primarily olefins (Table 2; Figure 2).

n-Alkanes present were *n*-C$_{23}$, *n*-C$_{25}$, *n*-C$_{26}$, *n*-C$_{27}$, *n*-C$_{28}$, *n*-C$_{29}$, and *n*-C$_{30}$. As in *N. mona*, *n*-C$_{25}$ and *n*-C$_{27}$ were the most abundant, comprising

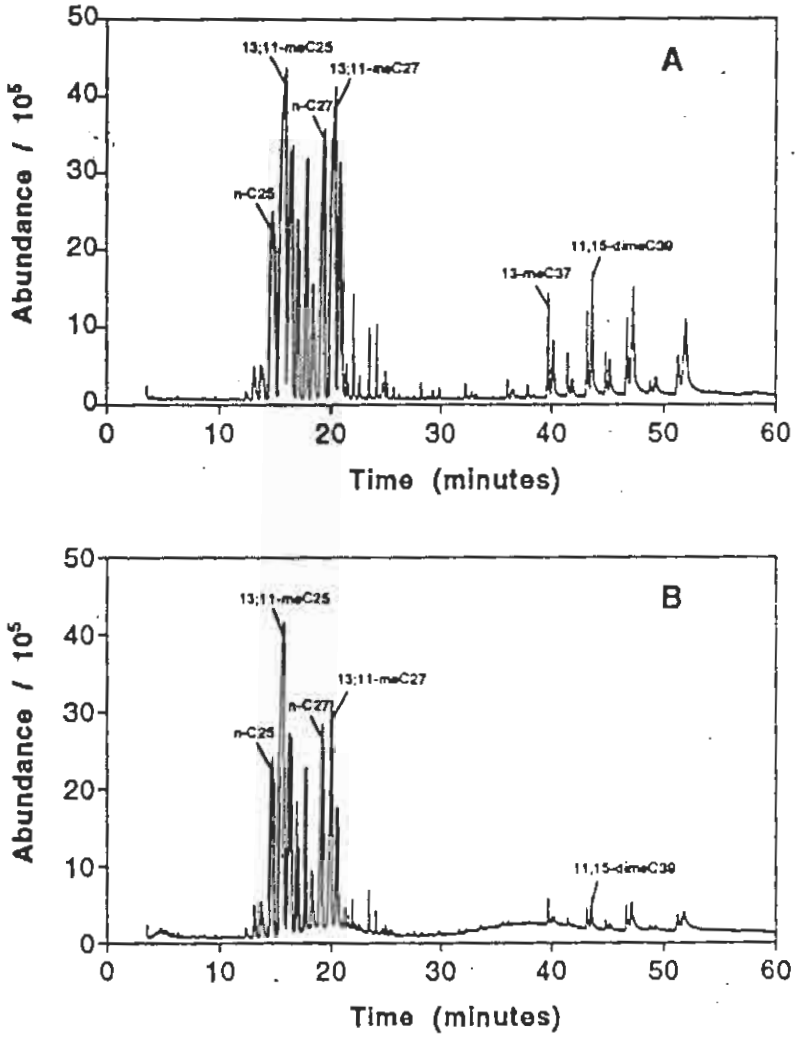

FIG. 1. Total ion chromatogram of the cuticular hydrocarbons from *Neotermes mona* from Guana Island. A = pseudergates, nymphs, and larvae; B = alates.

about 13% and 12%, respectively, of the total hydrocarbons from the sample of larvae, nymphs and pseudergates. All of the other *n*-alkanes combined represented no more than 5.5% of the total hydrocarbons.

Only isomeric mixtures of internally branched monomethylalkanes with parent carbon chains of C_{25}, C_{27}, C_{37}, C_{39}, and C_{41} were found. The early eluting components (C_{25} and C_{27}) of this class of hydrocarbons represented

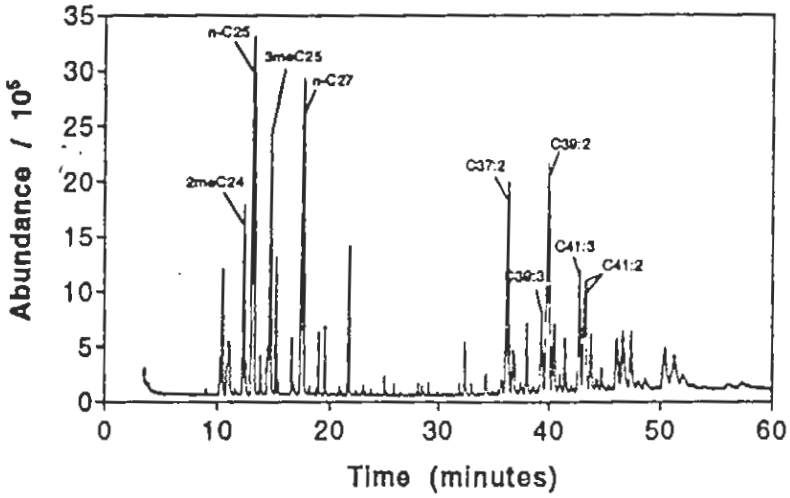

FIG. 2. Total ion chromatogram of the cuticular hydrocarbons from nymphs, pseudergates, and large larvae of *Cryptotermes brevis* from Honolulu, Hawaii.

<1.0% of the total hydrocarbon. The later eluting components (C_{37}, C_{39}, and C_{41}) coeluted with a diene and could not be separately quantified.

2- and 3-Methylalkanes were identified from C_{23} to C_{29}. These terminally branched monomethylalkanes comprised about 17% of the total hydrocarbon. In *C. brevis* the 2- and 3-methylalkanes almost always occurred in pairs. When the parent chain of these hydrocarbons contained an even number of carbons, the 2-methylalkane predominated; when the parent chain contained an odd number of carbons, the 3-methylalkane was more abundant (Figure 2).

No internally branched dimethylalkanes were found in our sample. Terminally branched dimethylalkanes were not very abundant (<0.3% of the total hydrocarbon) and occurred only at C-25 and C-31. All were found to have the first methyl branch on carbon 3. No trimethylalkanes were identified.

Alkenes, alkadienes, and alkatrienes were the predominant class of cuticular hydrocarbons, representing approximately 51% of the total hydrocarbons. The number of carbons ranged from 31 to 45 (Table 2).

Procryptotermes corniceps (Snyder). *P. corniceps* is moderately abundant in the BVI (Collins et al., 1997). Darlington (1992) and Krishna (1962) reported an extention of the known range of *P. corniceps* to Antigua, Guadeloupe, Jamaica, Montserrat, and Puerto Rico; Scheffrahn et al. (1990) added Turks and Caicos Islands. Soldiers of *P. corniceps* are distinctive with proportionally long, strongly curved, sharply pointed mandibles. The heads of the soldiers slope steeply in front and have short hornlike projections from the frons near the outer edge of the bases of the mandibles (Krishna, 1962).

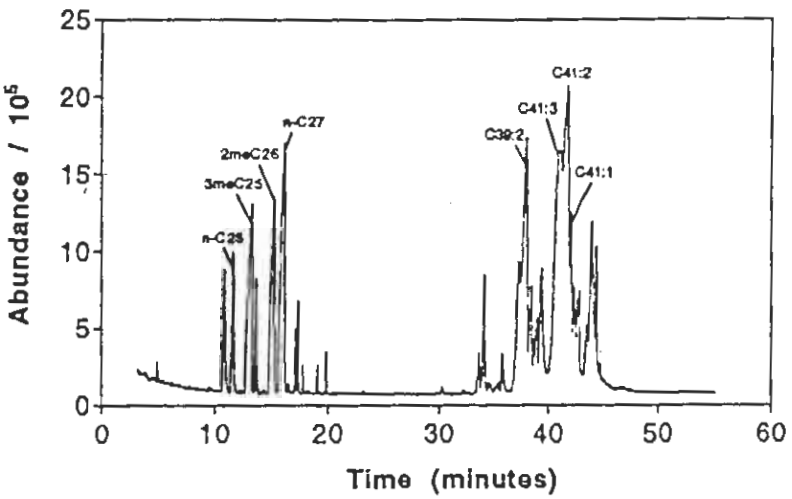

FIG. 3. Total ion chromatogram of the cuticular hydrocarbons from 20 pseudergates of *Procryptotermes corniceps* from Great Camino.

The cuticular hydrocarbon mixture of *P. corniceps* was very similar in gross comparison to that of *C. brevis*. The early-eluting components were almost exclusively *n*-alkanes and terminally branched monomethylalkanes, and the late-eluting compounds were primarily olefins (Table 2; Figure 3).

n-Alkanes present were *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{27}, *n*-C_{28}, and *n*-C_{29}. The most abundant hydrocarbons were *n*-C_{25} and *n*-C_{27}, comprising about 6.3% and 11.7% of the total hydrocarbon, respectively. All of the other *n*-alkanes represented <2.5% of the total hydrocarbons.

Internally branched monomethylalkanes were not common in *P. corniceps*. 13-MeC_{27} occurred in trivial amounts. The isomeric mixture of 15-; 13-MeC_{39} coeluted with $C_{40:2}$. In most samples 15-; 13-MeC_{41} made up an average of about 0.9% of the total hydrocarbons. 2- and 3-Methylalkanes were identified from C_{24} to C_{28}. These terminally branched monomethylalkanes comprise about 23% of the total hydrocarbon. As in *C. brevis* the 2- and 3-methylalkanes almost always occurred in pairs. When the parent chain of these hydrocarbons contained an even number of carbons, the 2-methylalkane predominated; when the parent chain contained an odd number of carbons, the 3-methylalkane was more abundant (Figure 3). Di- and trimethylalkanes were not detected.

As with *C. brevis*, the alkenes, alkadienes, and alkatrienes were the predominant class of cuticular hydrocarbons, comprising over 55% of the total hydrocarbons. The number of carbons ranged from 25 to 43. One sample contained a significant amount of pentacosadiene (>15% of the total hydrocarbons); this compound was not seen in any of the other samples.

Incisitermes spp. Members of the genus *Incisitermes* are the most common kalotermitds found in the BVI. Collins et al. (1997) and Scheffrahn et al. (1994) list *I. snyderi* (or *I.* nr *snyderi*) from all of the islands in the BVI sampled by M. S. Collins, except for Eustatia, and *I. incisus* from Beef Island, Eustatia, Guana Island, and Virgin Gorda. Because of the morphological variation and the uncertainty of the taxonomy of *Incisitermes* in the BVI, we were unable to unambiguously assign specimens used for hydrocarbon characterization to a specific taxon within *Incisitermes* (Collins et al., 1997).

Specimens identified as *I.* nr *snyderi* were the most common found in the BVI. They live in sound, dead trees of many species, as well as in structural timber throughout much of its range (Light, 1993; Harris, 1961). Specimens identified as *I. incisus* were collected in the mangrove swamps fringing Beef Island and in trees on the wetter side of Guana Island. No records of attack on buildings were available for *I. incisus.*

After comparing the cuticular hydrocarbons of over 20 colonies of *Incisitermes* from the BVI including samples from Eustatia identified as *I. incisus*, we concluded that the mixtures of the cuticular hydrocarbons of these samples were as variable as the morphology of the termites. The general "patterns" of these chromatograms varied from one similar to *N. mona*, where >95% of the hydrocarbons have 23–29 carbons (Figure 4), to one similar to the kalotermitids found in drier conditions, where a significant proportion (>30%) of the hydrocarbons were olefins with 33 or more carbons (Figure 5). We also found *Incisitermes* specimens with hydrocarbon mixtures that were intermediate to these extremes (Figure 6). Because of this broad variation, we report the extremes and discuss the classes of hydrocarbons in general terms, describing the range that we observed (Table 2).

n-Alkanes present were *n*-C_{23}, *n*-C_{24}, *n*-C_{25}, *n*-C_{26}, *n*-C_{27}, *n*-C_{28}, and *n*-C_{29}. The most abundant ones were *n*-C_{25} and *n*-C_{27} in all *Incisitermes* samples. Internally branched monomethylalkanes were rare and were found for C_{25}, C_{27}, C_{28}, C_{29}, C_{35}, C_{37}, and C_{39}; they were not detectable in most of the samples. Methyl branches were found on carbons, 7, 9, 11, 13, 15, and 17, occasionally in isomeric mixtures. The 2- and 3-methylalkanes were identified for C_{24}–C_{27}, with 2-MeC_{25} being the most abundant. Of the 3-methylalkanes, 3-MeC_{25} was the most abundant. These terminally branched monomethylalkanes comprised from about 16–32% of the total hydrocarbon.

Di- and trimethylalkanes were rare in *Incisitermes* from the BVI. Most of the dimethylalkanes were late-eluting (carbon numbers in the parent chain from 33 to 37). The dimethylalkanes were all internally branched with the first methyl branch occurring on 9, 11, or 13 carbon. The position of the first methyl branch tended to be more internal as the parent chain length increases. The second methyl group was usually separated from the first by nine methylene units.

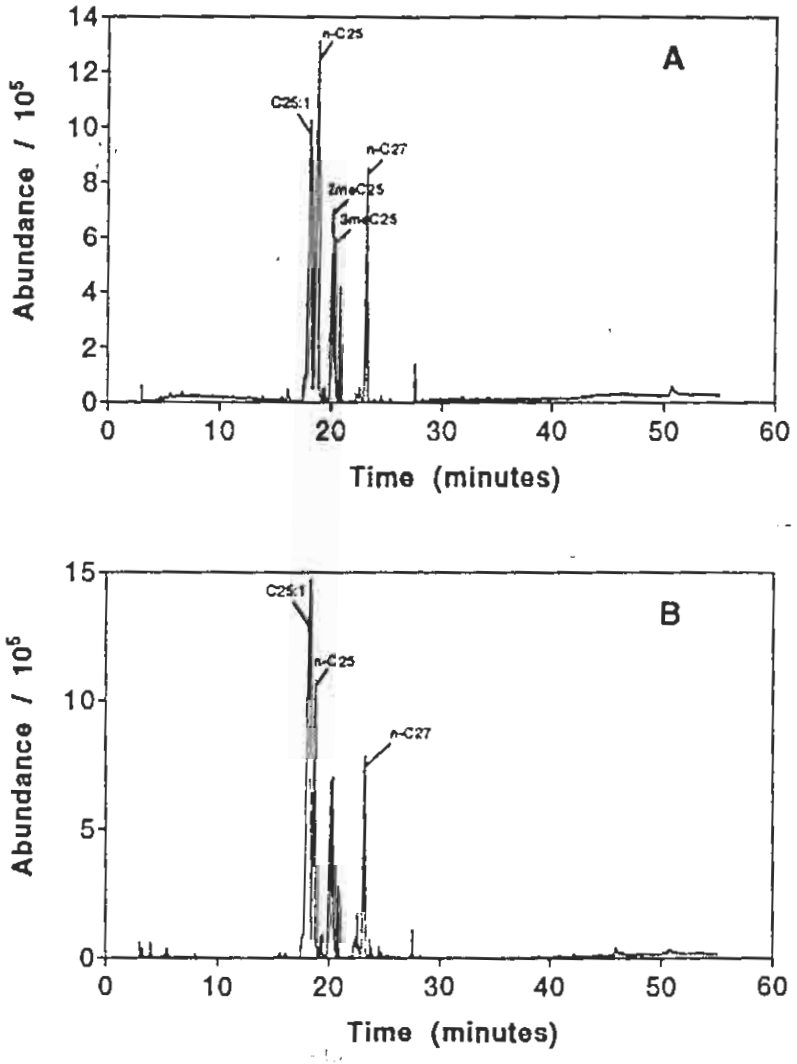

FIG. 4. Total ion chromatogram of cuticular hydrocarbons from pseudergates from two separate colonies of *Incisitermes* from Guana Island.

Trimethylalkanes were found at C_{33}, C_{35}, and C_{37} in very few samples and were trivial in abundance when present.

The unsaturated components constituted about 30–60% of the total hydrocarbon. $C_{25:1}$ was the predominant olefin comprising as much as 25% of the

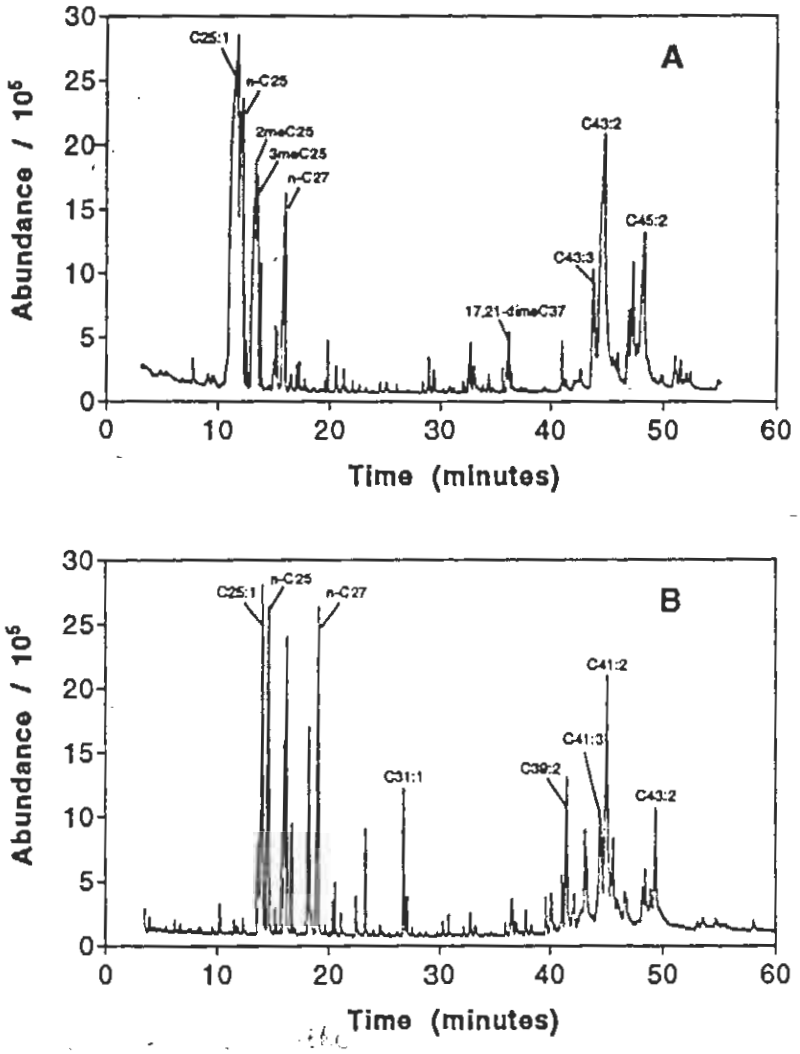

FIG. 5. Total ion chromatogram of cuticular hydrocarbons from pseudergates of *Incisitermes* from Scrub Island (A) and Anegada (B).

total (Figures 4–6). Late-eluting olefins were either totally absent (Figure 4) or constituted 25–35% of the total hydrocarbons (Figures 5 and 6).

The results of our studies of *Incisitermes* from the BVI were equivocal. Cuticular hydrocarbons have proven useful in discriminating species of many groups of termites (Haverty et al., 1988, 1990, 1991, 1992; Howard et al., 1978, 1982, 1988; Thorne and Haverty, 1989; Thorne et al., 1993), but they

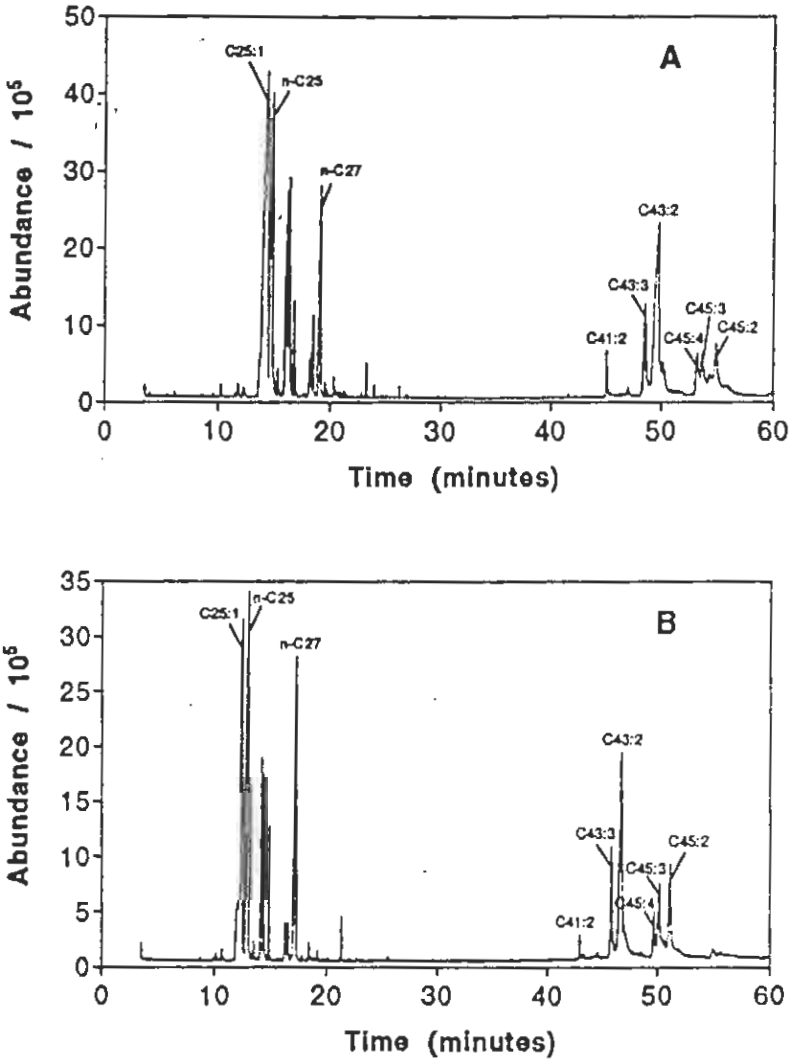

FIG. 6. Total ion chromatogram of cuticular hydrocarbons from pseudergates and/or nymphs of *Incisitermes* from Guana Island (A) and Tortola (B).

were not diagnostic characters for sorting taxa in *Incisitermes* from the BVI. We saw no distinct differences between samples identified as *I. incisis* and many of those identified as *I.* nr *snyderi*. Further collections from the US Virgin Islands, the Greater Antilles, and mainland North America will be necessary to resolve the taxonomy of this refractory genus.

Rhinotermitidae

Thus far, only one taxon from this family has been collected in the British Virgin Islands.

Heterotermes (sp.) Samples of this genus are so morphologically variable that Snyder questioned whether the genus comprises a single, highly variable species or a complex of closely related species (Scheffrahn et al., 1994). There are three described species recorded from this region: *H. tenuis* (Hagen), *H. convexionotatus* (Snyder), and *H. cardini* (Snyder). Until the taxa are resolved, members of this genus collected in the BVI will be referred to as *Heterotermes* sp.

The cuticular hydrocarbon mixture of *Heterotermes* sp. from the BVI included mostly compounds with 26–31 carbons in the parent chain (Table 2; Figure 7). Late-eluting compounds accounted for only about 1% of the total hydrocarbons. Mono- and dimethylalkanes with 27 or 29 carbons in the parent chain accounted for >76% of the total. *Heterotermes* sp. from the BVI had a mixture of hydrocarbons very similar to that of *H. aureus* (Snyder) from the Sonoran Desert near Tucson, Arizona (Haverty and Nelson, unpublished observations).

n-Alkanes present were *n*-C_{26}, *n*-C_{27}, *n*-C_{28}, *n*-C_{29}, *n*-C_{30}, and *n*-C_{31}. *n*-C_{27} was the most abundant normal alkane comprising about 4.5% of the total hydrocarbon. The others combined represented <4% of the total. We identified

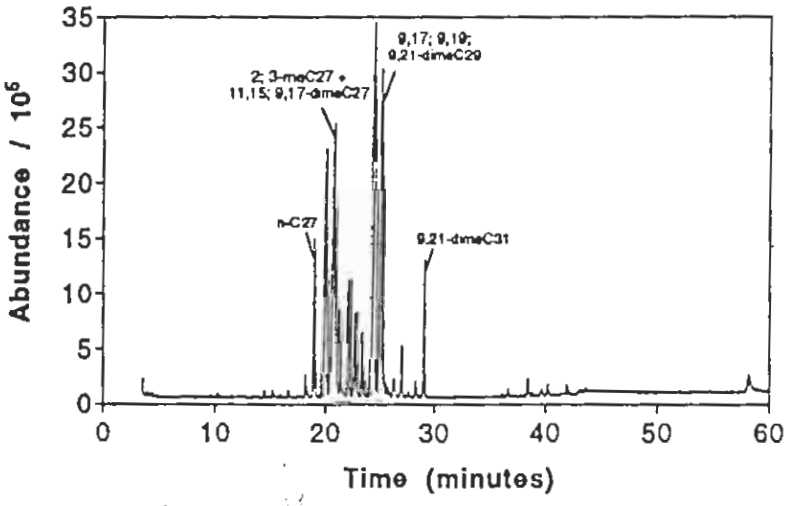

FIG. 7. Total ion chromatogram of cuticular hydrocarbons from workers of *Heterotermes* sp. from Guana Island.

isomeric mixtures of internally branched monomethylalkanes with parent carbon chains ranging from C_{27} to C_{31}, and C_{37}. This class of compounds was very abundant, representing nearly 44% of the total. Two isomeric mixtures, 13-; 11-; 9-; 7-MeC_{27} and 13-; 11-; 9-; 7-MeC_{29}, were predominant and accounted for >37% of the total hydrocarbon. We identified 2- and 3-methylalkanes for C_{26} and C_{27}. Only the 2-methyl isomer occurred at C_{28}. Terminally branched monomethylalkanes comprise only 1.1% of the total hydrocarbon.

Internally branched dimethylalkanes predominated and accounted for over 45% of the total hydrocarbon fraction. The most abundant dimethylalkanes occurred in isomeric mixtures with 27 or 29 carbons in the parent chain (Figure 7). Only one terminally branched dimethylalkane (3,21-DimeC_{29}) was identified, and it coeluted with 7,13,21-TrimeC_{29} in trace amounts. Various isomers of trimethylnonacosane were detected, but in trivial amounts. Only two olefins were present, $C_{37:1}$ and $C_{39:1}$, constituting <1% of the total hydrocarbon.

Termitidae

Three species of termitids, belonging to two genera, were collected on Guana Island and nearby islands of the BVI complex.

Parvitermes wolcotti (Snyder). *P. wolcotti* is a small nasute that forages in dead wood or on the ground in areas with fairly dense tree cover on Guana Island, BVI (Collins et al., 1977). We were not able to collect large samples until a fortuitous collection of 173 workers from a colony on Peter Island allowed us to document the hydrocarbon mixture of this species. The pattern of the hydrocarbon mixture of *P. wolcotti* was an inverse of *N. mona* in that nearly 80% of the hydrocarbons were late-eluting olefins with 41–45 carbons (Figure 8).

n-Alkanes present were *n*-C_{27}, *n*-C_{28}, and *n*-C_{29}. In total the *n*-alkanes comprised only 6.5% of the total hydrocarbon. One internally branched monomethylalkane was detected, 5-MeC_{29}, and represented only 1% of the total hydrocarbon. 2- and 3-Methylalkanes were identified for C_{27} and C_{29}, but 2-MeC_{28} was the most abundant internally branched monomethylalkane (Figure 8). The five compounds in this group comprised over 11% of the total hydrocarbons. One dimethyalkane was noted (as isomeric mixture 5,19-; 5,17-DimeC_{29}) and amounted to <1.7% of the cuticular hydrocarbon fraction. No trimethylalkanes were observed.

Unsaturated components comprised the predominant class of hydrocarbons in *P. wolcotti*, making up nearly 80% of the total hydrocarbons present. All of the olefins had an odd number of carbons (41, 43, and 45) and possessed three or four double bonds.

Nasutitermes costalis (Holmgren). *N. costalis* is the less common of the two carton nest-building nasutes found in the BVI. It occurs primarily in wetter

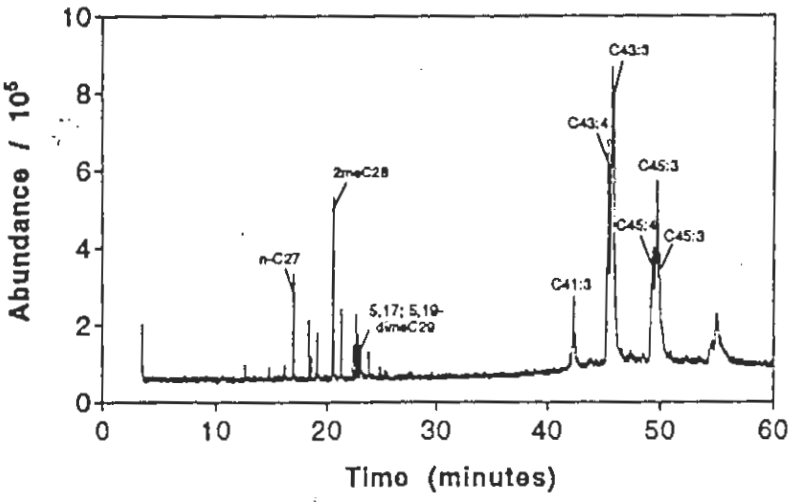

FIG. 8. Total ion chromatogram of cuticular hydrocarbons from workers of *Parvitermes wolcotti* from Peter Island.

localities in the BVI; Tortola and Guana Island are the only islands where this species has been found (Collins et al., 1997; Scheffrahn et al., 1994). *N. costalis* occurs on many of the islands in the Greater and Lesser Antilles, from Cuba south to Trinidad and Tobago (Snyder, 1949; Araujo, 1977; Scheffrahn et al., 1994). The relative scarcity of *N. costalis* in the BVI may be related to the lower moisture availability on most of the islands of the complex. Krecek (1970) found that *N. costalis* distribution patterns and nest composition on Cuba indicated a relatively higher moisture demand than that shown by the other common nasute, *N. rippertii* (Rambur).

All of the cuticular hydrocarbons of *N. costalis* had parent chains ranging from 25 to 33 carbons. Those with 29–31 carbons in the parent chain comprised over 88% of the total hydrocarbon mixture (Figure 9).

n-Alkanes present were *n*-C_{25}, *n*-C_{27}, *n*-C_{28}, and *n*-C_{29}. *n*-C_{29} was the most abundant, comprising 1.9% of the total hydrocarbon. The other three *n*-alkanes represented only 3.2% of the total hydrocarbons.

We identified isomeric mixtures of internally branched monomethylalkanes with parent carbon chains ranging from C_{27} to C_{33}. Positions of methyl branches ranged from carbon 9 to 15. Methyl branches located on even-numbered carbons were found only when the parent chain of the hydrocarbon had an even number of carbons, while branches on odd-numbered carbons were found to occur on hydrocarbons with either odd or even numbers of carbons in the parent chain. Internally branched monomethylalkanes were one of the most abundant classes

C-9 to C-15? see page 936, line 32

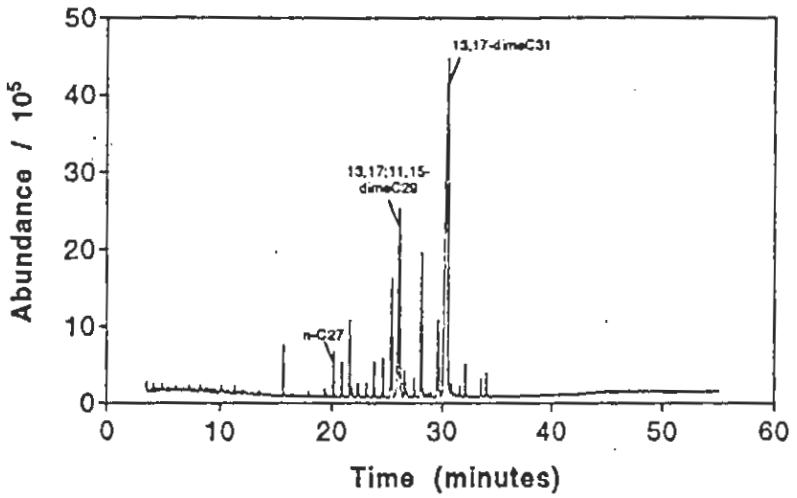

FIG. 9. Total ion chromatogram of cuticular hydrocarbons from workers of *Nasutitermes costalis* from Tortola.

of hydrocarbons produced by *N. costalis*, representing about 15% of the total hydrocarbon. Two isomeric mixtures, 15-; 13-; 11-MeC_{29} and 15-; 13-; 11-MeC_{31}, accounted for nearly 80% of this class of hydrocarbon.

3-Methylalkanes were identified for C_{27} and C_{29}. 2-MeC_{27} coeluted with 11,15-DimeC_{27}. These terminally branched monomethylalkanes comprised at least 0.45% of the total hydrocarbons, but certainly less than 3.0%.

Internally branched dimethylalkanes were the predominant hydrocarbon class and constituted 78% of the total cuticular hydrocarbon fraction of *N. costalis* (Figure 9). All of these internally branched dimethylalkanes had three methylene groups separating the methyl branches. Dimethylalkanes had carbon numbers in the parent chain ranging from 27 to 33. Dimethylalkanes with 29 and 31 carbons in the parent chain accounted for nearly 64% of the total hydrocarbon complement. Terminally branched dimethylalkanes were not encountered.

Only two trimethylalkanes were identified. Each had three methylene groups between the methyl branches. Combined, they totaled $<1\%$ of the total hydrocarbon; $C_{27:1}$ was the only olefin found and represented only 0.24% of the total hydrocarbon.

Nasutitermes acajutlae (Holmgren). *N. acajutlae* is the most conspicuous, and apparently the most abundant, species of termite in the BVI complex. Colonies of this species construct enormous nests (up to 1.5 m in diameter, up to 2.0 m in height) composed of dark to silvery brown, delicate, friable, parch-

mentlike outer walls enclosing the variously sized, heavier-walled cells of the carbon matrix. Nests are usually ellipsoidal or irregularly rounded.

N. acajutlae was recently resurrected as a species morphologically distinguishable from *N. nigriceps* (Thorne et al., 1994). Termites in the *N. acajutlae*/*N. nigriceps* complex range from Mexico south into South America, then east and north through the Caribbean. Members of the two species have a tolerance for wide variations in moisture availability and use a variety of foods and nesting sites. Mature nests and individuals of *N. acajutlae* are larger than those of *N. costalis*. Soldiers of *N. acajutlae* have reddish to dark brown heads; alates are relatively large and chestnut brown in color (Thorne et al., 1994).

N. acajutlae was found on every island surveyed and has been found on even the smallest of islands, such as Carrot Rock (Scheffrahn et al., 1994; Collins et al., 1997). We obtained hydrocarbon samples of this species from 11 islands (Table 1). We extensively sampled workers and soldiers from 13 colonies on Guana Island, and collected alates when we encountered them (Haverty et al., 1996). To further assess interisland variability, we sampled *N. acajutlae* from diverse habitats on Tortola in 1994.

We identified 33 hydrocarbons from workers, 45 from soldiers, and 43 from alates of *N. acajutlae* (Haverty et al., 1996). The hydrocarbons found in all three castes aggregated into two distinct groups. The early-eluting components were primarily *n*-alkanes, methyl-branched alkanes, and a few normal alkenes. The late-eluting compounds consisted almost exclusively of unsaturated components, with chain lengths of 37–45 carbons and one to six double bonds, and a few monomethyl alkanes in trace amounts (especially in alates). Soldiers had considerably greater proportions of the early-eluting compounds (23–29 carbons) than did workers or alates (Figures 10 and 11). Whereas workers and alates had an average of 88–96% of the cuticular hydrocarbons with 33 or more carbons, soldiers had an average of about 69% of these late-eluting compounds. The predominant class of hydrocarbons was the olefin fraction, comprising greater than 89% of the total hydrocarbon component in workers and alates and about 76% in soldiers.

n-Alkanes present ranged from *n*-C_{23} to *n*-C_{33}. Usually *n*-C_{25} and *n*-C_{27} were the most abundant for workers, soldiers, and alates. The *n*-alkanes were least abundant in alates (1.8–4.3% of total), moderately abundant in workers (4–7.3% of total), and most abundant in soldiers (17.4–18.8% of the total hydrocarbon) (Figures 10 and 11).

Internally branched monomethylalkanes were nearly always encountered, in trivial amounts in workers (0.4–0.9% of total), and in significant amounts in soldiers (about 5.5% of total). Internally branched monomethylalkanes were usually present in alates, but accounted for only 0.4–1.7% of the total hydrocarbon. Only trivial amounts of 2- and 3-methylalkanes occurred in workers and soldiers. Alates usually had a significant component of 2-methylalkanes, com-

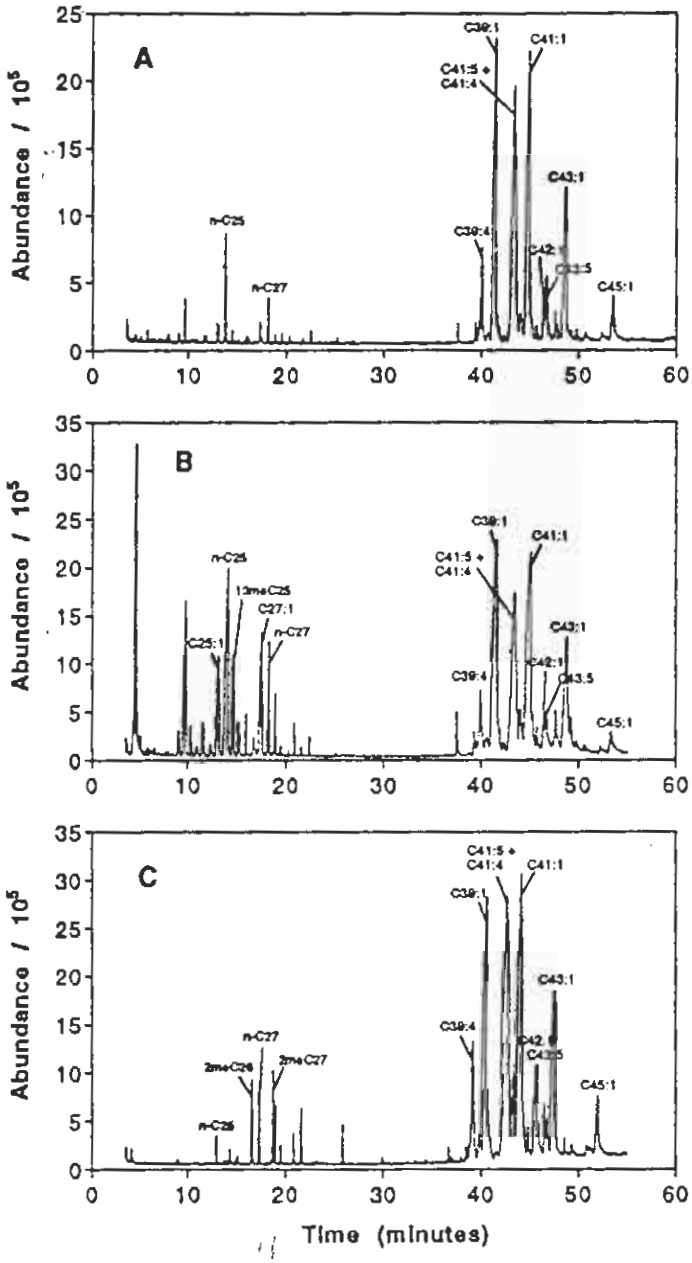

FIG. 10. Total ion chromatogram of cuticular hydrocarbons from *Nasutitermes acajutlae* from Guana Island. A = workers, B = soldiers, C = alates.

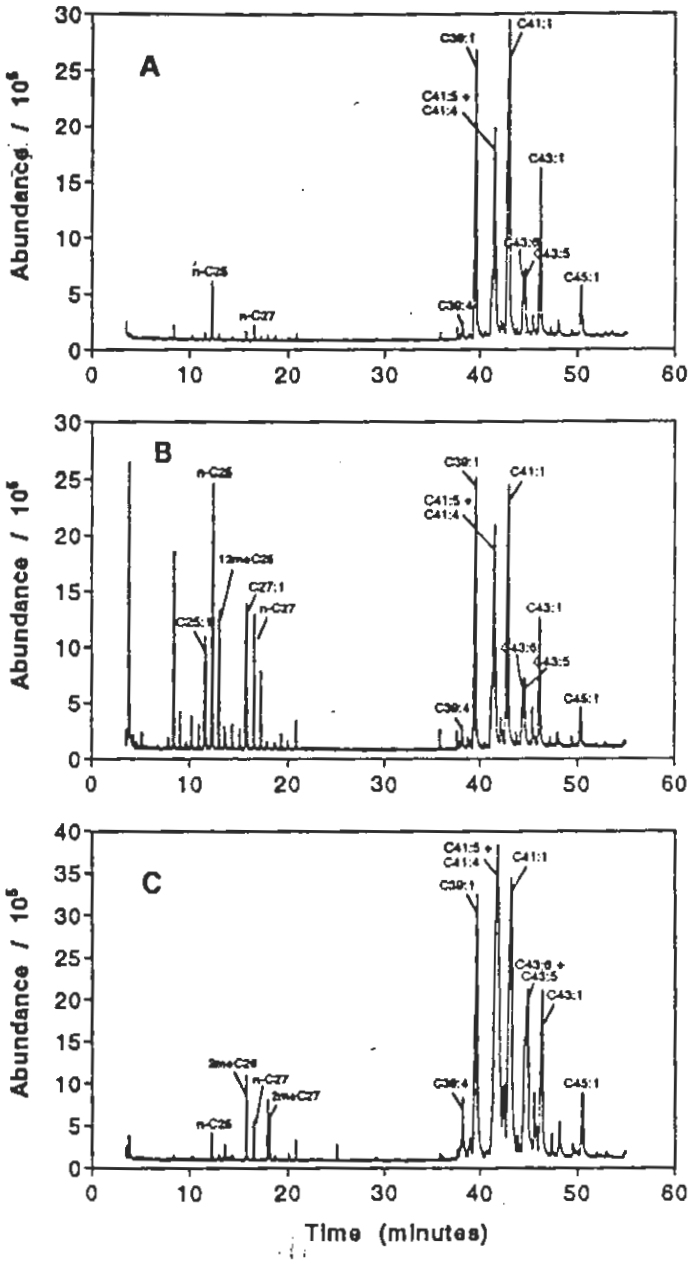

FIG. 11. Total ion chromatogram of cuticular hydrocarbons of *Nasutitermes acajutlae* from Tortola, BVI. A = workers, B = soldiers, C = alates.

prising 1.8–4.6% of the total hydrocarbon, and were always found in the early-eluting constituents (Figures 10 and 11). No dimethyl- or trimethylalkanes were found in workers or soldiers; only 5,17-DimeC_{25} was found in alates in trivial amounts.

The unsaturated component was the paramount class of hydrocarbons in the cuticular lipids of *N. acajutlae*. Olefins comprised an average of 90–96% of the total hydrocarbon in workers and alates. Since soldiers contained a larger proportion of early-eluting *n*-alkanes, the olefin component amounted to about 76% of the total hydrocarbon in this caste. For all castes, $C_{39:1}$, $C_{41:5}$, $C_{41:4}$, $C_{41:1}$, and $C_{43:1}$ accounted for at least 70% of the total olefin (Haverty et al., 1996).

Intercaste Variation in N. acajutlae. We observed consistent intercaste differences in hydrocarbon mixtures of *N. acajutlae*. Workers and alates produced proportionally more olefins than did soldiers. Conversely, soldiers made proportionally more of the early-eluting hydrocarbons, such as *n*-alkanes and monomethylalkanes, than did workers and alates.

Cuticular hydrocarbons of workers, soldiers, and alates were not qualitatively identical. Soldiers had some early eluting compounds (11-; 9-MeC_{23}, 3-MeC_{23}, $C_{24:1}$, 11-MeC_{24}, 3-MeC_{25}, $C_{26:1}$, and 13-; 12-; 11-MeC_{26}) that were not found in workers or alates from Guana Island or Tortola (Tables 4 and 5; Figures 10 and 11). Samples of alates from both Guana Island and Tortola included hydrocarbons (5-MeC_{25}, 2-MeC_{25}, 5,17-DimeC_{25}, 2-MeC_{26}, and 3-MeC_{27}) not found in either workers or soldiers (Tables 4 and 5). Alates from Guana Island contained some abundant hydrocarbons ($C_{40:5}$, 15-MeC_{43}, and $C_{45:2}$) and trace hydrocarbons not seen in workers or soldiers from the same island (Table 4). Alates, workers, and soldiers from Tortola have these latter hydrocarbons as well as significant amounts (0.6–0.7%) of $C_{45:5}$, which was not seen in the Guana Island samples (Tables 4 and 5). Alates from both Guana Island and Tortola were also missing a few hydrocarbon components ($C_{25:1}$, $C_{27:1}$, and 13-; 11-MeC_{27}) that were commonly observed in workers and soldiers (Tables 4 and 5). $C_{40:1}$ and $C_{42:1}$ were not detected in alates from Guana Island, but were frequently found in workers and soldiers (Table 4).

$C_{43:6}$ was detected only in alates from Guana Island that were collected in 1993; however, this hydrocarbon was found in all castes collected on Tortola in 1994. It is curious that in preliminary work $C_{43:6}$ was also seen in workers, soldiers, and alates from specimens taken in 1994 from some of the same colonies on Guana Island that we sampled in 1993 (Haverty, Thorne, and Nelson, unpublished observations).

These obvious year-to-year differenees could, in fact, represent distinct annual variation. They may be an artifact resulting from variations in handling of samples (for example, minor differences in drying technique or storage before and after processing). Whether these differences are real or an artifact of pro-

TABLE 4. RELATIVE QUANTITIES (MEAN AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS OF SAMPLES OF WORKERS, SOLDIERS, AND ALATES OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND[a]

Hydrocarbon	Workers (mean ± SD)	Soldiers (mean ± SD)	Alates (mean ± SD)
$C_{23:1}$	0.32 ± 0.32	0.41 ± 0.17	0.00 ± 0.00
C_{23}	0.97 ± 0.33	4.04 ± 0.91	0.08 ± 0.04
11-; 9-MeC_{23}[b]	0.00 ± 0.00	0.51 ± 0.15	0.00 ± 0.00
3-MeC_{23} + $C_{24:1}$[c]	0.00 ± 0.00	0.18 ± 0.15	0.00 ± 0.00
C_{24}	0.22 ± 0.22	0.66 ± 0.19	0.00 ± 0.00
11-MeC_{24}	0.00 ± 0.00	0.37 ± 0.32	0.00 ± 0.00
$C_{25:1}$	0.68 ± 0.27	2.96 ± 0.69	0.00 ± 0.00
C_{25}	3.05 ± 1.14	9.16 ± 1.28	0.55 ± 0.14
13-; 11-MeC_{25}[b]	0.16 ± 0.17	1.98 ± 0.43	0.12 ± 0.03
5-MeC_{25}	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.01
2-MeC_{25}	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.03
3-MeC_{25} + $C_{26:1}$[c]	0.00 ± 0.00	0.57 ± 0.13	0.00 ± 0.00
5,17-$DimeC_{25}$	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.01
C_{26}	0.26 ± 0.27	0.59 ± 0.16	0.14 ± 0.02
13-; 12-; 11-MeC_{26}[b]	0.00 ± 0.00	0.42 ± 0.35	0.00 ± 0.00
2-MeC_{26}	0.00 ± 0.00	0.00 ± 0.00	1.42 ± 0.16
$C_{27:1}$	0.86 ± 0.42	3.88 ± 0.63	0.00 ± 0.00
C_{27}	1.90 ± 1.60	3.67 ± 0.92	1.44 ± 0.28
13-; 11-MeC_{27}[b]	0.07 ± 0.11	1.14 ± 0.21	0.00 ± 0.00
5-MeC_{27}	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04
2-MeC_{27}	0.15 ± 0.13	0.14 ± 0.09	1.67 ± 0.29
3-MeC_{27}	0.00 ± 0.00	0.00 ± 0.00	0.72 ± 0.13
C_{28}	0.15 ± 0.19	0.13 ± 0.12	0.30 ± 0.08
2-MeC_{28} + $C_{29:1}$[c]	0.00 ± 0.00	0.19 ± 0.10	0.53 ± 0.11
C_{29}	0.73 ± 0.56	0.56 ± 0.25	1.08 ± 0.25
5-MeC_{29}	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.05
2-MeC_{29}	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03
3-MeC_{29}	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.05
C_{30}	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.04
C_{31}	0.06 ± 0.15	0.02 ± 0.04	0.58 ± 0.08
C_{33}	0.00 ± 0.00	0.01 ± 0.03	0.12 ± 0.03
$C_{35:1}$	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.06
13-MeC_{35}	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.06
$C_{37:1}$	0.40 ± 0.17	0.65 ± 0.17	0.40 ± 0.14
$C_{38:1}$	0.53 ± 0.20	0.58 ± 0.13	0.47 ± 0.07
$C_{39:5}$[d]	0.36 ± 0.33	0.28 ± 0.25	0.00 ± 0.00
$C_{39:4}$[d]	2.00 ± 0.73	1.33 ± 0.61	3.89 ± 0.78
$C_{39:2}$	0.43 ± 0.48	1.29 ± 0.56	1.14 ± 0.11
$C_{39:1}$	20.01 ± 2.20	15.77 ± 2.79	14.40 ± 1.26
15-MeC_{39}	0.04 ± 0.12	0.36 ± 0.27	0.00 ± 0.00
$C_{40:5}$	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 0.35
$C_{40:1}$	2.51 ± 0.42	2.89 ± 0.62	0.00 ± 0.00

TABLE 4. Continued

Hydrocarbon	Workers (mean ± SD)	Soldiers (mean ± SD)	Alates (mean ± SD)
$C_{41:4}$ + $C_{41:3}$[e]	18.77 ± 3.81	11.01 ± 3.92	21.72 ± 2.41
$C_{41:2}$	1.21 ± 0.63	1.50 ± 0.49	3.31 ± 0.20
$C_{41:1}$	24.43 ± 2.74	17.42 ± 3.52	20.24 ± 1.18
15-MeC_{41}	0.13 ± 0.23	0.46 ± 0.24	0.76 ± 0.15
$C_{42:1}$	1.60 ± 0.65	1.99 ± 1.03	0.00 ± 0.00
$C_{43:6}$ + $C_{43:5}$[f]	4.35 ± 1.37	2.41 ± 1.09	6.24 ± 1.11
$C_{43:2}$	0.83 ± 0.52	1.14 ± 0.43	2.63 ± 0.27
$C_{43:1}$	10.40 ± 1.61	7.66 ± 1.48	10.28 ± 1.09
15-MeC_{43}	0.00 ± 0.00	0.00 ± 0.00	0.43 ± 0.11
$C_{45:2}$	0.00 ± 0.00	0.00 ± 0.00	0.32 ± 0.11
$C_{45:1}$	2.41 ± 0.82	1.69 ± 0.64	3.26 ± 0.87

[a] Three subsamples of 100 workers from each of 13 colonies. Four subsamples of ca. 4 ml of soldiers from each of 13 colonies. Two subsamples of 25–31 alates from four colonies.
[b] An isomeric mixture. These monomethylalkanes coelute.
[c] This monomethylalkane and the olefin coelute.
[d] These two isomers did not completely resolve in alates. Therefore, the areas for the two isomers were summed for alates only.
[e] These two isomers did not completely resolve. Therefore, the areas for the two isomers were summed.
[f] $C_{43:6}$ was identified only in alates. There were two isomers of $C_{43:5}$ that did not completely resolve. Therefore, the areas for the two isomers were summed.

tocol, we must consider them when evaluating minor variations in hydrocarbon mixtures for taxonomic studies of termites.

Island-to-Island Variation in N. acajutlae. Our collections of *N. acajutlae* were much more extensive, both in numbers of samples and geographic coverage (Table 1), than for any other termite taxon in the British Virgin Islands. We observed qualitative differences between samples of soldiers from Guana Island and Tortola, two islands separated by a channel approximately 2 km wide (Tables 4 and 5). Soldiers from collections on Guana Island did not have $C_{40:5}$ or $C_{45:2}$, whereas soldiers from Tortola possessed small amounts of these olefins. We did not have samples of soldiers from other islands, and our alate collections from other islands were limited. However, comparison of hydrocarbon mixtures from workers indicated a range in variation, both qualitative and quantitative, possible within one species (Tables 4 to 6).

Workers from islands more distant from Tortola and Guana tended to have more of the terminally branched monomethylalkanes (Tables 4–6; Figures 10–12). Two samples in particular, those from Scrub Island and Great Camino, displayed hydrocarbon mixtures more similar to soldiers (Figure 12); however,

TABLE 5. RELATIVE QUANTITIES (MEAN AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS OF SAMPLES OF WORKERS, SOLDIERS, AND ALATES OF *Nasutitermes acajutlae* (Holmgren) FROM TORTOLA[a]

Hydrocarbon	Workers (mean ± SD)	Soldiers (mean ± SD)	Alates (mean ± SD)
$C_{23:1}$	0.00 ± 0.00	0.38 ± 0.13	0.00 ± 0.00
C_{23}	0.47 ± 0.16	3.99 ± 0.56	0.08 ± 0.01
11-; 9-MeC_{23}[b]	0.00 ± 0.00	0.66 ± 0.11	0.00 ± 0.00
3-MeC_{23} + $C_{24:1}$[c]	0.00 ± 0.00	0.32 ± 0.06	0.00 ± 0.00
C_{24}	0.07 ± 0.08	0.65 ± 0.05	0.01 ± 0.03
11-MeC_{24}	0.00 ± 0.00	0.46 ± 0.10	0.00 ± 0.00
$C_{25:1}$	0.50 ± 0.18	3.23 ± 0.46	0.00 ± 0.00
C_{25}	2.11 ± 0.59	8.41 ± 0.86	0.57 ± 0.20
13-; 11-MeC_{25}[b]	0.27 ± 0.09	2.52 ± 0.38	0.07 ± 0.06
5-MeC_{25}	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.03
2-MeC_{25}	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.03
3-MeC_{25} + $C_{26:1}$[c]	0.00 ± 0.00	0.62 ± 0.07	0.00 ± 0.00
5,17-$DimeC_{25}$	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02
C_{26}	0.06 ± 0.09	0.49 ± 0.08	0.08 ± 0.02
13-; 12-; 11-MeC_{26}[b]	0.00 ± 0.00	0.35 ± 0.09	0.00 ± 0.00
2-MeC_{26}	0.00 ± 0.00	0.00 ± 0.00	1.04 ± 0.24
$C_{27:1}$	0.54 ± 0.17	3.47 ± 0.56	0.00 ± 0.00
C_{27}	0.74 ± 0.24	3.25 ± 1.00	0.47 ± 0.10
13-; 11-MeC_{27}[b]	0.08 ± 0.10	1.08 ± 0.26	0.00 ± 0.00
2-MeC_{27}	0.24 ± 0.15	0.12 ± 0.09	0.61 ± 0.17
3-MeC_{27}	0.00 ± 0.00	0.00 ± 0.00	0.45 ± 0.10
C_{28}	0.13 ± 0.10	0.00 ± 0.00	0.07 ± 0.02
2-MeC_{28}	0.07 ± 0.10	0.00 ± 0.00	0.12 ± 0.02
$C_{29:1}$	0.00 ± 0.00	0.13 ± 0.11	0.00 ± 0.00
C_{29}	0.33 ± 0.09	0.62 ± 0.43	0.41 ± 0.14
C_{31}	0.04 ± 0.07	0.02 ± 0.04	0.28 ± 0.05
C_{33}	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.05
$C_{37:1}$	0.24 ± 0.12	0.38 ± 0.09	0.09 ± 0.02
$C_{38:1}$	0.39 ± 0.08	0.36 ± 0.09	0.26 ± 0.02
$C_{39:5}$	0.25 ± 0.15	0.15 ± 0.12	0.42 ± 0.08
$C_{39:4}$	1.36 ± 0.67	1.02 ± 0.46	2.60 ± 0.69
$C_{39:2}$	0.29 ± 0.29	0.59 ± 0.18	0.87 ± 0.10
$C_{39:1}$	18.06 ± 1.64	12.66 ± 1.68	12.40 ± 0.28
$C_{40:5}$	0.66 ± 0.30	0.44 ± 0.24	1.44 ± 0.29
$C_{40:1}$ + $C_{41:4}$ + $C_{41:5}$[d]	20.77 ± 3.01	18.98 ± 3.25	30.53 ± 2.19
$C_{41:2}$	1.43 ± 0.15	1.60 ± 0.22	3.39 ± 0.26
$C_{41:1}$	24.21 ± 2.06	15.27 ± 1.84	18.13 ± 0.29
15-MeC_{41}	0.39 ± 0.29	0.46 ± 0.25	0.83 ± 0.04
$C_{43:6}$	2.55 ± 0.76	2.05 ± 0.36	3.18 ± 0.64
$C_{43:5}$[e]	5.77 ± 1.19	4.07 ± 1.14	7.18 ± 0.90
$C_{43:2}$	1.12 ± 0.32	1.39 ± 0.15	2.17 ± 0.19
$C_{43:1}$	10.69 ± 0.80	6.77 ± 0.58	8.04 ± 0.34

TABLE 5. Continued

Hydrocarbon	Workers (mean ± SD)	Soldiers (mean ± SD)	Alates (mean ± SD)
15-MeC_{43}	0.20 ± 0.19	0.21 ± 0.10	0.43 ± 0.12
$C_{43:5}$	0.69 ± 0.37	0.59 ± 0.28	0.66 ± 0.31
$C_{45:2}$	0.19 ± 0.15	0.33 ± 0.06	0.26 ± 0.07
$C_{45:1}$	3.09 ± 0.31	1.94 ± 0.09	2.52 ± 0.36

[a] Three subsamples of 200 workers from each of seven colonies. Four subsamples of ca. 4 ml of soldiers from each of six colonies. Two subsamples of 25–31 alates from three colonies.
[b] An isomeric mixture. These monomethylalkanes coelute.
[c] This monomethylalkane and the olefin coelute.
[d] These three isomers did not completely resolve. Therefore, the areas for the isomers were summed.
[e] There were two isomers of $C_{43:5}$ that did not completely resolve. Therefore, the areas of the two isomers were summed.

TABLE 6. RELATIVE QUANTITIES (MEAN AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS OF SAMPLES OF WORKERS AND ALATES OF *Nasutitermes acajutlae* (HOLMGREN) FROM BRITISH VIRGIN ISLANDS, EXCLUSIVE OF GUANA ISLAND AND TORTOLA[a]

Hydrocarbon	Workers (mean ± SD)	Alates (mean ± SD)
C_{23}	0.63 ± 0.47	0.15 ± 0.31
C_{24}	0.08 ± 0.15	0.00 ± 0.00
2-MeC_{24} + $C_{25:1}$[b,c]	0.32 ± 0.75	0.00 ± 0.00
$C_{25:1}$[c]	1.73 ± 4.45	0.00 ± 0.00
C_{25}	3.65 ± 2.47	0.67 ± 0.83
13-; 11-MeC_{25}[d]	0.27 ± 0.21	0.00 ± 0.00
2-MeC_{25}	0.56 ± 1.59	0.03 ± 0.06
3-MeC_{25} + $C_{26:1}$[b]	0.48 ± 1.29	0.00 ± 0.00
C_{26}	0.20 ± 0.36	0.00 ± 0.00
2-MeC_{26}	0.32 ± 0.60	0.78 ± 0.50
$C_{27:1}$	0.36 ± 0.44	0.00 ± 0.00
C_{27}	1.78 ± 1.22	0.51 ± 0.29
13-; 11-MeC_{27}	0.14 ± 0.18	0.00 ± 0.00
2-MeC_{27}	0.48 ± 0.42	0.63 ± 0.45
3-MeC_{27}	0.04 ± 0.13	0.34 ± 0.23
C_{28}	0.10 ± 0.12	0.04 ± 0.07
C_{29}	0.42 ± 0.31	0.38 ± 0.12
C_{31}	0.00 ± 0.00	0.09 ± 0.10

TABLE 6. Continued

Hydrocarbon	Workers (mean ± SD)	Alates (mean ± SD)
$C_{37:1}$	0.11 ± 0.13	0.05 ± 0.10
$C_{38:1}$	0.29 ± 0.21	0.24 ± 0.16
$C_{39:5}$	0.00 ± 0.00	0.19 ± 0.25
$C_{39:4}$	1.53 ± 1.00	2.08 ± 1.73
$C_{39:3}$	0.16 ± 0.28	0.24 ± 0.48
$C_{39:2}$	0.13 ± 0.43	0.63 ± 0.48
$C_{39:1}$	20.92 ± 4.61	18.18 ± 2.68
15-MeC_{39}	0.07 ± 0.23	0.21 ± 0.42
$C_{40:1}$ + $C_{41:4}$ + $C_{41:5}$[e]	22.28 ± 5.61	30.18 ± 3.38
$C_{41:3}$	0.24 ± 0.79	0.00 ± 0.00
$C_{41:2}$	0.83 ± 1.07	2.81 ± 1.93
$C_{41:1}$[c]	27.65 ± 5.53	26.30 ± 2.54
15-MeC_{41}	0.17 ± 0.34	0.14 ± 0.28
$C_{42:x}$[f]	0.00 ± 0.00	0.25 ± 0.31
$C_{42:1}$	0.77 ± 0.43	0.79 ± 0.55
$C_{43:5}$[g]	3.49 ± 1.14	4.86 ± 0.99
$C_{43:3}$	0.23 ± 0.77	0.00 ± 0.00
$C_{43:2}$	0.81 ± 1.71	0.76 ± 0.55
$C_{43:1}$	8.73 ± 2.67	8.50 ± 1.89

[a] Mean for workers is derived from 11 samples from colonies of *N. acajutlae*: three from Necker Is. and one each from Great Camino, Scrub Is., Eustatia, Virgin Gorda, Lesser Jost Van Dyke, Greater Jost Van Dyke, Great Thatch, and Cooper. Mean for alates is derived from four samples from colonies of *N. acajutlae*: one each from Lesser Jost Van Dyke, Greater Jost Van Dyke, Great Thatch, and Necker Is.

[b] This monomethylalkane and this olefin coelute.

[c] Two separate isomers of $C_{25:1}$ occur in the samples from Great Camino and Scrub Island. This abundant, second isomer (maximum value of 15.1% in the Great Camino sample) was found only in these two samples.

[d] An isomeric mixture. These monomethylalkanes coelute.

[e] These three isomers did not completely resolve. Therefore, the areas for the three isomers were summed.

[f] An isomeric mixture. The exact number of double bonds is difficult to determine. In some instances two or three peaks were present with identical spectra. The areas for all isomers of the same olefin were summed.

[g] This olefin had two isomers that did not completely resolve. The areas for both isomers were summed.

examination of the extracted voucher specimens confirmed that only workers were extracted in these samples. The single sample from Great Camino displayed some distinct differences from samples collected on other islands. The later-eluting isomer of $C_{25:1}$ was present in great abundance, its peak area exceeding that of *n*-C_{25} (Figure 12B). This sample from Great Camino was the only one in which two isomers of $C_{43:3}$ were detected in measurable amounts.

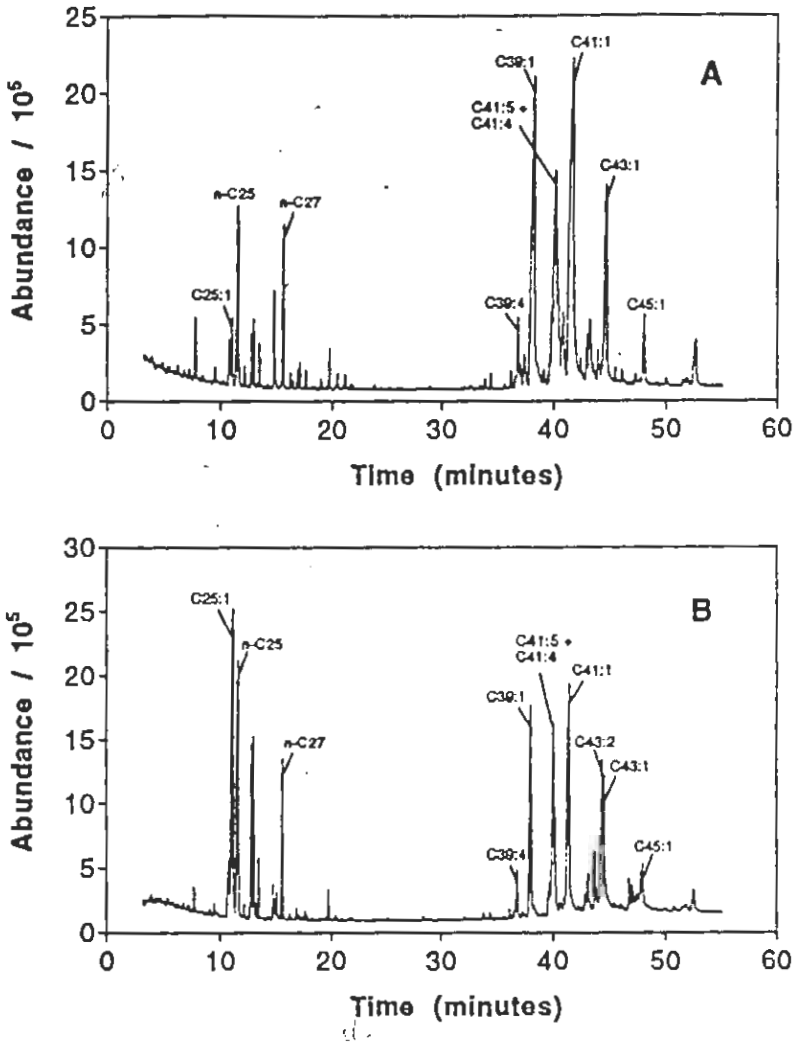

FIG. 12. Total ion chromatogram of cuticular hydrocarbons from workers of *Nasutitermes acajutlae* from (A) Scrub Island and (B) Great Camino.

The retention time of $C_{43:2}$ in this sample indicated that this compound was a different isomer of $C_{43:2}$ than was seen in other samples of *N. acajutlae*. In the Great Camino sample, $C_{43:2}$ was not completely resolved from $C_{43:1}$; this is true for $C_{45:2}$ and $C_{45:1}$ as well (Figure 12B). The existence of island-to-island differences in hydrocarbon mixtures within one species should alert us to exercise caution when evaluating the use of cuticular hydrocarbons in termite taxonomy.

TABLE 7. DIAGNOSTIC CUTICULAR HYDROCARBONS (>1.0% OF TOTAL HYDROCARBON) FROM PSEUDERGATES (LARVAE AND NYMPHS) OR WORKERS OF 8 TERMITE TAXA FROM BRITISH VIRGIN ISLANDS[a]

Hydrocarbon	Termite species[b]							
	N mon	C bre	P cor	I spp[c]	H sp	P wol	N cos	N aca
C_{25}	+++	+++	+++	+++	0	0	++	+++
C_{26}	+++	++	++	++	tr	0	0	tr
C_{28}	tr	+	tr	tr	++	++	+	tr
C_{29}	+	++	+	+	++	++	++	+
13-; 11-MeC_{25}	+++	+	0	+	0	0	0	tr
13-; 12-MeC_{26}	+++	0	0	0	0	0	0	0
13-; 11-; 9-; 7-MeC_{27}	+++	tr	tr	tr	+++	0	+	tr
14-; 13-; 12-; 9-; 7-MeC_{28}	+	0	0	0	+++	0	+	0
15-; 13-; 11-; 9-; 7-; 5-MeC_{29}	tr	0	0	0/+	+++	+	+++	0
15-; 13-; 11-; 9-MeC_{31}	tr	0	0	0	+	0	+++	0
2-MeC_{24}	++	+++	+++	+++	0	0	0	0
2-MeC_{25}	+++	++	+++	+++	0	0	0	0
3-MeC_{25}	+++	+++	+++	+++	0	0	0	0
2-MeC_{26}	++	+	+++	+/++	+	0	0	0
3,X-$DimeC_{25}$	++	tr	0	0	0	0	0	0
11,15-$DimeC_{27}$	++	0	0	0	+++	0	++	0
9,17-$DimeC_{27}$	0	0	0	0	+++	0	0	0
9,X-$DimeC_{28}$	0	0	0	0	+++	0	0	0
13,17-; 11,15-$DimeC_{29}$	0	0	0	0	0	0	+++	0
9,19-; 9,17-$DimeC_{29}$	0	0	0	0/+	+++	0	0	0
7,21-$DimeC_{29}$	0	0	0	0	+++	0	0	0
11,15-; 12,16-; 13,17-$DimeC_{31}$	0	0	0	0	0	0	+++	0
11,15-$DimeC_{39}$	++	0	0	0	0	0	0	0
13,17-$DimeC_{41}$	++	0	0	0	0	0	0	0
$C_{25:1}$	0	0	++	+++	0	0	0	+
$C_{27:1}$	tr	0	0	+++	0	0	tr	+
$C_{37:2}$	0	+++	++	0	0	0	0	0
$C_{39:2}$	0	+++	+++	0/+	0	0	0	+
$C_{39:1}$	0	++	++	tr	tr	0	0	+++
$C_{40:1}$	0	0	0	0	0	0	0	+++
$C_{41:5}$	0	0	0	0	0	0	0	+++
$C_{41:4}$	0	0	0	0	0	0	0	+++
$C_{41:3}$	0	+++	+++	0/+	0	+++	0	0
$C_{41:2}$	0	+++	+++	0/++	0	0	0	++
$C_{41:1}$	0	++	+++	0/+	0	0	0	+++
$C_{43:4}$	0	0	+	0	0	+++	0	+++
$C_{43:3}$	0	++	++	0/+++	0	+++	0	0
$C_{43:2}$	0	++	++	+++	0	0	0	+
$C_{43:1}$	0	++	0	0/++	0	0	0	+++

TABLE 7. Continued

Hydrocarbon	Termite species[b]							
	N mon	C bre	P cor	I spp[c]	H sp	P wol	N cos	N aca
$C_{45:4}$	0	0	0	0/+	0	+++	0	0
$C_{45:3}$	0	++	0	0/++	0	+++	0	0
$C_{45:1}$	0	0	0	0/+++	0	0	0	++

[a] Relative proportions of the total hydrocarbon mixture for each species. +++ = >3.0%; ++ = 1.0–3.0%; + = 0.3–0.99%; and tr = <0.3%; 0 = not detected.

[b] N mon = *Neotermes mona*; C bre = *Cryptotermes brevis*; P cor = *Procryptotermes corniceps*; I spp = *Incisitermes* species; H spp = *Heterotermes* species; P wol = *Parvitermes wolcotti*; N cos = *Nasutitermes costalis*; N aca = *Nasutitermes acajutlae* (from Guana Island).

[c] *Incisitermes* spp. displayed a wide range of hydrocarbon mixtures. For example, 0/+++ would denote the range from absent to above 3%.

Taxonomic and Biogeographic Value of Cuticular Hydrocarbon Profiles. One of the objectives of our studies of the termite fauna of the British Virgin Islands was to begin to build a library of cuticular hydrocarbon profiles correlated with species determinations based on morphological characters. Much of this work will be published separately as in-depth studies of individual taxa (genera or species complexes) from the Caribbean Basin. BVI termite species that were readily identifiable on the basis of morphological characters of the soldiers or alates also had diagnostic cuticular hydrocarbon mixtures. Using only the consistently abundant hydrocarbons, one could unambiguously identify species based on characterization of the hydrocarbons of workers, larvae, pseudergates, or nymphs without the sometimes rare soldiers and alates needed for morphological diagnoses (Table 7). Separation of closely related taxa has been demonstrated for species of *Nasutitermes* from the Caribbean Basin and *Zootermopsis* from western North America using the consistently abundant hydrocarbons (Haverty et al., 1988, 1992).

Cuticular hydrocarbons may eventually help resolve one of the more difficult taxonomic problems among the termite species of the British Virgin Islands, i.e., separation of species within the genus *Incisitermes*. *Incisitermes* from the Cayman Islands identified as *I. tabogae* (Snyder) possess a distinct cuticular hydrocarbon mixture clearly separable from the *Incisitermes* examined from the BVI (Haverty et al., unpublished observations). Similarly, the taxonomy of *Heterotermes* might be clarified if consistent hydrocarbon mixtures can be used to presort specimens for morphological study.

CONCLUSIONS

All classes of hydrocarbons are seen among the eight termite taxa characterized from the BVI. All taxa have normal alkanes present in their cuticular hydrocarbon mixture: n-C_{25} and n-C_{27} are the most abundant. Internally branched monomethylalkanes are not commonly seen or are present in very small amounts relative to all other hydrocarbons; only *Neotermes mona* and *Heterotermes* sp. incorporate these components in relatively large quantities. Terminally branched monomethylalkanes are much more common in most of the species, but are present only in trace amounts in *Nasutitermes costalis* and *N. acajutlae*. Dimethylalkanes are present in relatively large quantities only in *N. mona*, *Heterotermes* and *N. costalis*, species with rather high moisture requirements, and are absent or present only in trace amounts in the other taxa. Trimethylalkanes are quite rare; they are completely absent in six of the taxa, present in trace amounts in *Incisitermes* and in small amounts in *N. costalis*.

In general, olefins are the most common of the hydrocarbons found in the termites of the BVI. Early-eluting alkenes are abundant in *Incisitermes* spp., but absent or rare in all other taxa. Late-eluting olefins, especially those with 39, 41, and 43 carbons, are quite abundant for many of the species. These late-eluting olefins have one to six double bonds. The positions of these double bonds were not determined, but for the purposes of this paper we feel it is not essential to know their location.

Polyunsaturated hydrocarbons are common in the termites of the BVI. This degree of unsaturation is not common in termites we have sampled from temperate or subtropical locations. For the termites that live in above-ground nests in dry habitats or entirely within dry wood, cuticular hydrocarbon mixtures consist of generally larger molecules, reflecting the moisture demands of this habitat (Collins et al., 1997; Hadley, 1980, 1985). Termites that live within wood on live trees or in situations with more available moisture generally have a hydrocarbon mixture composed mostly of lower-molecular-weight components with carbon numbers ranging from 23 to 33 (Collins et al., 1997).

Consistently abundant hydrocarbons can be used as taxonomic characters for separating the termites of the BVI. Variation in hydrocarbon components was shown for *N. acajutlae* from different islands, but the differences were relatively minor. *Incisitermes* presented the greatest challenge; the variation in cuticular hydrocarbons was as great as that of soldier morphology.

Acknowledgments—We thank J. D. Lazell and the Conservation Agency for support and discussion throughout this project. R. H. Scheffrahn reviewed an earlier version of this paper. We extend our sincere thanks to Dr. Henry and Gloria Jarecki and the staff of the Guana Island Club for their support and hospitality during the course of this project. This research was made possible by NSF grant BSR-8607407 to B.L.T. and a cooperative agreement between the University of Maryland and the Pacific Southwest Research Station.

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Journal of Chemical Ecology, Vol. 22, No. 11, 1996

HYDROCARBONS OF *Nasutitermes acajutlae* AND COMPARISON OF METHODOLOGIES FOR SAMPLING CUTICULAR HYDROCARBONS OF CARIBBEAN TERMITES FOR TAXONOMIC AND ECOLOGICAL STUDIES

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(Received October 30, 1995; accepted July 2, 1996)

Abstract—Using data from the arboreal nesting *Nasutitermes acajutlae* (Holmgren), we propose standard collection and extraction methodology for characterization of cuticular hydrocarbons of termites under field conditions in the tropics. Specifically, we evaluated: (1) the effect of the duration and the number of extractions; (2) the effect of drying termites before extraction; (3) the effect of sample size; (4) the effect of solvents (ethanol versus hexane) on cuticular hydrocarbon profiles. Olefins comprise ca. 70% of the cuticular hydrocarbons of *N. acajutlae*. Hydrocarbons consist of two distinct groups: early-eluting components, primarily *n*-alkanes and methyl-branched alkanes, and late-eluting compounds, which consist almost exclusively of unsaturated components with one to six double bonds. Soldiers have more early-eluting compounds than workers or alates. Nests from the same island had qualitatively similar, but quantitatively dissimilar hydrocarbon mixtures. Brief extractions of 300 live workers in 10 ml of hexane for only 20 sec produced a hydrocarbon mixture equivalent to a 10-min extraction. Long-term extraction of 300 workers in hexane for two years resulted in different mixtures of hydrocarbons. Drying workers tended to enhance extraction of the less abundant unsaturated compounds such as $C_{41:4}$ and $C_{41:5}$. A single extraction of a

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0098-0331/96/1100-2081$09.50/0

minimum of 100 workers (live or dried), with hexane for 20 sec to 10 min is best; these extraction regimes resulted in mixtures of hydrocarbons that are quantitatively very similar. For quantitative comparisons, extracts from dried samples should not be compared to those from live samples. Storage in ethanol caused numerous unidentified, nonhydrocarbon compounds to be extracted either from the cuticle or from internal tissues.

Key Words—*Nasutitermes acajutlae*, chemotaxonomy, Isoptera, Termitidae, tropical termites, gas chromatography, cuticular hydrocarbons, olefins, mass spectrometry.

INTRODUCTION

Termites play an essential role in the ecological dynamics of many tropical ecosystems, recycling nutrients and aerating soils in forests, mangrove swamps, and grasslands. Some species of termites are also economically important as structural pests in urban, as well as rural, areas of the tropics. The termite fauna of tropical regions is known to be diverse, but species diagnosis remains equivocal in many groups. Cuticular hydrocarbons are useful for discriminating termite species in tropical (Haverty et al., 1990b, 1991a, 1992, 1997; Howard et al., 1988) and temperate (Haverty et al., 1988, 1991a; Howard et al., 1978, 1982a,b; Thorne and Haverty 1989; Thorne et al., 1993) regions. Species sorting and diagnosis based on such chemical separation may then be used to facilitate discovery of morphological criteria for discriminating species (Haverty et al., 1988; Thorne and Haverty, 1989) and delimiting geographic distributions (Thorne et al., 1993).

Comparative research for characterizing cuticular hydrocarbons for taxonomic or ecological studies will necessitate uniform protocols among investigators. Field circumstances must also be considered. In the tropics, termites are usually collected far from laboratory facilities. Hydrocarbon extractions must therefore be delayed until well after the insects have been collected. Standard preservation techniques, such as freezing, are usually impractical. Keeping subcolonies, groups, or samples of a specific size alive and healthy for more than a few hours is very difficult. Hot sun and predacious ants usually render field samples unusable, and live cultures are prone to problems with humidity, food stress, and pathogenic fungi. Collection of specimens directly into 70% or 85% ethanol may partially extract hydrocarbons or include some debris and potential contaminants. Long-term retention of specimens in ethanol or in a solvent such as hexane for extended periods, although potentially quite convenient, may also extract internal hydrocarbons and confound the characterization of cuticular hydrocarbons.

We have collected termites from the islands in the Caribbean and have also received specimens collected by colleagues, for characterization of cuticular

hydrocarbons. The method of collection has varied among these efforts. To interpret these data we must know if different collection/extraction regimes are equivalent or comparable. We also want to recommend the most appropriate technique for a given taxon to collaborating scientists.

In the earliest studies of termites that introduced the concept of species specificity of cuticular hydrocarbons, it was not specified how the insects were handled before extraction (Howard et al., 1978, 1982a). In later studies of chemical mimicry by termitophilous staphylinids, Howard et al. (1980, 1982b) separated the termitophiles from the termites and froze the beetles at −20°C before extraction. Haverty et al. (1988) froze *Zootermopsis* spp. individuals, then subsequently thawed them to room temperature before extraction. The termites were brought from the field to the laboratory alive (Haverty et al., 1988). However, after extracting a few live termites, one of us (L.J.N.) noticed that the termites convulsed and emptied their gut contents into the hexane during the process. Freezing the insects, followed by extraction of the specimens at ambient temperature, prevented this possible source of contamination.

Subsequent studies of cuticular hydrocarbons of termites have used live termites extracted in the field or in the laboratory [Howard et al., 1988 (for *Nasutitermes*); Haverty et al., 1990b (for *Nasutitermes*), 1991a, unpublished (for *Reticulitermes*)], or termites dried in the field and later extracted in the laboratory [Haverty et al., 1990a, 1996b (for *Coptotermes*), 1991a, 1992 (for *Reticulitermes* and *Coptotermes*)]. Our preliminary results comparing extraction of live versus dried *Nasutitermes acajutlae* (Holmgren) indicated that the resulting hydrocarbon mixtures were not equivalent.

Many of our colleagues find it difficult to dry termites while collecting termites in the tropics because of logistical problems. Ovens, heating lamps, and electricity are not always available. Since drying is often impractical, many researchers prefer to collect termites directly into alcohol or hexane. Collectors also do not want to divert their efforts away from maximizing the number of samples they can collect while in the field. The usual field technique is to place termites directly into 80% or 100% ethanol for later transmittal to the museum or laboratory. Detailed comparisons of cuticular hydrocarbon patterns derived from extractions of live or dried termites or termites stored for extended periods in ethanol or hexane are thus warranted and are one focus of this study.

Another factor affecting the assessment of the cuticular hydrocarbon pattern is the number of insects that are extracted or quantity of hydrocarbon extracted. In some studies a variable number of individuals (and mixture of castes) was included. Haverty et al. (1991a) used from 15 to 200 *Reticulitermes* spp. workers per sample in their preliminary study of this genus. In most of our studies an exact number of termites was extracted: individual *Zootermopsis* spp. pseudergates, nymphs, soldiers, or alates (Haverty et al., 1988); 100 *Coptotermes formosanus* Shiraki workers or soldiers (Havery et al., 1990a); 200 *C. formo-*

sanus workers or 50 soldiers (Haverty et al. 1996b); and 100 *Nasutitermes costalis* (Holmgren) or *N. ephratae* (Holmgren) large workers (Haverty et al., 1990b).

Extraction of too few individuals can result in a diluted extract and require a deviation from a standard technique. Characterization of hydrocarbons from dilute extracts would likely underestimate or not detect the less abundant compounds and overestimate the abundant compounds. We know of no published studies to determine the minimum number of individuals necessary to characterize the cuticular hydrocarbons of any termite taxon.

One of the primary reasons we began studies of extraction methodology was to remedy a problem encountered during studies of the cuticular hydrocarbons of *N. corniger* (Motchulsky), *N. ephratae*, and *N. costalis* (Haverty et al., 1990b; Howard et al., 1988). Haverty et al. (1990b) experienced difficulty obtaining a sufficiently large hydrocarbon sample by extracting 100 large workers of *N. costalis* or *N. ephratae* in the field in Trinidad. The samples taken during that study were barely sufficient to allow quantification of the hydrocarbon mixtures. Thus, we designed a first set of experiments in 1989 to better understand the difficulties experienced with the characterization of cuticular hydrocarbons from *N. costalis* and *N. ephratae*.

Given that any taxonomic or ecological study entails processing a large number of samples, it is essential to keep the method of analysis as consistent as possible. We have developed chromatographic conditions and mass spectral acquisition parameters that give optimal results for the vast majority of insect samples. We therefore focused our efforts on comparing various collection and extraction regimes using our standard analytical technique.

In the present study, we attempted to define a standard methodology for collecting and extracting termites under field conditions. Our field work was based at our research site on Guana Island, British Virgin Islands (BVI) (Thorne et al., 1994; Haverty et al., 1997). This island has a diverse termite fauna consisting of nine species in three families. The most conspicuous, and apparently abundant, species is the arboreal nesting *N. acajutlae* (Thorne et al., 1994; Scheffrahn et al., 1994). This species, as well as a closely related species, *N. nigriceps* (Holmgren), is numerically and ecologically conspicuous on many of the Caribbean islands (Thorne et al., 1994). A better understanding of the appropriate procedure(s) for sampling and extracting cuticular hydrocarbons of *N. acajutlae* is important to our understanding of the taxonomy, ecology, and biogeography of this and other *Nasutitermes* species. The methodology described here is optimal for sampling and extracting cuticular hydrocarbons for this species and should be applicable to most termites in the tropics. Very small termites, such as *Parvitermes* spp., will likely require larger samples, whereas the larger termites, such as *Neotermes* spp. and *Incisitermes* spp., will require fewer individuals.

For taxonomic studies we consider cuticular hydrocarbons useful primarily for separating species within a genus; obvious and consistent morphological characters usually suffice to separate taxa at higher levels (Haverty et al., 1991b). Hydrocarbons beneficial for taxonomic studies should be abundant, not minor components (at least 1.0%, but preferably 5.0% of the total hydrocarbon component). However, if a hydrocarbon is consistently detected, even in quantities below 1.0%, we would consider it a useful taxonomic character. Useful hydrocarbons should also be unique or present in only a few of the species, or conversely, they should be common in most of the species yet completely absent, rare, or of insignificant quantities in one or a few species. Furthermore, they should have a unique elution time so that they do not coelute with another hydrocarbon in the same species, nor should they elute at a time similar to that of a different hydrocarbon in a different species (Haverty et al., 1991b). From a set of hydrocarbons with these characteristics, dichotomous keys can be constructed. For ecological or biogeographic studies of the same species, examinations of the quantitative differences of the hydrocarbon mixture would be of paramount importance.

In this paper we report the results of studies conducted in 1989 and 1993 to compare and improve our sampling and extraction techniques for the characterization of cuticular hydrocarbons of tropical termites. In 1989 we examined influences of the number and duration of extractions. In 1993 we tried to design studies that would ultimately provide us with an optimal field method for characterizing both the composition and relative abundance of the cuticular hydrocarbons of *N. acajutlae*. We compare methodologies and suggest standard and alternative, acceptable methodologies for both chemotaxonomic and ecological studies of this termite. Specifically, we evaluate: (1) the effect of the duration and the number of extractions; (2) the effect of drying termite specimens before extraction; (3) the effect of sample size; and (4) the effect of solvents (ethanol versus hexane) on the reproducibility of cuticular hydrocarbon profiles.

METHODS AND MATERIALS

Collecting and Sorting Termites. We collected workers and soldiers of *N. acajutlae* during two separate trips (1989 and 1993) to Guana Island, BVI. During each trip different collection techniques were used. In 1989 two nests were sampled near White Sands Beach. Portions of nest material containing workers and soldiers were returned to the laboratory. Nest material was placed in pans and folded cardboard index cards were placed on top of this material. Workers and soldiers climbed onto the cards and then were tapped off into trays, where they were sorted, counted, and prepared for hydrocarbon analyses as described below.

In 1993 we selected 13 colonies from the same area, and we used a modification of a technique demonstrated to us by Dr. Jan Krecek. This is the best technique for collecting large numbers of workers and soliders with the least effect on nest structure. A tangential slice, 2–3 cm deep and 15–20 cm in diameter, was removed from the more fragile exterior portion of a nest and discarded. Over this breach we placed one or two 20-cm × 20-cm squares of moist corrugated cardboard. If we were able to cut the slice from the top of a nest, the corrugated cardboard squares were secured against the surface of the nest with a stone. If the slice was taken from the side of the nest (often we could not reach the top of a nest) the corrugated cardboard squares were secured to the nest with 7.5 cm, galvanized, finishing nails.

As soon as the slice of nest was removed and the corrugated cardboard squares put in place, soldiers swarmed out of the breach and covered the squares; workers immediately retreated into the nest. In less than 60 sec the squares were removed and a collection of nearly pure soldiers was tapped into a collection pan. To readily obtain a nearly pure sample of hundreds to thousands of soldiers, this process can be repeated several times. We then visually scanned all individuals in the collection pan and removed the few workers in the sample.

Workers were collected by leaving the moist squares of corrugated cardboard on the nest for up to 60 min. Once the alarm reaction of the soldiers began to dissipate, workers ventured to the underside of the squares and began to repair the breach. When the cardboard was gently removed, a dense sample of workers adhered to its surface. In contrast to the technique for collecting soldiers, the squares of corrugated cardboard containing the workers (and relatively few soldiers) were placed in a collection pan and returned to the field laboratory for sorting, counting, and preparation of workers for hydrocarbon analyses as described below.

Preparation of Termites for Extraction. Because soldiers squirt glue over the containers and themselves, they were difficult to count individually. For the purposes of cuticular hydrocarbon analyses, we measured ca. 5–8 ml of soldiers (about 500 individuals) in 20-ml scintillation vials (Wheaton scintillation vials, foil-lined caps) for extraction or drying. Workers were separated and individually counted into 20-ml scintillation vials for extraction or drying of the appropriate number of individuals. Alates were occasionally present; when collected, they were individually counted into vials and handled in exactly the same way as the workers.

Additional termites from each of the sampled colonies (with soldiers and alates, when available, as diagnostic castes) were placed in 80% ethanol (from Quantum Chemical Corp., 200-proof punctilious dehydrated alcohol) to serve as voucher specimens. These voucher specimens are kept at the Pacific Southwest Research Station, Albany, California, and the Department of Entomology, University of Maryland, College Park, Maryland.

Standard Extraction Process. Cuticular lipids were extracted by immersing termites in *n*-hexane (EM Science OmniSolv, suitable for HPLC, GC, and residue analysis). Our usual procedure has been a 10-min extraction of 100 termites in 10 ml of *n*-hexane. In this study, we used this procedure as the standard to evaluate the various extraction regimes described in the following sections. The lipid extracts resulting from each of the various methods were pipetted through 4 cm of activated BioSil-A (silica gel, BioRad Laboratories, 100–200 mesh) in Pasteur pipet minicolumns (5 mm ID) in order to isolate the hydrocarbon components. The resulting hydrocarbon extracts were evaporated to dryness under nitrogen and redissolved in 60 μl of *n*-hexane for gas chromatography–mass spectrometry (GC-MS) analyses.

Duration and Number of Extractions. In 1989 we evaluated the following extraction regimes using *N. acajutlae* from Guana Island, BVI, to determine whether we could improve upon extracting 100 live workers in 10 ml of *n*-hexane for 10 minutes:

A. Extract 300 workers in 10 ml *n*-hexane for 10 min (standard technique with an increased sample size).

B. Extract 300 workers three times in 10 ml of *n*-hexane for 10 min, keeping each extract separate to determine if the standard technique left significant quantities of hydrocarbon on the sample.

C. Extract 300 workers in 10 ml of *n*-hexane for 20 sec followed by a 10-min extraction in 10 ml of *n*-hexane, keeping each extract separate to determine whether a brief extraction would produce a mixture of hydrocarbons comparable to the standard, A, extraction.

D. Extract 300 workers in 10 ml of *n*-hexane for 20 sec followed by extracting for 24 hr in 10 ml *n*-hexane, keeping each extract separate (same as extraction B with an extended second extraction) to determine whether an extended extraction would remove additional hydrocarbons from the cuticle or from other tissues.

E. Extract 300 workers in 10 ml of *n*-hexane for 24 hr to allow for a less stringent extraction schedule.

F. Long-term extraction of 300 workers in 10 ml of *n*-hexane for two years to allow field collection with subsequent laboratory storage for an extended period.

Effect of Drying. In 1993 six samples of 100 *N. acajutlae* workers from each of 13 nests were allocated to evaluate the effect of drying termites prior to extraction. The cuticular hydrocarbon profiles of the dried termites were compared to those generated from an extraction of live termites. Live termites were extracted at the Guana Island, BVI, field laboratory by placing each of three samples from each colony directly into separate 20-ml scintillation vials and extracting these samples in 10 ml of *n*-hexane for 10 min (standard procedure A). The hexane from each of 39 vials was then decanted into separate 20-ml

scintillation vials and subsequently returned to our laboratory in California for purification and analysis of cuticular hydrocarbons.

The other three samples of *N. acajutlae* workers from each of 13 colonies were dried by placing 20-ml scintillation vials, each containing 100 individuals, in a wire box over a single 75-W, reflecting, incandescent light. The amount of time required to completely dry termites varied slightly as a function of the position of the vials over the bulb. The position of the vials over the bulb was changed in an attempt to make the drying time similar for each sample. Once termites were completely dried, we attempted to keep them dry by tightly sealing the vials. The vials were returned to our laboratory in California for extraction, purification, and analysis of cuticular hydrocarbons. Until they were extracted, dried samples were kept in a freezer at −20°C in California to prevent possible fungal growth.

Effects of Sample Size. In 1993, samples of 25, 50, 100, or 200 *N. acajutlae* workers from five different colonies were extracted either live or dried as described above. Each combination (sample size × live versus dried × colony) was replicated three times.

Effects of Solvent. In 1993, for each of five colonies, three replicates of 200 workers were placed in 20 ml of 100% ethanol and left in the solvent for 60 days. The ethanol extract was decanted and evaporated to dryness under nitrogen. The extract was then redissolved in 10 ml of hexane and processed by the standard procedure. After the ethanol was drained from the termites, the insects were dried and extracted with hexane following the standard procedure.

Characterization of Cuticular Hydrocarbons. Each sample was analyzed by GC-MS in order that the presence of all compounds could be verified by mass spectral data. These data allowed us to obtain information about coeluting compounds in particular. GC-MS analyses were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a HP 5970B Mass Selective Detector interfaced with HP Chemstation software. The GC-MS was equipped with an HP-1, fused silica capillary column (25 m × 0.2 mm ID) and operated in split mode (with a split ratio of 8:1). A 3-μl aliquot was injected into the GC-MS. Each mixture was analyzed using a temperature program from 200°C to 320°C at 3°C/min with a final hold of 16 min. Electron impact (EI) mass spectra were obtained at 70 eV. *n*-Alkanes were identified by their mass spectra. Mass spectra of methylalkanes were interpreted as described by Blomquist et al. (1987) to identify methyl branch locations. Alkenes were identified by their retention times relative to *n*-alkanes and/or mass spectra; the latter only for olefins with 35 or more carbons. A typical alkene mass spectrum shows a molecular ion and a series of fragments at 14-mass unit intervals (e.g., 69, 83, 97), similar to those displayed by *n*-alkanes, less 2 mass units. Interpretation of the mass spectra of dienes and polyunsaturated hydrocarbons was extrapolated from this pattern, i.e., for each double bond, the molecular ion is decreased by 2 mass units.

In the text and tables, we use shorthand nomenclature to identify individual hydrocarbons or mixtures of hydrocarbons. This shorthand uses a descriptor for the location of methyl groups (X-Me), the total number of carbons (subscript XX) in the hydrocarbon component excluding the methyl branch(es), and the number of double bonds following a colon (subscript Y). Thus, pentacosane becomes n-C_{25}; 11-methylpentacosane becomes 11-MeC_{25}; and pentacosene becomes $C_{25:1}$. Hydrocarbons are presented in the tables in the order of elution on our GC-MS system.

Integration of the total ion chromatogram was performed by the data analysis software (HP59974J Rev. 3.1.2) in the HP Chemstation. GC-MS peak areas were converted to percentages of the total hydrocarbon fraction. These percentages for each hydrocarbon peak were the response variables used to make statistical comparisons among preparation and extraction techniques.

Statistical Analyses. The effect of drying termites before extraction was assessed by a series of t tests of the differences between the mean percentage of a given hydrocarbon (three samples from each colony were averaged, which resulted in 13 replicates, one for each colony) from the two conditions (Steel and Torrie, 1960). The significance of the calculated t value was tested at $\alpha = 0.00227$ [0.05/22, the number of consistently abundant (0.3% or greater of the total) hydrocarbon peaks for workers]. Our null hypothesis was that the 10-min extraction of 100 dried termites was not significantly different from the standard 10-min extraction of 100 live termites.

The effect of group size was tested with an analysis of variance for each hydrocarbon (Steel and Torrie, 1960). Each treatment combination (sample size × colony) was replicated three times. The four sample sizes were compared separately for termites extracted live or dried. Our null hypothesis was that all sample sizes provide extracts with the same percentages of each hydrocarbon. The significance of the F statistic was also tested with $\alpha = 0.00227$. Significant differences among mean percentages for each hydrocarbon were determined by Tukey's honestly significant difference (HSD) procedure. We were looking for the smallest sample size that results in a hydrocarbon profile equivalent in percentage of hydrocarbons to that of the next greatest sample size. A sample size was considered inadequate if it yielded a hydrocarbon mixture that had significantly different percentages from that of the next larger sample size.

RESULTS AND DISCUSSION

Cuticular Hydrocarbons of N. acajutlae

We identified 33 hydrocarbons from workers, 45 from soldiers, and 43 from alates of *N. acajutlae*; a total of 60 different hydrocarbons or isomeric mixtures in this species (Table 1; Figure 1). The hydrocarbons found in all three

TABLE 1. RELATIVE ABUNDANCE (MEAN PERCENT AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS OF WORKERS, SOLDIERS, AND ALATES OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND, BVI[a]

	Workers		Soldiers		Alates	
Hydrocarbon[b]	Mean	SD	Mean	SD	Mean	SD
$C_{23:1}$	0.32	0.32	0.41	0.17	0.00	0.00
C_{23}	0.97	0.33	4.04	0.91	0.08	0.04
11-; 9-MeC_{23}[c]	0.00	0.00	0.51	0.15	0.00	0.00
3-MeC_{23} + $C_{24:1}$[d]	0.00	0.00	0.18	0.15	0.00	0.00
C_{24}	0.22	0.22	0.66	0.19	0.00	0.00
11-MeC_{24}	0.00	0.00	0.37	0.32	0.00	0.00
$C_{25:1}$	0.68	0.27	2.96	0.69	0.00	0.00
C_{25}	3.05	1.14	9.16	1.28	0.55	0.14
13-; 11-MeC_{25}[c]	0.16	0.17	1.98	0.43	0.12	0.03
5-MeC_{25}	0.00	0.00	0.00	0.00	0.07	0.01
2-MeC_{25}	0.00	0.00	0.00	0.00	0.20	0.03
3-MeC_{25} + $C_{26:1}$[d]	0.00	0.00	0.57	0.13	0.00	0.00
5,17-DimeC_{25}	0.00	0.00	0.00	0.00	0.08	0.01
C_{26}	0.26	0.27	0.59	0.16	0.14	0.02
13-; 12-; 11-MeC_{26}[c]	0.00	0.00	0.42	0.35	0.00	0.00
2-MeC_{26}	0.00	0.00	0.00	0.00	1.42	0.16
$C_{27:1}$	0.86	0.42	3.88	0.63	0.00	0.00
C_{27}	1.90	1.60	3.67	0.92	1.44	0.28
13-; 11-MeC_{27}[c]	0.07	0.11	1.14	0.21	0.00	0.00
5-MeC_{27}	0.00	0.00	0.00	0.00	0.08	0.04
2-MeC_{27}	0.15	0.13	0.14	0.09	1.67	0.29
3-MeC_{27}	0.00	0.00	0.00	0.00	0.72	0.13
C_{28}	0.15	0.19	0.13	0.12	0.30	0.08
2-MeC_{28} + $C_{29:1}$[d]	0.00	0.00	0.19	0.10	0.53	0.11
$C_{29:1}$[e]						
C_{29}	0.73	0.56	0.56	0.25	1.08	0.25
5-MeC_{29}	0.00	0.00	0.00	0.00	0.07	0.05
2-MeC_{29}	0.00	0.00	0.00	0.00	0.03	0.03
3-MeC_{29}	0.00	0.00	0.00	0.00	0.04	0.05
C_{30}	0.00	0.00	0.00	0.00	0.05	0.04
C_{31}	0.06	0.15	0.02	0.04	0.58	0.08
C_{33}	0.00	0.00	0.01	0.03	0.12	0.03
$C_{35:1}$	0.00	0.00	0.00	0.00	0.11	0.06
13-MeC_{33}	0.00	0.00	0.00	0.00	0.12	0.06
$C_{37:1}$	0.40	0.17	0.65	0.17	0.40	0.14
$C_{38:1}$	0.53	0.20	0.58	0.13	0.47	0.07
$C_{39:5}$[f]	0.36	0.33	0.28	0.25		
$C_{39:4}$[f]	2.00	0.73	1.33	0.61	3.89	0.78
$C_{39:2}$	0.43	0.48	1.29	0.56	1.14	0.11
$C_{39:1}$	20.01	2.20	15.77	2.79	14.40	1.26
15-MeC_{39}	0.04	0.12	0.36	0.27	0.00	0.00

TABLE 1. Continued

Hydrocarbon[a]	Workers		Soldiers		Alates	
	Mean	SD	Mean	SD	Mean	SD
$C_{40:5}$	0.00	0.00	0.00	0.00	0.90	0.35
$C_{40:1}$	2.51	0.42	2.89	0.62	0.00	0.00
$C_{41:6} + C_{41:5}$[c]	18.77	3.81	11.01	3.92	21.72	2.41
$C_{41:2}$	1.21	0.63	1.50	0.49	3.31	0.20
$C_{41:1}$	24.43	2.74	17.42	3.52	20.24	1.18
15-MeC_{41}	0.13	0.23	0.46	0.24	0.76	0.15
$C_{42:1}$	1.60	0.65	1.99	1.03	0.00	0.00
$C_{43:6} + C_{43:5}$[h]	4.35	1.37	2.41	1.09	6.24	1.11
$C_{43:2}$	0.83	0.52	1.14	0.43	2.63	0.27
$C_{43:1}$	10.40	1.61	7.66	1.48	10.28	1.09
15-MeC_{43}	0.00	0.00	0.00	0.00	0.43	0.11
$C_{45:5}$[e]						
$C_{45:2}$	0.00	0.00	0.00	0.00	0.32	0.11
$C_{45:1}$	2.41	0.82	1.69	0.64	3.26	0.87

[a] Three subsamples of 100 workers from each of 13 colonies. Four subsamples of ca. 4 ml of soldiers from each of 13 colonies. Two subsamples of 25–31 alates from four colonies.

[b] This shorthand (X-MeC_{XX} and $C_{XX:Y}$) uses descriptors for the location of the methyl group (X-Me), the total number of carbons (XX), excluding the methyl branch(es), and the number of double bonds (Y). Locations of double bonds are undetermined.

[c] An isomeric mixture. These monomethylalkanes coelute.

[d] This monomethylalkane and the olefin coelute.

[e] These hydrocarbons were not detected in samples from Guana Island, but were found in samples from Tortola, BVI. $C_{29:1}$ was found in trace amounts in soldiers only; $C_{45:5}$ was found in all three castes, but in amounts averaging $< 1.0\%$.

[f] These two isomers occasionally did not resolve in alates. Therefore, the areas for the two isomers were summed for alates only.

[g] These two isomers occasionally did not resolve. Therefore, the areas for the two isomers were summed.

[h] $C_{43:6}$ was identified only in alates. There were two isomers of $C_{43:5}$ that occasionally did not resolve. Therefore, the areas for the two isomers were summed.

castes segregate into two distinct groups. The early-eluting components are primarily *n*-alkanes, methyl-branched alkanes, and a few normal alkenes. The late-eluting compounds consist almost exclusively of unsaturated hydrocarbons with chain lengths of 37–45 carbons and one to six double bonds and a few monomethyl alkanes in trace amounts (especially in alates). Soldiers have considerably greater proportions of the early-eluting compounds (23–29 carbons) than do workers or alates (Table 1; Figure 1). Whereas workers and alates have at least 88% of the cuticular hydrocarbons with 31 or more carbons, soldiers have only about 69% of these late-eluting compounds. The predominant class

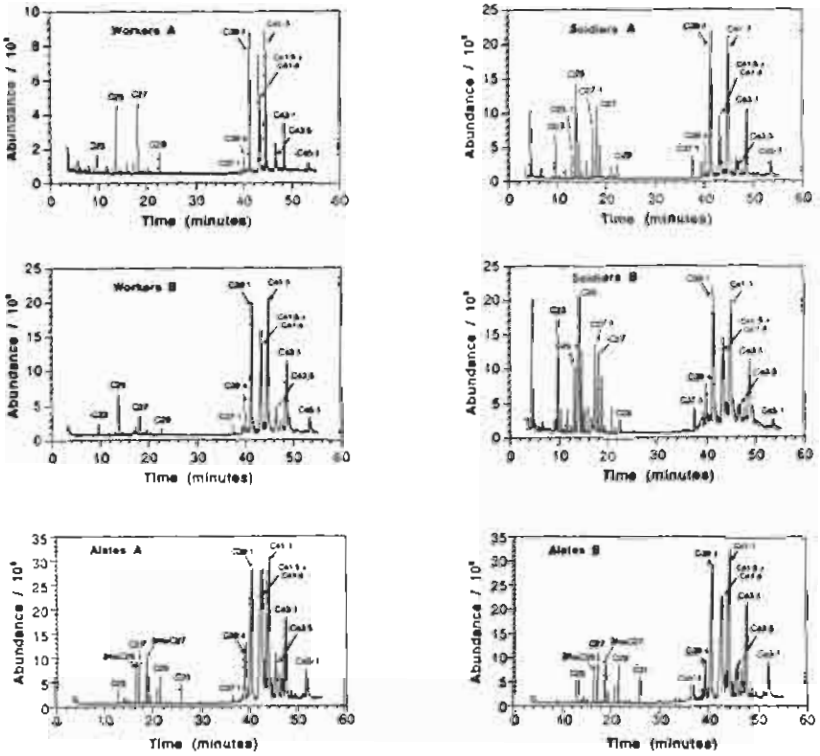

FIG. 1. Total ion chromatograms of the cuticular hydrocarbons of workers, soldiers, and alates of *Nasutitermes acajutlae* (Holmgren) from two colonies (A and B) from Guana Island, BVI. The cuticular hydrocarbon extracts were derived from dried samples of 100 workers, ca. 8 ml of soldiers, and 25 alates collected in October 1993.

of hydrocarbons in all three castes is the olefin fraction (Table 1). No dimethyl- or trimethylakanes were found in workers or soldiers.

n-Alkanes. Normal alkanes ranged from n-C_{23} to n-C_{33}; n-C_{25} and n-C_{27} were usually the most abundant. The n-alkanes were least abundant in alates, moderately abundant in workers, and most abundant in soldiers (Tables 1; Figure 1).

Internally Branched Monomethylalkanes. This class of hydrocarbon was nearly always encountered, in trivial amounts in workers and in significant amounts in soldiers (5.2% of the total hydrocarbon), and accounted for only 1.4% of the total hydrocarbon in alates. For soldiers most of this class of hydrocarbon eluted early, while those found in alates were in the late-eluting fraction (Table 1).

Terminally Branched Monomethylalkanes. The 2- and 3-methylalkanes occurred in trivial amounts in workers and soldiers. Alates usually had a significant component of 2-methylalkanes. These hydrocarbons were always found in the early-eluting constituents (Table 1; Figure 1).

Dimethyl- and Trimethylalkanes. Only one dimethylalkane was identified: 5,17-dimeC_{25}. It was found only in trivial amounts in alates (Table 1).

Olefins. The unsaturated component is the paramount class of hydrocarbons in the cuticular lipids of *N. acajutlae*. Olefins comprise over 90% of the total hydrocarbons in workers and alates. Because soldiers contain a larger proportion of early-eluting *n*-alkanes, the olefin component amounts to an average of 75.6% of the total hydrocarbons in this caste. For all castes, $C_{39:1}$, $C_{41:5}$, $C_{41:4}$, $C_{41:1}$, and $C_{43:1}$ combined account for >70% of the total olefin fraction (Table 1).

We are confident in our identification of the late-eluting monounsaturated alkenes. The spectra of $C_{39:1}$ and $C_{41:1}$ display the parent ion (546 and 574, respectively) as well as the characteristic fragmentation pattern with the predominant peaks of fragments (83 and 97) being 2 mass units less than would be expected from *n*-alkanes and exceeding fragment 67 in abundance (Figure 2). The polyunsaturated components, for example, $C_{41:4}$ and $C_{41:5}$, are identified by the presence of parent ions (568 and 566, respectively) which are 8 and 10 mass units less than the normal alkane with the same number of carbons (Figure 3).

Polyunsaturated alkenes have not been commonly reported from the cuticular hydrocarbons of termites. However, Moore (1969) was the first to describe the cuticular lipids from a termite, *Nasutitermes exitiosis* (Hill), from Australia. He found a complex mixture of unsaturated components with the predominant component identified as a quadrupally unsaturated, unbranched hydrocarbon, $C_{39:4}$. Similarly, we have identified high-molecular-weight dienes, trienes, and tetraenes from the cuticular lipids of *Cryptotermes brevis* (Walker), *Procryptotermes corniceps* (Snyder), *Incisitermes* spp., *Parvitermes wolcotti* (Snyder), and *N. acajutlae*, as well as other species of *Nasutitermes* from the Caribbean Basin (Haverty et al., 1992, 1997).

Effect of Duration and Number of Extractions

Early in our research on the chemotaxonomy of termites we extracted live or recently frozen individuals. The standard 10-min extraction of 300 live workers of *N. acajutlae* (Figure 4A) allowed us to resolve and characterize most of the components identified for this species (Tables 1 and 2). Additional components were identified by drying termites first (see Table 1 and *Effects of Drying* below). We also discovered that different nests of this species from the same island produced qualitatively similar (not identical), but quantitatively dissimilar, hydrocarbon mixtures (Figure 1; and unpublished observations). Further-

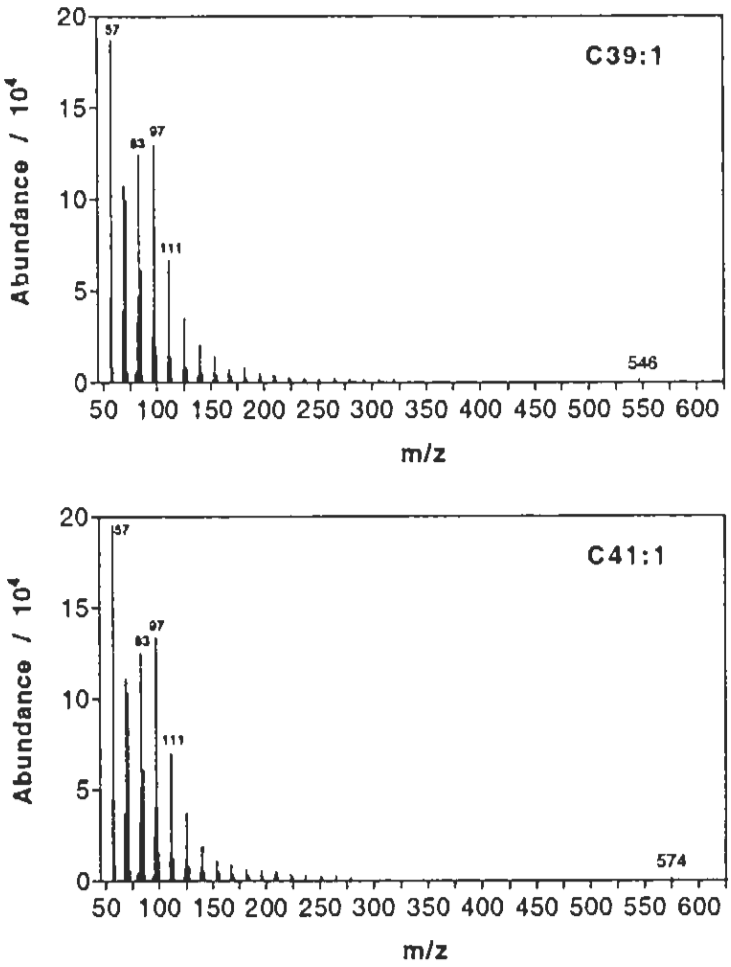

FIG. 2. Mass spectra of nonatriacontene ($C_{39:1}$) and hentetracontene ($C_{41:1}$) from cuticular hydrocarbons from workers of *Nasutitermes acajutlae* from Guana Island, BVI.

more, these colony-specific hydrocarbon analyses are repeatable; when a second group of 300 live workers was extracted using the standard procedure, the hydrocarbon mixtures from each colony were qualitatively identical and quantitatively quite similar (Figure 4A and B1; Table 2). A second 10-min extraction of the same 300 workers resulted in a hydrocarbon mixture that was quite similar to the mixture from the first extraction (Figure 4B2), although the ion abundances of the peaks were about half those in the first extract. A third, 10-min extraction resulted in an extract that did not resemble those from either of the

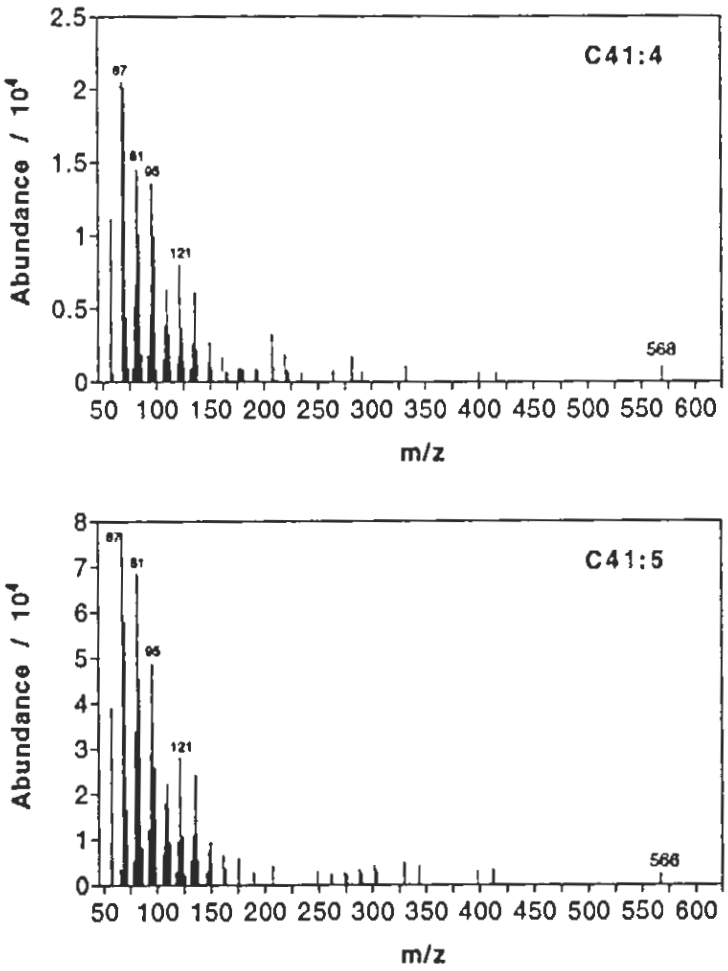

FIG. 3. Mass spectra of hentetracontatetraene ($C_{41:4}$) and hentetracontapentaene ($C_{41:5}$) from cuticular hydrocarbons from workers of *Nasutitermes acajutlae* from Guana Island, BVI.

first two extractions; only the predominant peaks (C_{25}, C_{27}, $C_{39:1}$, and $C_{41:1}$) were detected (Table 2; Figure 4B3).

An extraction of 300 live workers in 10 ml of hexane for only 20 sec appeared to be equivalent to a 10-min extraction (Table 2; Figure 4A, C1, and D1). Furthermore, the 20-sec extraction was repeatable. A subsequent 10-min extraction of the same workers produced a hydrocarbon mixture similar to that of a 20-sec extraction, although the ion abundances of the peaks were about

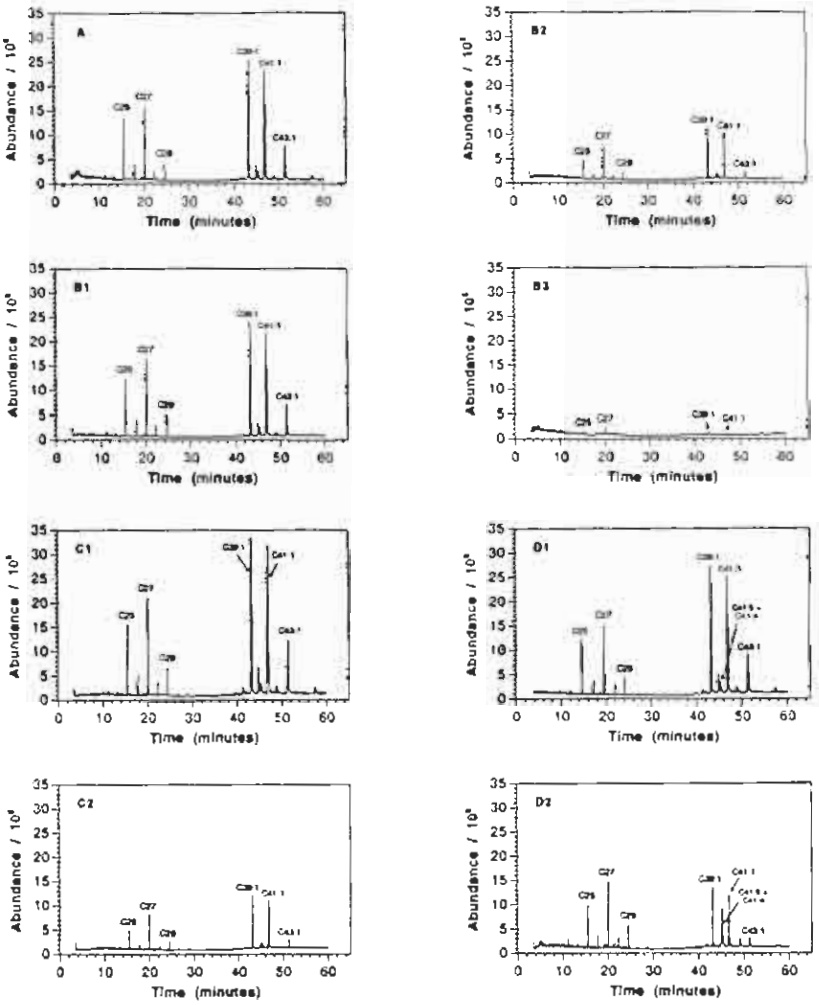

FIG. 4. Chromatograms of cuticular hydrocarbons extracted from 300 live workers from one nest of *Nasutitermes acajutlae* from Guana Island, BVI. A = extraction with 10 ml hexane for 10 min (standard); B1 = first extraction with 10 ml hexane for 10 min (equivanent to the standard); B2 = second extraction of the same termites as B1 with 10 ml hexane for 10 min; B3 = third extraction of the same termites as B1 and B2 with 10 ml hexane for 10 min; C1 = first extraction with 10 ml hexane for 20 sec; C2 = second extraction of the same termites as C1 with 10 ml hexane for 10 min; D1 = first extraction with 10 ml hexane for 20 sec (equivanent to C1); D2 = second extraction of the same termites as D1 with 10 ml hexane for 24 hr; E = extraction with 10 ml hexane for 24 hr; F = extraction with 10 ml hexane for two years. Samples A–F were each derived from independent groups of 300 workers.

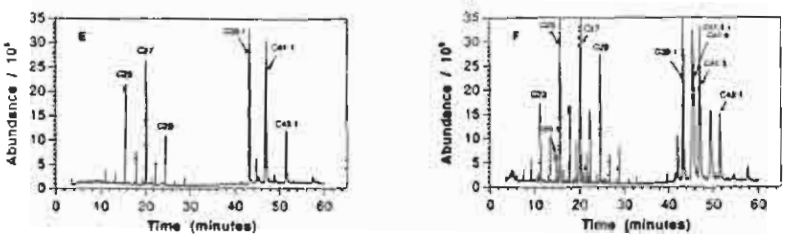

FIG. 4. Continued.

TABLE 2. RELATIVE ABUNDANCE (MEAN PERCENT FROM TWO COLONIES) OF CUTICULAR HYDROCARBONS OF WORKERS OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND, BVI, RESULTING FROM DIFFERENT EXTRACTION REGIMES[a]

Hydrocarbon[b]	A	B1	B2	B3	C1	C2	D1	D2	E
C_{23}	0.3	0.4	0.0	0.0	0.2	0.0	0.3	1.5	0.6
C_{24}	0.3	0.3	0.0	0.0	0.2	0.0	0.2	0.5	0.3
$C_{25:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C_{25}	4.7	4.7	5.3	18.4	3.5	4.9	3.9	7.5	5.6
C_{26}	0.9	1.0	0.9	0.0	0.7	0.9	0.7	1.4	1.1
2-MeC_{26} + $C_{27:1}$	0.4	0.3	0.0	0.0	0.1	0.0	0.2	1.2	0.5
C_{27}	5.1	6.1	7.5	20.1	4.2	7.5	4.3	9.5	6.7
C_{28}	0.4	0.6	0.8	0.0	0.4	0.8	0.4	1.3	0.7
C_{29}	1.0	1.3	1.8	0.0	0.9	1.8	0.8	3.0	1.7
$C_{37:1}$	0.2	0.2	0.2	0.0	0.3	0.0	0.3	0.0	0.3
$C_{38:1}$	0.4	0.6	0.7	0.0	0.8	0.0	0.6	0.2	0.4
$C_{39:4}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
$C_{39:1}$	32.0	30.0	31.7	34.3	31.3	32.6	31.4	23.0	28.4
$C_{40:1}$	2.7	2.9	2.4	0.0	2.9	1.6	3.0	1.8	2.5
$C_{41:5}$	0.7	0.7	1.6	0.0	0.7	1.2	0.7	3.9	0.7
$C_{41:4}$	0.9	0.9	2.4	0.0	0.9	2.3	1.5	8.0	0.7
$C_{41:1}$	37.1	35.4	35.9	27.2	37.7	38.2	37.2	25.9	35.3
$C_{42:1}$	0.9	1.1	0.4	0.0	1.2	0.0	1.2	0.0	1.0
$C_{43:5}$	0.0	0.1	0.0	0.0	0.0	0.0	0.4	2.0	0.0
$C_{43:1}$	11.2	11.2	8.0	0.0	11.8	8.3	11.3	6.3	10.9
$C_{45:1}$	0.6	1.5	0.4	0.0	1.6	0.0	1.7	0.1	1.4

[a] Extraction regimes A–F are outlined in the Methods and Materials and in the legend of Figure 4. Extraction procedure F was not included because of numerous unique, unknown, undetermined, or different peaks.

[b] This shorthand (X-MeC_{XX} and $C_{XX:Y}$) uses descriptors for the location of the methyl group (X-Me), the total number of carbons (XX), excluding the methyl branch(es), and the number of double bonds (Y). Locations of double bonds are undetermined.

half those in the first extract (Table 2; Figure 4C1 and C2). Extraction of 300 workers for 24 hr after a 20-sec extraction resulted in an extract that was different from the standard extraction (10 ml for 10 min) or the 20-sec extraction. Proportional relationships changed dramatically: C_{25} and C_{27} were much more prominent as were $C_{41:4}$ and $C_{41:5}$, in extracts from the second extraction (Table 2; Figure 4A, D1, and D2).

Extraction of 300 workers for 24 hr resulted in a mixture of hydrocarbons that appeared similar to the standard 10-min extraction or to a 20-sec extraction (Table 2; Figure 4A, C1, D1, and E). Holding a sample of 300 workers in hexane for a period of two years provided a radically different mixture of hydrocarbons than the standard 10-min extraction (Figure 4F). Many hydrocarbons that we rarely see in *N. acajutlae* workers (such as C_{22}, $C_{23:1}$, $C_{24:1}$, two isomers of $C_{25:1}$, $C_{26:1}$, 11- and 13-MeC_{25}, $C_{27:2}$, 2-MeC_{26}, $C_{29:2}$, 5-MeC_{29}, $C_{31:1}$, and $C_{43:2}$) were present in quantities exceeding trace ($\bar{X} < 0.3\%$) amounts. One isomer of $C_{25:1}$ was identified in extractions of workers of *N. acajutlae* in 1993. However, the additional isomer (same mass spectrum with a different retention time) was not seen in the 1993 extractions. Furthermore, some compounds (C_{25}, C_{27}, $C_{41:5}$, and $C_{41:4}$) were present in much greater proportions. As a result of these qualitative and quantitative differences, we feel that a characteristic mixture of cuticular hydrocarbons can be obtained with extractions lasting from 20 sec to 24 hr. We do not consider the hydrocarbon mixtures from two-year extractions to be comparable to the standard 10-min extraction.

Effect of Drying

Mixtures of cuticular hydrocarbons extracted from live or dried workers were quantitatively different from one another. Of the 32 hydrocarbon peaks, the percentages of 20 of them were significantly different (Table 3). The most striking differences were exhibited in the late-eluting alkenes (Table 3; Figure 5). Drying the workers before extraction resulted in highly significant differences in the relative amounts of $C_{41:4}$ and $C_{41:5}$. Related to the apparent increased efficiency of the extraction of these compounds was the apparent decrease in the relative amounts of the most abundant compounds, $C_{39:1}$, $C_{41:1}$, and $C_{43:1}$.

In general, drying workers prior to extraction tended to enhance extraction of the less abundant unsaturated compounds. Extraction of 100 dried workers did not result in equivalent mixtures of hydrocarbons when compared to extraction of 100 live workers; these extraction regimes are not comparable for taxonomic or ecological purposes. Using either one of these extraction regimes exclusively would suffice for characterization of cuticular hydrocarbons for ecological studies, because the primary consideration would be for comparing the relative or absolute quantities of hydrocarbons. However, for taxonomic studies, the goal is to identify hydrocarbons that are consistently present even in quan-

TABLE 3. RELATIVE ABUNDANCES (MEAN PERCENT AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS FROM SAMPLES OF 100 WORKERS OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND, BVI, EXTRACTED ALIVE OR AFTER DRYING

Hydrocarbon[a]	Extracted live[b]		Extracted dry[b]		
	Mean	SD	Mean	SD	t value[c]
C_{23}	0.80	0.29	0.97	0.33	−3.029
C_{24}	0.45	0.27	0.22	0.22	3.348
$C_{25:1}$	0.36	0.35	0.68	0.27	−4.445
C_{25}	2.64	0.95	3.05	1.14	−2.618
C_{26}	0.53	0.32	0.26	0.27	4.453
$C_{27:1}$	0.34	0.33	0.86	0.42	−6.610
C_{27}	1.40	0.95	1.90	1.60	−2.706
C_{28}	0.37	0.22	0.15	0.19	3.251
C_{29}	0.53	0.34	0.73	0.56	−2.438
$C_{37:1}$	0.45	0.22	0.40	0.17	1.724
$C_{38:1}$	0.63	0.25	0.53	0.20	3.537
$C_{39:4}$	0.54	0.40	2.00	0.73	−7.526
$C_{39:2}$	0.08	0.36	0.43	0.48	−3.272
$C_{39:1}$	28.12	2.49	20.01	2.20	17.314
$C_{40:1}$	2.84	0.27	2.51	0.42	3.928
$C_{41:4} + C_{41:5}$	6.23	3.61	18.77	3.81	−16.929
$C_{41:1}$	33.77	2.73	24.43	2.74	15.720
$C_{42:1}$	1.46	0.50	1.60	0.65	−1.650
$C_{43:5}$	1.01	1.13	4.35	1.37	−9.746
$C_{43:2}$	0.04	0.12	0.83	0.52	−5.876
$C_{43:1}$	13.15	1.41	10.40	1.61	11.519
$C_{45:1}$	3.44	0.66	2.41	0.82	6.233

[a] This shorthand (X-MeC_{XX} and $C_{XX:Y}$) uses descriptors for the location of the methyl group (X-Me), the total number of carbons (XX), excluding the methyl branch(es), and the number of double bonds (Y). Locations of double bonds are undetermined.

[b] Three subsamples of 100 workers from 13 colonies were either placed in a scintillation vial alive, then extracted for 10 min with 10 ml of hexane or placed in a vial, dried over an incandescent light, then extracted for 10 min with 10 ml of hexane.

[c] The critical t for $\alpha = 0.00227$ with 12 df is 3.859 for each comparison for each hydrocarbon. If $|t| > t_c$ then the difference is significant.

tities as low as 0.3% of the total hydrocarbon. Drying termites prior to the extraction greatly increases the chance of consistently detecting hydrocarbons of low abundance, especially the olefin fraction; therefore we feel dried samples should be used for taxonomy.

There is the possibility that these differences are due to the extraction of hydrocarbons from internal tissues. By definition, these hydrocarbons are not

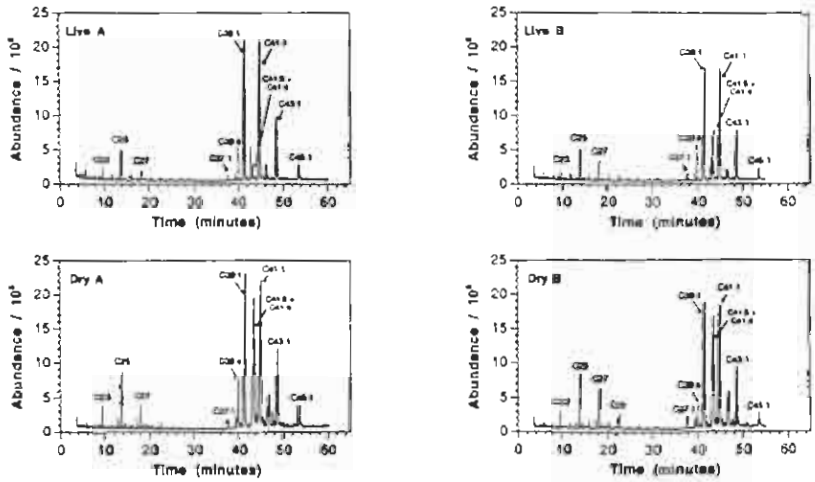

FIG. 5. Chromatograms of cuticular hydrocarbons extracted from 100 workers from two nests (A and B) of *Nasutitermes acajutlae* from Guana Island, BVI. Workers were extracted with 10 ml of hexane for 10 min either alive or after drying.

cuticular lipids, although the components may be the same (de Renobales et al., 1991). Dried termites are more fragile and often lose legs, antennae, or even heads during shipment, and also, the cuticle can become cracked. These conditions could allow the hexane to penetrate and extract lipids from the inner layers of cuticle and internal tissues. It is conceivable that some of the hydrocarbons extracted from our dried samples are not surface hydrocarbons, but those deposited on the external surface of the cuticle of the next instar (Howard et al., 1995). We found no evidence that any abundant hydrocarbons were unique to dried samples. However, the minor components $C_{41:2}$ and $C_{43:2}$ may have been unique to dried samples ($\bar{X} < 0.1\%$ in live samples; $\bar{X} > 0.8\%$ in dried samples for each component).

After examining hundreds of samples of termites extracted alive or after drying, it appears to us that the chromatograms from dried individuals are sharper and have a flatter baseline than those from live, field-extracted individuals. Three possible reasons are: (1) live termites void gut contents when placed in hexane and thus introduce contaminants, (2) live insects have a higher water content in the cuticle and less hydrocarbon is extracted because hexane is hydrophobic, or (3) hexane extracts in vials solubilize contaminants from the vial lids during transit from the field to the laboratory. The vials used were lined with foil, not Teflon. When we stored clean hexane in vials that were upside down, the resulting chromatogram had an uneven baseline similar to that resulting from extraction of live termites; however, no hydrocarbon peaks were seen. Further study of this phenomenon is warranted.

Effects of Sample Size

Statistically significant differences (α = 0.00227) in the percentage of hydrocarbon components among sample sizes were found for 16 hydrocarbon peaks from workers extracted alive (Table 4). The most abundant components, $C_{39:1}$ and $C_{41:1}$, were not significantly different among sample sizes. Samples of 25 or 50 workers were found to be significantly different in only three cases: C_{26}, C_{28}, and C_{29}. Samples of 25 workers yielded extracts with hydrocarbon proportions significantly different from samples of 100 workers in 13 cases and from samples of 200 workers in 16 cases (Table 4). Samples of 50 workers yielded extracts with hydrocarbon proportions significantly different from sam-

TABLE 4. RELATIVE ABUNDANCE (MEAN PERCENT AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS FROM FOUR SAMPLE SIZES OF WORKERS FROM FIVE NESTS OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND, BVI, EXTRACTED ALIVE[a]

	25 workers		50 workers		100 workers		200 workers		
Hydrocarbon[b]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*
C_{23}	1.33a	0.50	1.26ab	0.40	0.85bc	0.20	0.74c	0.14	10.82
C_{24}	1.18a	0.59	0.99a	0.25	0.41b	0.18	0.41b	0.09	20.52
$C_{25:1}$	0.00a	0.00	0.01a	0.05	0.33b	0.30	0.25b	0.13	15.31
C_{25}	4.73a	1.31	4.05ab	1.15	2.84b	0.48	2.78b	0.44	15.66
C_{26}	1.49a	0.53	1.01b	0.24	0.49c	0.18	0.45c	0.13	39.79
$C_{27:1}$	0.14a	0.44	0.11a	0.21	0.42a	0.31	0.36a	0.15	4.03
C_{27}	2.48a	1.23	1.97a	0.73	1.59a	0.65	1.44a	0.66	4.44
C_{28}	1.17a	0.41	0.75b	0.19	0.32c	0.21	0.25c	0.10	41.75
C_{29}	1.18a	0.39	0.73b	0.15	0.46bc	0.26	0.35c	0.10	32.58
$C_{37:1}$	0.07a	0.18	0.14a	0.22	0.50b	0.18	0.48b	0.15	22.52
$C_{38:1}$	0.21a	0.38	0.33ab	0.27	0.68bc	0.23	0.80c	0.16	15.89
$C_{39:4}$	0.10a	0.22	0.15a	0.27	0.73b	0.34	0.81b	0.38	21.89
$C_{39:2}$	0.00a	0.00	0.00a	0.00	0.00a	0.00	0.08a	0.16	3.70
$C_{39:1}$	29.2a	2.47	28.7a	1.62	27.9a	2.70	26.4a	1.32	5.04
$C_{40:1}$	2.01a	0.85	2.47ab	0.40	2.87b	0.13	3.23c	0.52	14.10
$C_{41:6} + C_{41:5}$	5.47a	2.51	6.43a	2.44	7.92a	4.12	9.27a	2.52	4.70
$C_{41:1}$	35.5a	2.79	32.2a	8.88	32.4a	2.90	30.4a	1.98	2.79
$C_{42:1}$	0.99a	0.78	1.50ab	0.77	1.54ab	0.58	2.12b	0.66	6.50
$C_{43:5}$	0.23a	0.50	0.94ab	0.97	1.49ab	1.44	2.20b	0.71	11.14
$C_{43:2}$	0.00a	0.00	0.02a	0.08	0.03a	0.11	0.33b	0.11	49.69
$C_{43:1}$	10.7a	1.26	11.5ab	0.87	12.5b	1.48	12.4b	1.11	7.10
$C_{45:1}$	1.29a	1.10	1.96ab	0.84	3.21c	0.59	2.70bc	0.98	13.18

[a] Means are from three samples from each of five separate nests (N = 15). Means for a given hydrocarbon followed by the same letter are not significantly different at the α = 0.00227 level.

[b] This shorthand (X-MeC_{YY} and $C_{XX:Y}$) uses descriptors for the location of the methyl group (X-Me), the total number of carbons (XX), excluding the methyl branch(es), and the number of double bonds (Y). Locations of double bonds are undetermined.

ples of 100 workers in seven cases and from samples of 200 workers in 11 cases. Samples of 100 and 200 workers yielded extracts with hydrocarbon proportions significantly different in only two cases. If workers were extracted while alive, it appears that the cuticular hydrocarbon mixtures change the least in samples of 100 or more.

Statistically significant differences ($\alpha = 0.00227$) in the percentage of hydrocarbon components among sample sizes were found for 13 hydrocarbon peaks when workers were extracted after drying (Table 5). Contrary to the results from live workers, the most abundant hydrocarbons $C_{39:1}$ and $C_{41:1}$, did display statistically significant differences among sample sizes. Samples of 25 workers yielded no statistically significant differences from samples of 50 workers for any hydrocarbons. Samples of 25 workers yielded extracts with hydrocarbon proportions significantly different from samples of 100 in 12 cases and from 200 workers in only five cases. Samples of 50 workers yielded extracts with hydrocarbon proportions significantly different from samples of 100 or 200 workers in only one case each (Table 5). Samples of 100 and 200 workers were not found to be significantly different for any of the 22 hydrocarbons analyzed. Similar to the result of extracting live workers, extracting workers after drying changed the least in sample sizes of 100 or more; however, extracts of samples of 50 workers were significantly different only for $C_{39:2}$, which had a relative abundance of 0.0% in samples of 25 or 50 workers and ca. 0.7% in samples of 100 or 200 workers.

The less abundant or trace compounds from extractions of the standard group size (100) are either missing or infrequently recorded (resulting in a lower mean value) in samples of 25 or 50 workers (Tables 4 and 5; Figures 6 and 7). The most abundant compounds, such as $C_{39:1}$ and $C_{41:1}$, have a lower mean value in samples of 100 or 200 workers than in groups of 25 workers (Tables 4 and 5; Figures 6 and 7). This undoubtedly results from the greater contribution of the minor compounds to the total hydrocarbon mixtures in the larger sample sizes; many of these trace compounds are not recorded in the samples of 25 or 50 workers, and they do not add to the total hydrocarbons (Figures 6 and 7). Most of the less abundant components fall below the line of equality (dashed line).

Thus, for workers of *N. acajutlae*, 100 appears to be the optimum sample size for adequately characterizing the cuticular hydrocarbons for quantitative comparisons using our operating parameters, regardless of whether the workers are extracted while alive or after drying. If the goal is to characterize all hydrocarbon components, even though they might be present in trivial amounts, then a larger sample (200–300 workers) should be taken, the extracts should be concentrated beyond our standard 60 μl, or the split ratio of the gas chromatograph should be altered. Collecting fewer insects in the field would save time and add to the convenience of field workers, but altering the chromatographic

TABLE 5. RELATIVE ABUNDANCE (MEAN PERCENT AND STANDARD DEVIATION) OF CUTICULAR HYDROCARBONS FROM FOUR SAMPLE SIZES OF WORKERS FROM FIVE NESTS OF *Nasutitermes acajutlae* (HOLMGREN) FROM GUANA ISLAND, BVI, EXTRACTED AFTER DRYING[a]

	25 workers		50 workers		100 workers		200 workers		
Hydrocarbon[b]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*
C_{23}	1.20a	0.38	1.08a	0.28	0.93a	0.20	1.12a	0.26	2.36
C_{24}	0.34a	0.34	0.39a	0.28	0.24a	0.23	0.36a	0.20	0.89
$C_{25:1}$	0.24a	0.41	0.40ab	0.27	0.64b	0.21	0.59ab	0.19	6.29
C_{25}	5.25a	2.44	3.83ab	1.09	3.04b	0.77	3.69ab	0.81	6.20
C_{26}	0.41a	0.35	0.41a	0.31	0.35a	0.29	0.41a	0.26	0.14
$C_{27:1}$	0.33a	0.43	0.58ab	0.22	0.89b	0.56	0.74ab	0.25	5.71
C_{27}	2.75a	1.62	2.49a	1.47	2.13a	1.44	2.65a	1.46	0.49
C_{28}	0.05a	0.13	0.25a	0.20	0.26a	0.22	0.29a	0.17	5.55
C_{29}	0.62a	0.50	0.69a	0.42	0.84a	0.52	0.85a	0.50	0.81
$C_{37:1}$	0.12a	0.22	0.22ab	0.15	0.47b	0.07	0.43ab	0.15	16.98
$C_{38:1}$	0.26a	0.35	0.45ab	0.16	0.57b	0.10	0.63b	0.21	8.03
$C_{39:4}$	0.97a	0.75	1.30ab	0.43	2.00b	0.68	1.91ab	0.92	7.07
$C_{39:2}$	0.00a	0.00	0.00a	0.00	0.67b	0.46	0.69b	0.28	31.89
$C_{39:1}$	22.6a	1.99	21.3ab	1.57	19.6b	1.97	19.6b	1.79	9.23
$C_{40:1}$	1.90a	0.29	2.05ab	0.33	2.41b	0.19	2.27ab	0.55	5.85
$C_{41:4} + C_{41:5}$	18.6a	4.60	18.9a	3.05	19.4a	3.39	17.4a	2.43	0.87
$C_{41:1}$	28.7a	2.20	26.3ab	1.85	23.1b	1.77	23.4b	3.16	19.09
$C_{42:1}$	1.33a	1.02	1.75a	0.80	1.72a	0.52	1.44a	0.49	1.19
$C_{43:5}$	3.10a	1.58	4.17a	0.98	4.25a	1.92	4.40a	1.09	2.54
$C_{43:2}$	0.12a	0.47	0.48ab	0.32	0.91b	0.63	0.94b	0.37	10.62
$C_{43:1}$	9.58a	1.01	9.84a	0.76	10.0a	1.77	10.3a	1.30	0.78
$C_{45:1}$	1.35a	0.72	1.89ab	0.54	2.47b	0.55	2.26ab	0.83	7.99

[a] Means are from three subsamples from each of five separate nests ($N = 15$). Means for a given hydrocarbon followed by the same letter are not significantly different at the $\alpha = 0.00227$ level.

[b] This shorthand (X-MeC_{XX} and $C_{XX:Y}$) uses descriptors for the location of the methyl group (X-Me), the total number of carbons (XX), excluding the methyl branch(es), and the number of double bonds (Y). Locations of double bonds are undetermined.

[c] The *F* statistic has a $P < 0.00227$, yet the Tukey's procedure did not separate any of the means for this hydrocarbon.

parameters would increase the complexity and repeatability of laboratory operations. If a sample of 100 workers is not attainable, then concentration of the extract or altering the split ratio must be done or the sample will not be usable.

It is likely that our earlier problem with quantifying hydrocarbons from *N. costalis* and *N. ephratae* (Haverty et al., 1990b) resulted from two confounding problems. First, the workers were extracted live, in the field, which resulted in a less efficient extraction of the hydrocarbons, especially the less abundant components. Second, while 100 workers were used in the extractions, due to the

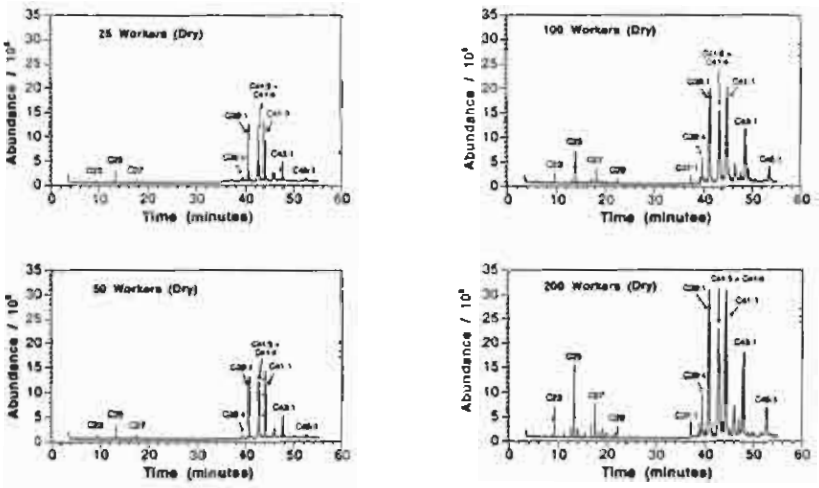

FIG. 6. Chromatograms of cuticular hydrocarbons extracted with 10 ml hexane for 10 min from four sample sizes (25, 50, 100, and 200) of dried workers from one colony of *Nasutitermes acajutlae* from Guana Island, BVI.

size of *N. ephratae*, these collections could very well have been below an acceptable size. Large workers of *N. ephratae*, (dry weight = 0.6 mg) are considerably smaller than large workers of *N. acajutlae* (dry weight = 1.3 mg) (Thorne, 1985); 100 large workers of the former species may not have had sufficient surface lipids to provide an adequate chromatogram.

Effects of Solvent

For field entomologists working in the tropics it would be convenient if storage of termites in 100% ethanol (EtOH) allowed for equivalent extraction of hydrocarbons. Preliminary observations of S. J. Seybold and L. J. Nelson (personal communication) indicated that EtOH will extract cuticular hydrocarbons of adult *Ips pini* (Say) (Coleoptera: Scolytidae). Furthermore, the longer beetles were in EtOH, the greater the extraction efficiency. Their work showed: (1) that the EtOH-extracted insects can be further extracted with hexane, (2) that the EtOH extract can be dried and the lipids redissolved in hexane, and (3) that the hexane solutions of the EtOH extract and the EtOH-extracted-insect extract can be recombined to provide a reconstituted extract for characterization. They have not shown whether the resulting hydrocarbon mixture is comparable to an extract gathered by a standard 10-min extraction with hexane of live or dead insects.

Unfortunately, the resulting extracts for both workers and soldiers of *N.*

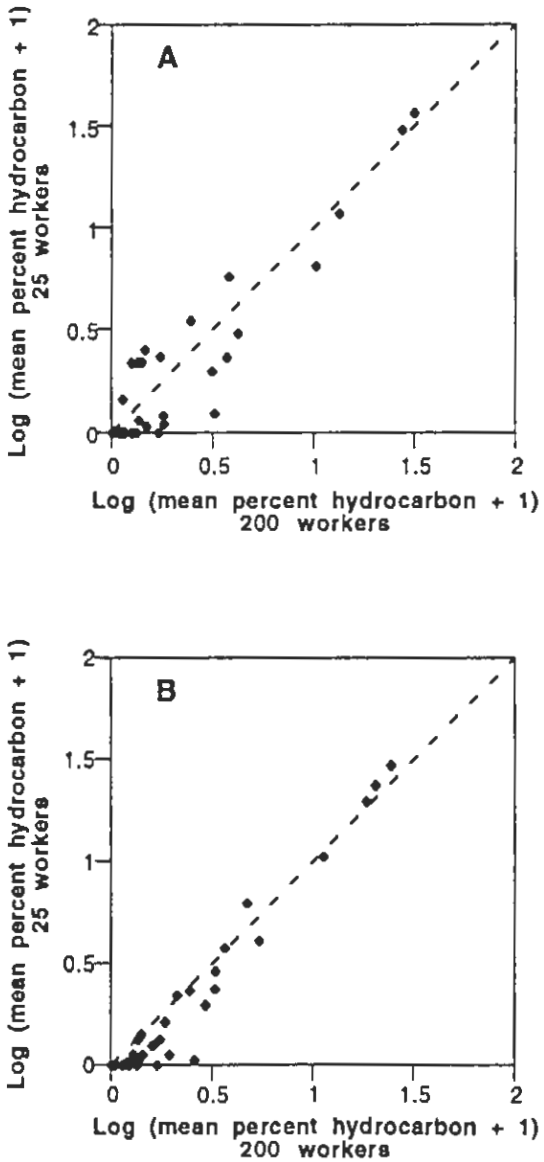

FIG. 7. Log (mean percent + 1) of 34 cuticular hydrocarbon components for groups of 25 and 200 workers extracted either alive (A) or after drying (B). Note: transformation was not done for statistical purposes, but to spread out the points for the less abundant components.

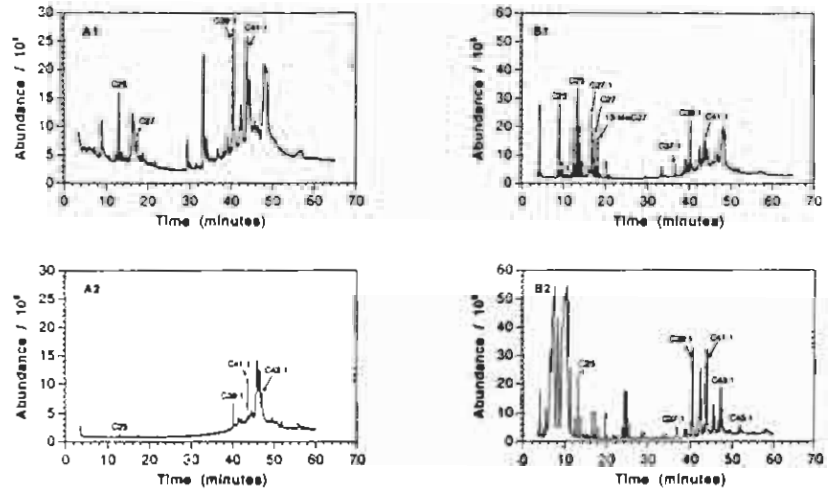

FIG. 8. Chromatograms of cuticular hydrocarbons (and other compounds) from 200 workers (A) or 200 soldiers (B) collected into 100% ethanol. For each caste the first chromatogram (A1 or B1) represents the pattern of compounds that were extracted by the ethanol. The second chromatogram for each caste (A2 or B2) represents the pattern of compounds extracted from the termites after they were stored in ethanol for 60 days, subsequently dried, then extracted with 10 ml of hexane for 10 min.

acajutlae stored in ethanol were not comparable to those where the standard technique was utilized. Storage in ethanol caused numerous, hexane-soluble, unidentified, nonhydrocarbon compounds to be extracted or these nonhydrocarbon peaks were in the ethanol as denaturing components (Figure 8). These compounds were not removed after evaporating the ethanol under nitrogen, redissolving in hexane, and pipetting through activated BioSil-A. Furthermore, enormous amounts of nitrogen and time were required to dry the ethanol samples. As a result of these problems, we did not characterize all 15 samples, because we presumed such a course would only waste resources and could affect our GC-MS. Therefore, unless a different cleanup procedure is developed, storage in ethanol is unacceptable for characterizing the hydrocarbons from *Nasutitermes*.

CONCLUSIONS

Different colonies of *N. acajutlae* produce qualitatively similar, but quantitatively dissimilar hydrocarbon mixtures. These colony-specific profiles appear to be reproducible; multiple extracts from separate samples of each colony are qualitatively identical and quantitatively quite similar. Only one extraction of a

sample of workers is necessary. A very brief extraction (in 10 ml of hexane for only 20 sec) of live workers produces a mixture of hydrocarbons equivalent to a 10-min or a 24-hr extraction. Holding a sample of workers (or soldiers) in hexane for a period of two years results in a radically different extract than the standard 10-min extraction and is not recommended.

Drying workers of *N. acajutlae* before extraction results in highly significant increases in the relative amounts of $C_{41:4}$ and $C_{41:5}$ and an apparent decrease in the relative amounts of the most abundant compounds, $C_{39:1}$, $C_{41:1}$, and $C_{43:1}$. In general, drying workers prior to extraction tends to enhance extraction of the less abundant unsaturated compounds and does not result in equivalent mixtures of hydrocarbons when compared to extraction of live workers. Extracting a minimum of 100 workers (live or dried) with hexane for 20 sec to 10 min seems to be the best method for characterizing cuticular hydrocarbons of *N. acajutlae*. For smaller species, samples of at least 200 would probably guarantee a satisfactory chromatogram.

For quantitative comparisons, the extraction technique should ideally be the same for all samples, i.e., hydrocarbon mixtures extracted from dried samples should not be compared to those extracted from live samples. Extraction of either live or dried termites would suffice for characterization of cuticular hydrocarbons for ecological studies but may not be comparable for taxonomic purposes. For quality of chromatograms and for several logistical reasons, we obtained the best results by drying at least 100 termites and then extracting them by the standard technique (10 min in 10 ml hexane) in the laboratory. Extraction of live termites in the field requires twice the number of vials and transportation of flammable liquids. Even with the potential logistical difficulties involved with drying termites in the tropics, we recommend this as the optimal technique to use.

Acknowledgments—We thank James A. Baldwin for his patient assistance in the statistical analysis of our hydrocarbon data. Gary Blomquist, Coby Schal, and James Lazell constructively reviewed an earlier version of this manuscript. We wish especially to thank the staff of the Guana Island Club and James Lazell for facilitating our studies of the termites of the Caribbean. This work was supported, in part, by National Science Foundation grant DEB-8607407 to B.L.T., a grant to the authors by The Conservation Agency through grants from the Falconwood Foundation, and by a cooperative agreement between the Pacific Southwest Research Station and the University of Maryland.

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SYSTEMATICS

Generic Revision of the Smaller Nasute Termites of the Greater Antilles (Isoptera, Termitidae, Nasutitermitinae)

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Ann. Entomol. Soc. Am. 89(6): 773–787 (1996)

ABSTRACT The generic status of the Greater Antillean smaller nasutes (all genera except *Nasutitermes*) is revised, paying special attention to anatomical characters of the workers. Two new monotypic genera, *Caribitermes* and *Antilliternes*, are created. *Terrenitermes* Spaeth, 1967, is synonymized with *Parviternes* Emerson (in Snyder 1949). The following new combinations are proposed: *Parviternes aequalis* (Snyder, 1924a) (formerly in *Obtusitermes*), *Parviternes antillarum* (Holmgren, 1910) (formerly in *Velociternes*), *Parviternes toussainti* (Banks, 1919) (formerly in *Terrenitermes*), *Caribitermes discolor* (Banks, 1919) and *Antilliternes subtilis* (Scheffrahn & Křeček, 1993) (both formerly in *Parviternes*). The 2 mainland species formerly assigned to *Parviternes* should be removed from this genus: one resulting new combination is *Velociternes laticephalus* (Snyder, 1926), whereas the actual taxonomic position of the species described as *Parviternes bacchanalis* Mathews, 1977, remains unclear. With the exception of a *Constrictotermes* species present in Cuba, the smaller nasute fauna of the Greater Antilles and adjacent Bahamas and Virgin Islands now appears endemic to this region at the genus level. We hypothesize that an ancestor related to *Obtusitermes* diversified in the Greater Antilles and gave rise to all *Parviternes*, *Caribitermes* and perhaps *Antilliternes* species.

KEY WORDS Termitidae, taxonomy, biogeography, new genera, West Indies, Neotropical Region

THE GREATER ANTILLES form an integrated biogeographic entity which comprises 4 major islands: Cuba, Hispaniola, Puerto Rico, and Jamaica. Geological evidence (Buskirk 1985, Burke 1988, Donnelly 1988, Ross and Scotese 1988, Perfit and Williams 1989) indicates that their origin resides in an island arc that arose between North and South America during the Cretaceous and started to migrate eastward ≈80 million years ago. Since then, Cuba, North Hispaniola, and Puerto Rico remained isolated from the mainland and at least partly emerged. Episodes of land connections or at least close proximity between these 3 islands are thought to have occurred until the Miocene (Buskirk 1985, Ross and Scotese 1988). Jamaica, as well as a former island now forming the southwestern peninsula of Hispaniola, was probably entirely submerged for a large part of the Tertiary, to rise again above sea level during the Early Miocene (Buskirk 1985). The Greater Antilles remained constantly isolated from the Lesser Antilles, almost entirely of Tertiary (Eocene–Miocene) volcanic origin, and from the Bahamas, situated on the North American continental platform, although the channel separating Cuba from the Bahamian Bank must have been considerably narrower during Pleistocene glaciations than today.

According to catalogs and faunal lists (Snyder 1949, 1956; Araujo 1977; Scheffrahn et al. 1994), the subfamily Nasutitermitinae is represented in the Greater Antilles by the genera *Nasutitermes* Dudley (in Dudley and Beaumont 1890), *Constrictotermes* Holmgren, 1910, *Velociternes* Holmgren, 1912, *Obtusitermes* Snyder, 1924a, *Parviternes* Emerson (in Snyder 1949), and *Terrenitermes* Spaeth, 1967. The genus *Nasutitermes* is pantropical and comprises 6 species in the Greater Antilles (Scheffrahn et al. 1994, Thorne et al. 1994). The other Nasutitermitinae recorded from the Greater Antilles belong to genera restricted to the Neotropics. They consist of small to medium-sized species, with more or less constricted soldier head capsules and variable patterns of soldier and worker polymorphism. None of them has until now been recorded from Jamaica, but at least 2 species are present in Puerto Rico (one of which reaches the Virgin Islands), 4 in Cuba (one of which reaches the Bahamas) and 8 in Hispaniola (Scheffrahn et al. 1994). The generic taxonomy and biogeog-

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0013-8746/96/0773–0787$02.00/0

raphy of these smaller nasutes will be the focus of the current work.

The genera *Velocitermes* and *Obtusitermes* were originally described from the neotropical mainland. Their type species are respectively *V. heteropterus* (Silvestri, 1901), from Brazil, and *O. panamae* (Snyder, 1925a), from Panama. According to current generic taxonomy, they are represented in the Greater Antilles by *V. antillarum* (Holmgren, 1910), from Hispaniola, and *O. aequalis* (Snyder, 1924a), from Cuba. The specimens listed by Scheffrahn et al. (1994) as *Velocitermes*? n. sp. A were found to be conspecific with *Parvitermes pallidiceps* (Banks) (see Scheffrahn and Roisin 1995), whereas the status of the species listed as *Velocitermes*? n. sp. B, from Hispaniola, will be discussed below.

The genus *Parvitermes* was created by Emerson (in Snyder 1949) to accommodate 6 species: *P. brooksi* (Snyder, 1925b) from Cuba and adjacent Bahamas Islands (type species), *P. discolor* (Banks, 1919) from Hispaniola and Puerto Rico, *P. flaveolus* (Banks, 1919) from Hispaniola, *P. pallidiceps* (Banks, 1919) from Hispaniola, *P. wolcotti* (Snyder, 1924b) from Puerto Rico and adjacent Virgin Islands, and with a question mark, *P. laticephalus* (Snyder, 1926) from Bolivia. A 7th species, *P. bacchanalis*, was described by Mathews (1977) from Brazil, and an 8th, *P. subtilis*, by Scheffrahn and Křeček (1993) from Cuba and Hispaniola. Recently, Scheffrahn and Roisin (1995) described from Hispaniola a 9th species, *P. collinsae* (called *Parvitermes*? n. sp. in Scheffrahn et al. 1994) and reported the discovery of a rare major soldier morph in this species as well as in *P. pallidiceps* and *P. wolcotti*. The genus *Terreniternes* was created by Spaeth (1967) for a single species from Hispaniola, *T. toussainti* (Banks, 1919).

The Antillean species *P. discolor*, *P. flaveolus*, *P. pallidiceps* and *T. toussainti* were originally placed in the genus *Constrictotermes* Holmgren, 1910 (type species *Eutermes cyphergaster* Silvestri, 1901), by Banks (1919). This genus now comprises 3 species from South America (Araujo 1977) and 1, *C. guantanamensis*, recently described from Cuba (Křeček et al. 1996). *P. brooksi*, *P. laticephalus*, and *P. wolcotti* were originally included in *Tenuirostritermes* Holmgren, 1912 (type species: *Termes tenuirostris* Desneux, 1904), at that time considered a subgenus of *Nasutitermes* (Snyder 1924b, 1925b, 1926). *Tenuirostritermes* has currently full generic status and includes 4 species confined to Central and North America (Araujo 1977).

Up to now, generic assignments of Antillean nasutes have been mostly based on soldier external morphology and polymorphism patterns. According to Spaeth (1967), the 2 soldier morphs of *Velocitermes* would be homologous to the minor and intermediate soldiers of the trimorphic mainland genus *Diversitermes* Holmgren, 1912, whereas the soldier morphs of *Terreniternes* and *Obtusitermes* would correspond to the intermediate and major soldiers of *Diversitermes*. Head capsule shape and number of antennal segments would separate *Terreniternes* from *Obtusitermes*. *Parviternes* would be characterized by monomorphic soldiers (Spaeth 1967).

It was not until Mathews (1977) that worker anatomy was used to provide taxonomic characters in neotropical termites. Fontes (1987a) proposed on this basis to transfer *P. bacchanalis* to *Obtusitermes* and to synonymize *Terreniternes* with *Parviternes*. [The work of Fontes (1987a) is a doctoral dissertation which does not constitute a publication in the sense of the *International Code of Zoological Nomenclature*, 3rd ed. (see Article 9 [11]). Therefore, the nomenclatorial modifications introduced therein should not be considered as published; Fontes himself (Fontes 1987a, p. 2) stated that these modifications needed to be proposed in the future.] The goal of the current study is to clarify the generic status and propose a modern basis to establish the phylogeny of all smaller nasutes from the Greater Antilles with the help of anatomical characters now known to be extremely important in Termitidae systematics (Noirot and Kovoor 1958; Kovoor 1969; Sands 1972; Johnson 1979; Miller 1986; Fontes 1987a, b).

Materials and Methods

Specimens of all Greater Antillean species of smaller nasutes and of species of special interest from the neotropical mainland were used for anatomical studies (see list in *Appendix 1*). For the sake of clarity, all nomenclatorial combinations used in *Appendix 1* and figure legends are those considered valid in the conclusion of the current study; former generic assignments are designated under parentheses by an equals sign. The digestive tube of large workers was observed in situ after removal of the abdominal wall and fat tissue under a dissecting microscope, then drawn at 50× magnification with the help of a camera lucida. P1 to P5 represent the different parts of the proctodeum, numbered as in Noirot and Noirot-Timothée (1969). Mandibles and split enteric valves were dehydrated and mounted on microscope slides. Specimens for scanning electron microscopy were prepared according to the method of Nation (1983), slightly modified: after dehydration by absolute ethanol, the specimens were left in hexamethyldisilazane for at least 12 h before being air dried and sputter coated with gold. Microphotographs of mandible molar plates were taken with an ISI DS-130 scanning electron microscope.

Systematics

Diagnostic Characters of Previously Described Genera. For all smaller nasute genera reported from the Greater Antilles, we review in this section the characteristics of the type species that

could be of diagnostic value in generic taxonomy. *Tenuirostritermes* is included for comparative purposes, although the Greater Antillean species originally described in this genus are currently placed in other genera.

Parviternes (type species: *P. brooksi*). Soldier monomorphic, with head capsule slightly constricted behind the antennal sockets. Antennal segments: 15 in the imago, 12–13 in the soldier, 13–14 in the worker. Soldier mandibles vestigial, with or without points. Digestive tube of the worker (Fig. 1A) with long mixed segment, all 4 Malpighian tubules inserted contiguously at mesenteroproctodeal junction. P1 extremely elongated toward the right side of the abdomen and forming ventrally a U-shaped loop. Enteric valve (Fig. 2A) of trilateral symmetry, 3 elongated, weakly sclerotized areas bearing long, curved spines, alternating with areas garnished with tiny spines. Worker mandibles (Fig. 3A): apical teeth approximately equal to first marginals; molar areas slightly concave, molar plate with 7–8 ridges (Fig. 4A).

Terrenitermes (type species: *T. toussainti*). Basically distinguished from *Parviternes brooksi* by its dimorphic soldiers. Soldier mandibles vestigial, with points. Antennal segments: imago 15–16, minor soldier 12, major soldier 13, worker 14–15. Worker digestive tube configuration (Fig. 1B), insertion of Malpighian tubules and enteric valve armature (Fig. 2D), and worker mandibles (Figs. 3B, 4B) very similar to those of *P. brooksi*.

Obtusitermes (type species: *O. panamae*). Soldiers dimorphic, minor soldier head strongly narrowed posteriorly. Soldier mandibles vestigial, without points. Antennal segments: minor and major soldiers 11, worker 12. Gut configuration of worker (Fig. 1C) as in *Parviternes*, except for mesenteric part of mixed segment, positioned ventrally rather than dorsally. Malpighian tubules contiguous. Enteric valve (Fig. 2H) very weakly armed: spines tiny, visible only with great difficulty. Worker mandibles resembling those of *Parviternes*, but with straighter molar plate.

Velociternes (type species: *V. heteropterus*). The description of this species by Silvestri (1901, 1903) was complemented by Mathews (1977) and Fontes (1987a). The species illustrated here is *V. barrocoloradensis* (Snyder, 1925c), whose anatomy closely resembles that of *V. heteropterus*. Soldiers dimorphic. Mandibles vestigial, with points. Appendages elongated. Worker gut (Fig. 1D) characterized by large crop, short mixed segment, P1 short and straight. According to Mathews (1977) and Fontes (1987a), Malpighian tubules inserted slightly before end of mesenteron in *V. heteropterus*, but this feature could not be ascertained in the type material available to us. In *V. barrocoloradensis*, Malpighian tubules in 2 pairs at mesentero-proctodeal junction. Enteric valve of both species composed of 3 major swellings alternating with 3 slightly smaller ones, all bearing small triangular spines (Fig. 2I). Worker mandibles (Fig. 3C) elongated, apical teeth as long as first marginals.

Constrictotermes (type species: *C. cyphergaster*). Medium-sized species. Soldier monomorphic with head capsule conspicuously constricted. Vestigial mandibles with points. Worker with well-pigmented head capsule and tergites. Appendages elongated. Enormous crop, mixed segment relatively short (≈1.5 times the diameter of the mesenteron), P1 shorter than mesenteron, Malpighian tubules inserted contiguously (for illustrations see Fontes 1987a, figures 181, 185–192). Apical tooth of mandibles deflected downward, molar area well developed, with numerous ridges (Fig. 3D).

Tenuirostritermes (type species: *T. tenuirostris*). Soldier monomorphic, with head capsule strongly constricted. Vestigial mandibles with points. Digestive tube of worker resembling that of *Velociternes*, but with mixed segment longer, about twice the width of the mesenteron (for illustrations see Fontes 1987a, figures 202–205). Malpighian tubules inserted in 2 pairs almost diametrally opposed. Worker mandibles elongated, curved at apex, resembling those of *Velociternes* but with narrower space between apical teeth and 1st marginals.

Species Assignments to Genus. *P. flaveolus* fits the description of *P. brooksi* closely in all respects. Although *O. aequalis* was suspected to possess dimorphic soldiers (Snyder 1924a), up to now a single soldier morph has been described in this species. This soldier possesses 11 antennal segments, as in *O. panamae*, or 12, as in *P. brooksi*. Vestigial points on mandibles are present in *P. flaveolus* and missing in *O. aequalis*, but this criterion seems of little value here because such points may be present or absent in soldiers of *P. brooksi*; even in a single soldier, the left and right mandibles may differ in this respect. Workers possess antennae with 13–14 segments, as in *P. brooksi*. The digestive tube configuration and enteric valve (Fig. 2B) of the workers of *O. aequalis* are not distinguishable from those of *P. brooksi* (Fig. 2A), but are distinct from those of *O. panamae* (Fig. 2H). We therefore propose to transfer *O. aequalis* to *Parviternes*.

The following species are extremely similar to *P. brooksi* by their worker mandibles, gut configuration and enteric valve: *P. wolcotti*, *P. collinsae*, a new species from Hispaniola (*Velocitermes*? n. sp. B), *P. pallidiceps* and *Velociternes antillarum* (Fig. 2C). An important feature by which these species differ from *P. brooksi* and among themselves is their caste system: whereas *P. brooksi*, *P. flaveolus*, and *P. aequalis* have monomorphic soldiers, the other species possess 2 soldier morphs. Major soldiers are very rare in *P. wolcotti* and *P. collinsae*, uncommon in *P. pallidiceps*, common in *Velocitermes*? n. sp. B and *V. antillarum*. To a varying degree, all soldiers show a constriction behind the antennal sockets. This constriction is weakest in the major soldier of *P. wolcotti*. Mandibular points are generally present, but are missing in *P. palli-*

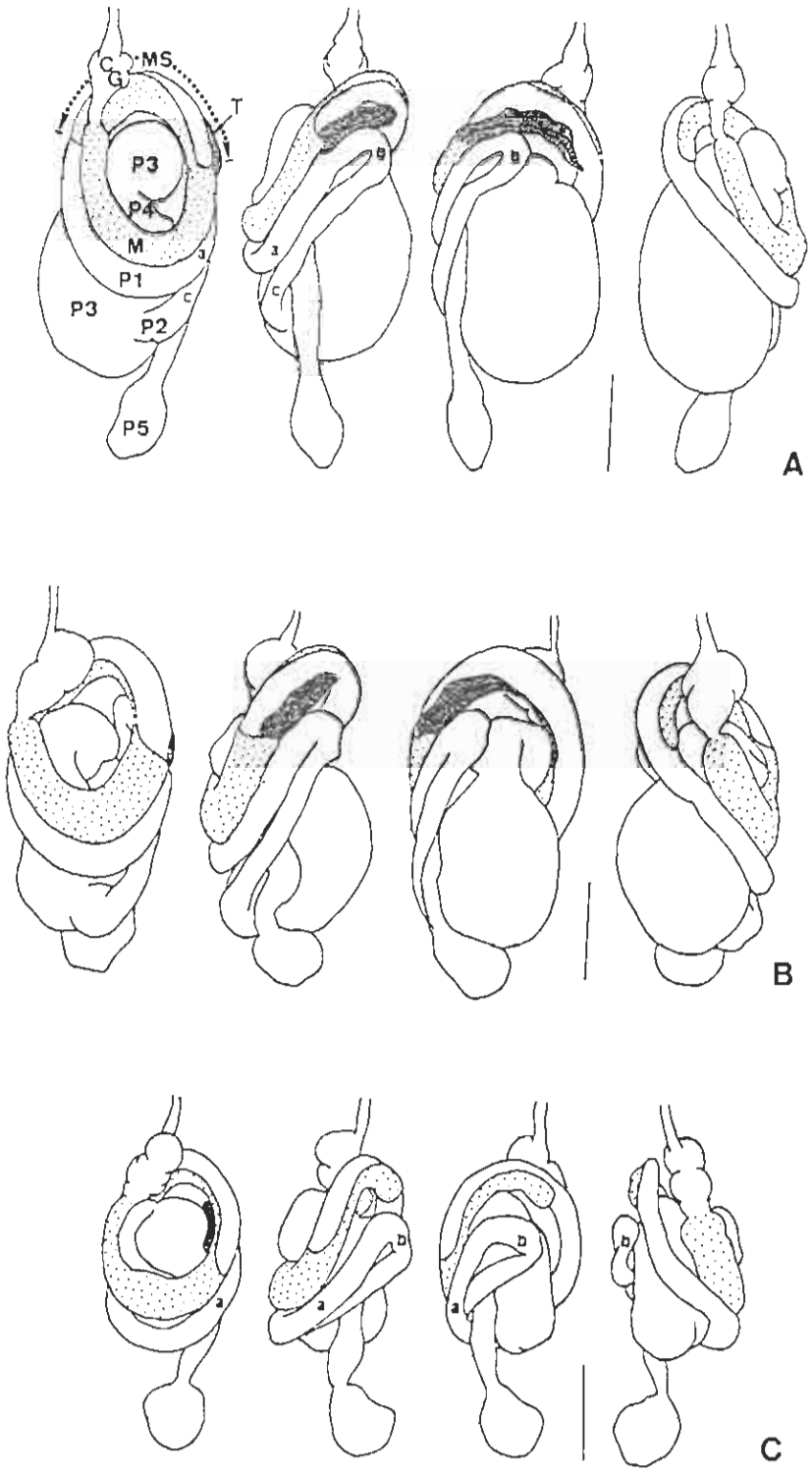
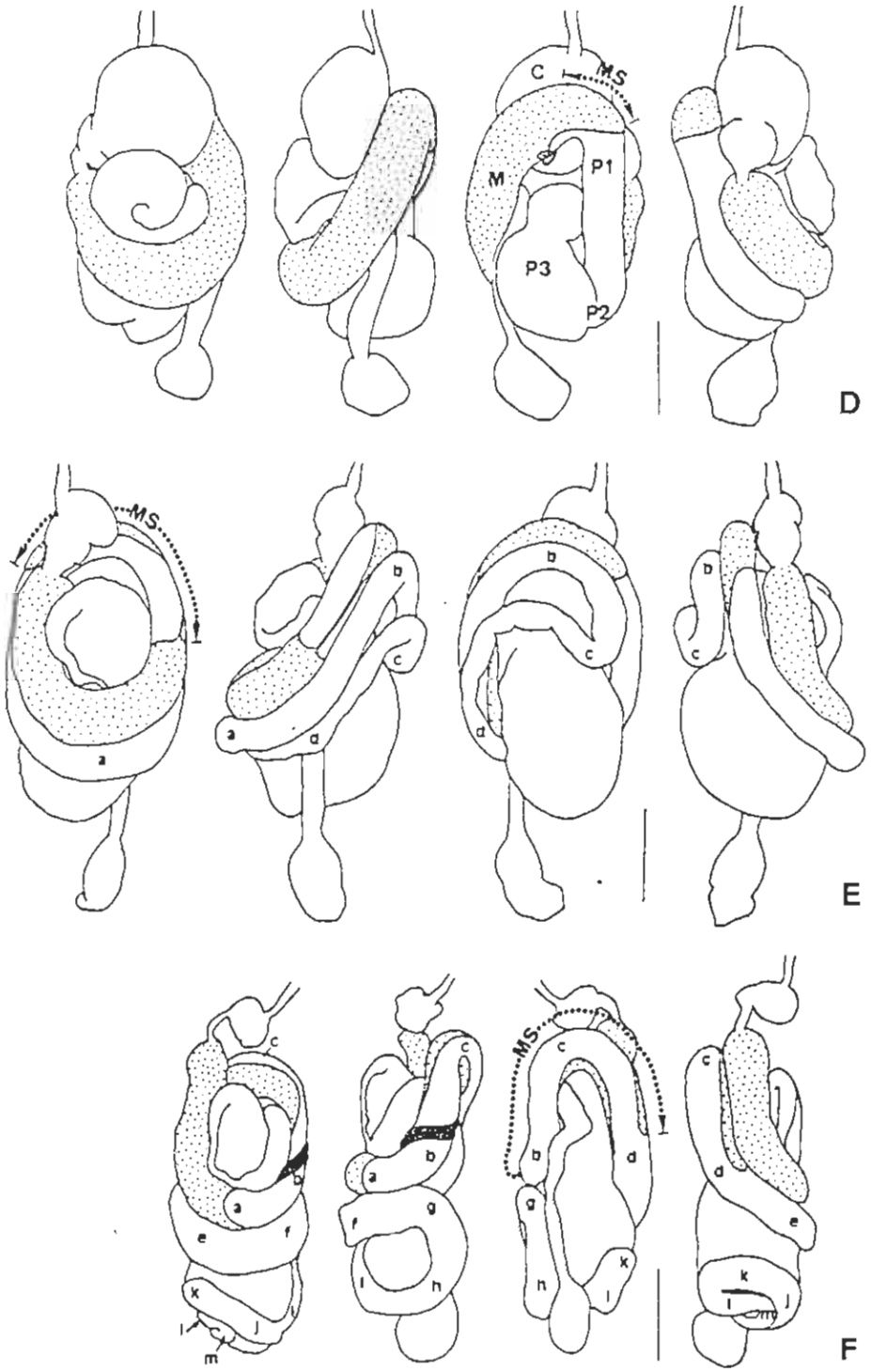


Fig. 1. Configuration of worker digestive tube *in situ*, seen from above, right, below, left. (A) *Parvitermes brooksi*. (B) *Parvitermes* (=*Terrenitermes*) *toussainti*. (C) *Obtusitermes panamae*. (D) *Velocitermes barrocoloradensis*. (E) *Caribitermes* (=*Parvitermes*) *discolor*. (F) *Antillitermes* (=*Parvitermes*) *subtilis*. C, crop; G, gizzard; M, mesenteron



(stippled); MS, mixed segment; P1 to P5, proctodeum; P1, 1st proctodeal segment; P2, enteric valve; P3, paunch; P4, colon; P5, rectum; T, Malpighian tubules (densely stippled). Lowercase letters are used to mark corresponding spots in different views of the digestive tube in the same individual. Scale bars = 0.5 mm.

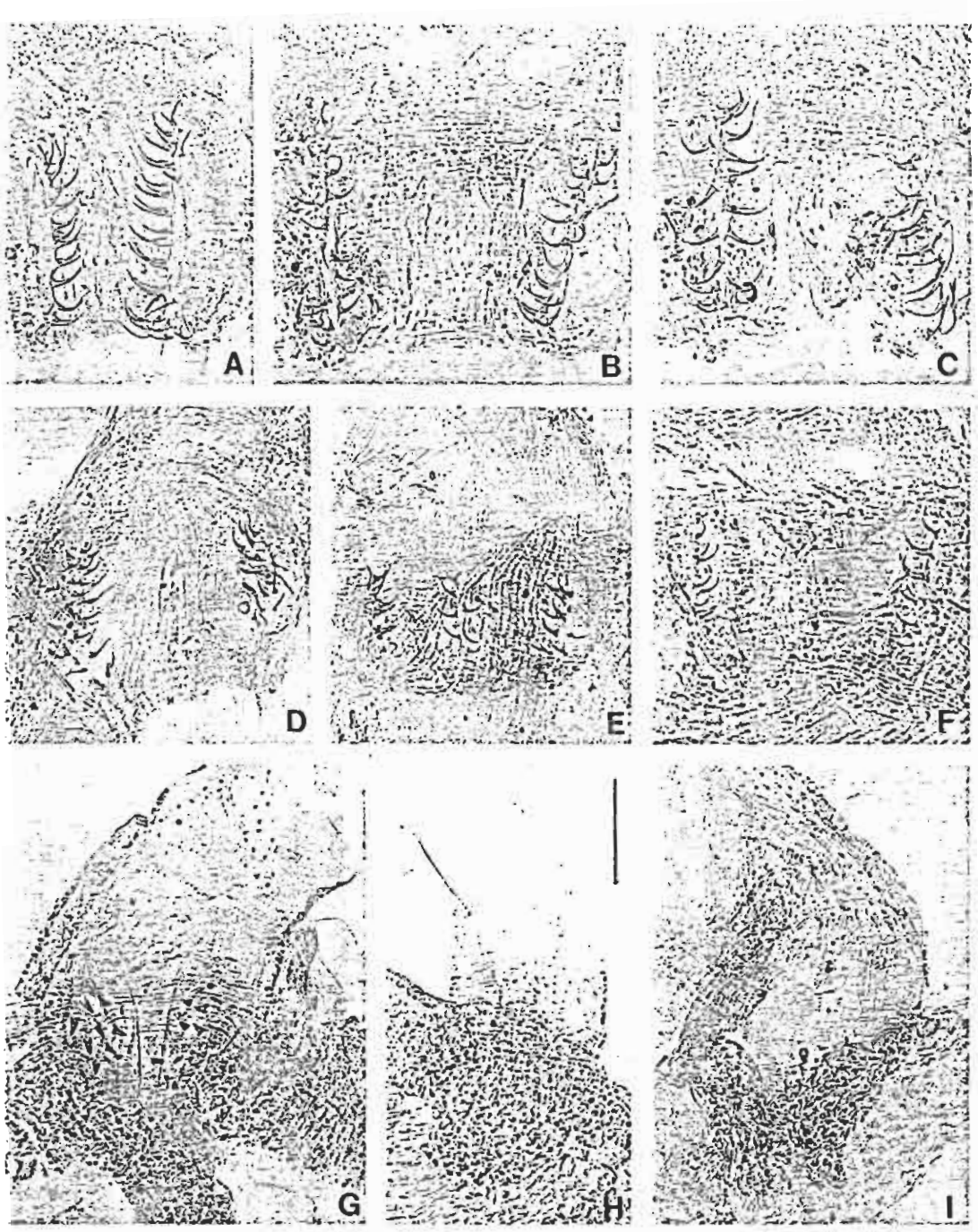


Fig. 2. Armature of worker enteric valve. (A) *Parvitermes brooksi*. (B) *Parvitermes* (=*Obtusitermes*) *aequalis*. (C) *Parvitermes* (=*Velocitermes*) *antillarum*. (D) *Parvitermes* (=*Terreniternes*) *toussainti*. (E) *Antillitermes* (=*Parviter-mes*) *subtilis*, paratype from North Hispaniola. (F) *Antillitermes* (=*Parvitermes*) *subtilis*, from Cuba. (G) *Caribitermes* (=*Parvitermes*) *discolor*. (H) *Obtusitermes panamae*. (I) *Velocitermes barrocoloradensis*. Enteric valves were cut longitudinally in half. Whenever the enteric valve comprised 3 major spiny areas alternating with minor ones, we chose to show the section showing 2 major areas (A–D, F, G, I). North Hispaniolan *A. subtilis* (E) possess 6 almost equal spiny swellings, whereas spiny areas are ill defined in *O. panamae* (H). Scale bar = 0.1 mm.

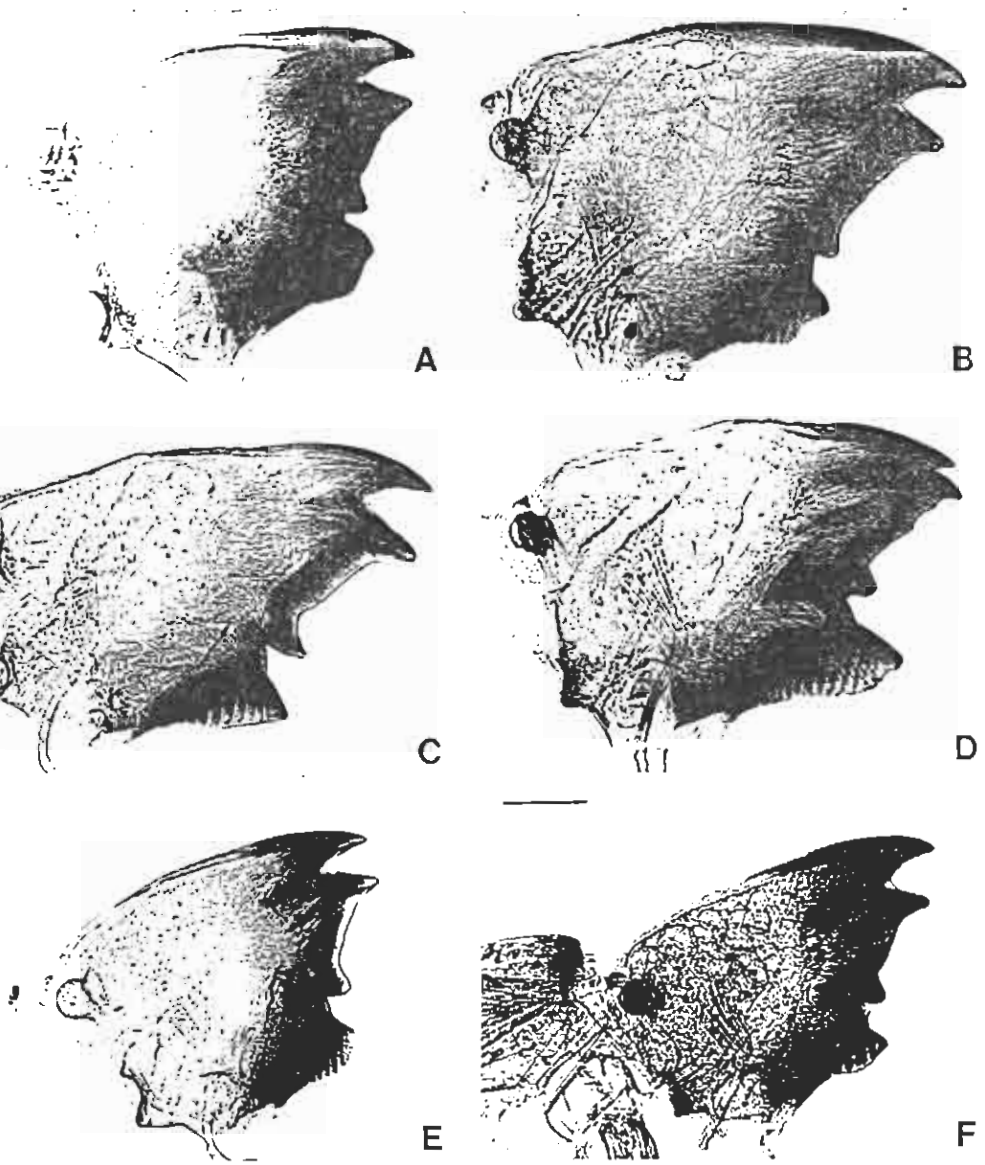


Fig. 3. Worker left mandible. (A) *Parviternes brooksi*. (B) *Parviternes* (=*Terrenitermes*) *toussainti*. (C) *Velociternes barrocoloradensis*. (D) *Constrictotermes cyphergaster*. (E) *Caribitermes* (=*Parviternes*) *discolor*. (F) *Antillitermes* (=*Parviternes*) *subtilis*. Scale bar = 0.1 mm.

diceps, some specimens of *P. brooksi* and rare individuals of *P. collinsae* and *Velocitermes?* n. sp. B. Antennal segments are 12 or 13 in the minor soldier, 14 or 15 in the major soldier, and 14 or 15 in the worker. The worker molar plate bears 4–5 ridges distal to the basal notch, 6–8 in total. There is thus no consistent morphological character which would justify the assignment of any of these species to a genus other than *Parviternes*. Although soldier caste patterns appear highly variable in this group, the striking anatomical uniformity of the workers favors the placement of these species in a single genus. *Velocitermes antillarum* should without doubt be transferred to *Parviternes*, as well as the undescribed species formerly cited as *Velocitermes?* n. sp. B.

Spaeth (1967) created the new genus *Terrenitermes* for *T. toussainti* on the basis of soldier dimorphism. This argument can no longer be upheld, because the 2 soldier castes of *T. toussainti* appear very similar to those found in *Parviternes* (formerly *Velocitermes?*) n. sp. B and *P. antillarum*. The dark pigmentation of *T. toussainti* resembles that of *P. antillarum*. By the morphology of its mandibles (Figs. 3B and 4B) and the anatomy of its digestive tube (Figs. 1B and 2D), the worker of

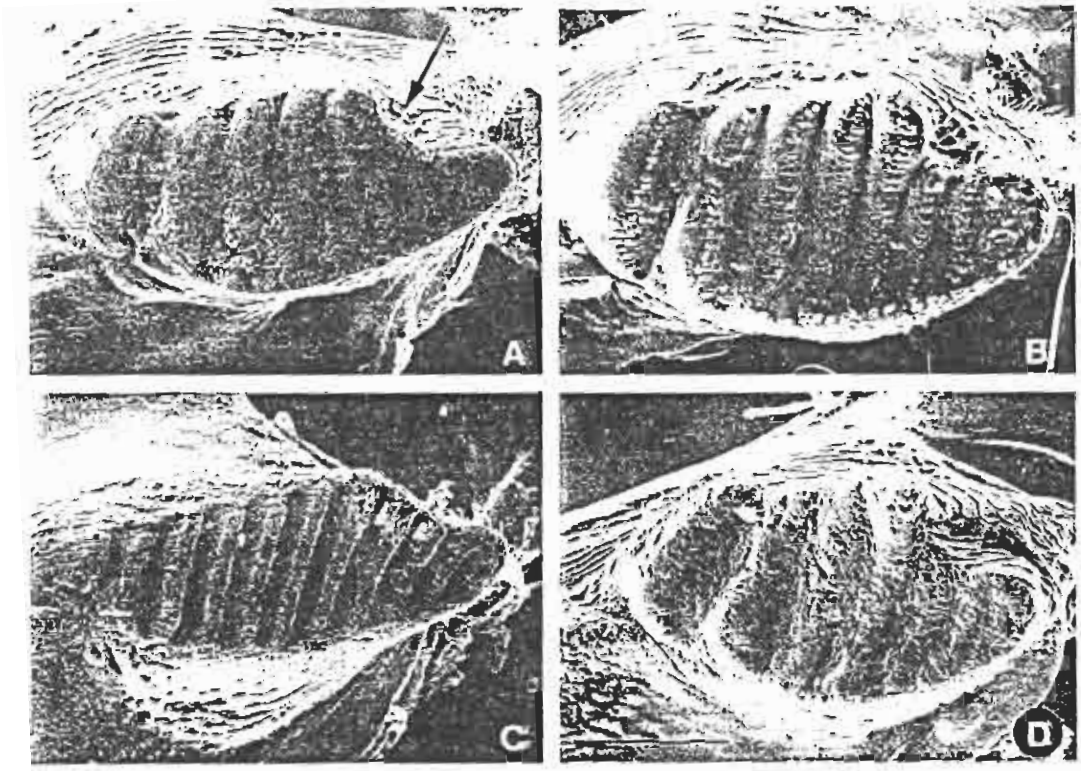


Fig. 4. SEM views of worker molar plate (right mandible). (A) *Parvitermes brooksi*. (B) *Parvitermes* (=*Terriditermes*) *toussainti*. (C) *Caribitermes* (=*Parvitermes*) *discolor*. (D) *Antilliternes* (=*Parvitermes*) *subtilis*. Arrow, basal notch. Scale bar = 50 μm.

T. toussainti fits the *Parvitermes* pattern closely. There is thus no consistent character that would justify maintaining *T. toussainti* in a separate genus. We therefore suggest, like Fontes (1987a), to transfer *T. toussainti* to *Parvitermes*. *Terriditermes* thereby becomes a junior subjective synonym of *Parvitermes*.

The Brazilian species *P. bacchanalis* shows a distinctive digestive tube configuration: P1 is long, forms a loop on the right side of the paunch, but this loop is shorter than in *O. panamae* and *P. brooksi* and directed anteriorly, so that the junction of the enteric valve with the paunch is situated below the mesenteron (see Fontes 1987a, figures 288–302). The enteric valve armature of *P. bacchanalis*, which Mathews (1977) unfortunately described among the generic characters of *Parvitermes*, is characterized by small stout spines. Removing this species from *Parvitermes* thus appears fully justified, although its transfer to *Obtusitermes*, suggested by Fontes (1987a), is dubious as well. Emerson (in Snyder 1949, p. 376) tentatively assigned the Bolivian species, *Nasutitermes* (*Tenuirostritermes*) *laticephalus* Snyder, 1926, to *Parvitermes*, although he suspected that it might actually belong to *Velocitermes*. Observation of workers from the type series of this species showed that their digestive tube is characterized by a short mixed segment and short, straight P1, thus fitting unambiguously the description of *Velocitermes* rather than that of *Parvitermes*.

No Greater Antillean species are thus left in the genera *Obtusitermes* and *Velocitermes*. None fits the description of *Tenuirostriternes*. However, anatomical observations confirmed the generic status of the species listed by Scheffrahn et al. (1994) as *Constrictotermes*? n. sp., which was discovered in southeastern Cuba and is now known as *C. guantanamensis* (Křeček et al. 1996).

Descriptions of New Genera. Two species, up to now known as *P. discolor* and *P. subtilis*, do not fit into any existing genus. Two new monotypic genera are erected below to accommodate them.

***Caribitermes*, new genus**

Type species. *Constrictotermes discolor* Banks, 1919.

Soldier monomorphic, fully nasute. Head capsule very slightly constricted behind antennae. Nasus slender. Mandibles vestigial, with points. Antennae of 12–13 segments.

Worker distinguished from *Parvitermes* by mixed segment (Fig. 1E), whose mesenteric part is isolated from the remainder of the mesenteron, connected to it only by proctodeal folds. Malpighian tubules inserted at mesentero-proctodeal junction in 2 contiguous pairs. Enteric valve (Fig.

...es (=*Terreni*-... Arrow, basal

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...*rmes* by ...steric part ...mesenteron, ...is. Malpi- ...proctodeal ...alve (Fig.

2G) with 3 swellings bearing short, thick, triangular spines. Mandibles (Fig. 3E) with apical teeth as long as or slightly shorter than 1st marginals. Molar plate flat (Fig. 4C), with 10–11 ridges. Antennae of 13–14 segments.

Antillitermes, new genus

Type species. *Parvitermes subtilis* Scheffrahn & Křeček, 1993. Soldier monomorphic. Head capsule pilosity dense, of short, parallel setae, leaning anteriorly. Head capsule slightly constricted behind antennae. Nasus slender. Mandibles vestigial, without points. Antennae of 12 segments.

Workers distinguished from all other genera by digestive tube configuration (Fig. 1F). Main body of mesenteron almost straight, short. Mixed segment very long (from a to d on Fig. 1F), much longer than remainder of mesenteron; P1 very long, forming a wide loop on right side of abdomen, then a complicated pattern of loops along posterior left and dorsal part of paunch. Malpighian tubules inserted at mesentero-proctodeal junction on small, bulbous swellings, in 2 pairs separated by a short space. Enteric valve with 3 major cushions garnished with long, curved spines. Three minor cushions alternate with the major ones. These minor cushions are barely less developed than the major ones in North Hispaniolan specimens (Fig. 2E), but are garnished with much smaller spines in South Hispaniolan and Cuban ones (Fig. 2F), thereby greatly resembling the *Parvitermes* pattern. Mandibles (Fig. 3F) more elongated than in *Parvitermes* species, apical teeth longer than 1st marginals, molar areas of both mandibles concave, molar plate with 6 weak ridges (Fig. 4D). Antennae of 13 segments.

Etymology. Both names recall the geographic distribution of the new genera. They are formed on Spanish "Caribe," "Antillas" and Latin "Termes," termite.

Discussion

The smaller nasute fauna of the Greater Antilles now appears almost completely endemic at the genus level. The only genus present on the neotropical mainland as well as in the Greater Antilles is *Constrictotermes*, represented by a single endemic species in Cuba (Křeček et al. 1996). The study of anatomical characters demonstrated that Greater Antillean species previously included in *Obtusitermes* or *Velocitermes* show only superficial resemblances with these genera and must be reassigned to *Parvitermes*. By contrast, the mainland species formerly known as *P. laticephalus* should be transferred to *Velocitermes*, and *P. bacchanalis* to another genus which is still to be determined. These transfers make *Parvitermes* itself a Greater Antillean endemic, apart from the incursion of *P. brooksi* into the Bahamas.

The most striking features of *Parvitermes* and *Caribitermes* is the configuration of their worker digestive tube: all species are characterized by a long loop of the 1st proctodeal segment (P1) on the right and ventral side of the abdomen. This loop is a clearly apomorphic character. The only non-Antillean genus to possess this feature is *Obtusitermes*. This suggests that *Parvitermes* and *Caribitermes* are descended from a single ancestral form and have the genus *Obtusitermes* as sister-group on the neotropical mainland.

Did the ancestors of the Greater Antillean genera *Parvitermes* and *Caribitermes* possess a dimorphic soldier caste? Soldier monomorphism, as found in *Tenuirostritermes*, most *Nasutitermes*, and many other genera within and outside the Nasutitermitinae, is generally considered plesiomorphic, although evolution can also proceed from soldier polymorphism to monomorphism (Emerson 1962). Because *Obtusitermes* possesses dimorphic soldiers, it is possible that this trait was already present in the common ancestor of *Obtusitermes*, *Parvitermes*, and *Caribitermes*. One soldier morph should then have disappeared during the evolution of *Caribitermes discolor* and 3 *Parvitermes* species (*P. brooksi*, *P. aequalis*, and *P. flaveolus*), while being retained in the other *Parvitermes*, although major soldiers are rare in some of them (Scheffrahn and Roisin 1995). The high variability of soldier polymorphism within groups of species very similar in other respects suggests that this polymorphism varies widely according to the ecology of each species and is of little value as a taxonomical character at higher levels. Attempts to use soldier polymorphism patterns to characterize genera have also met with great difficulties in the mainland complex comprising *Velocitermes* and *Diversitermes*, which superficially resemble *Parvitermes* (Mathews 1977). Although the occurrence of vestigial points on soldier mandibles may be of taxonomic value in other nasutitermitine lineages (Sands 1957), this character shows an erratic pattern in *Parvitermes*.

The worker mandibular morphology of *C. discolor* is that of a typical wood feeder (like most *Nasutitermes* species) and may represent the ancestral condition. *Parvitermes* species display a tendency toward more concave molar areas with fewer ridges, which probably indicates a diet of softer material such as decayed wood or herbaceous plants. The armature of the enteric valve is remarkably similar in all species of *Parvitermes*. Whereas the trilateral symmetry of the enteric valve is the rule in the lower termites and widespread in the Termitidae, and thus likely plesiomorphic (Noirot 1995), the marked difference between minor and major cushions of *Parvitermes* and the presence of long, curved spines on the latter are probably apomorphic.

Antillitermes subtilis is characterized by a unique digestive tube configuration. Although its origin and affinities are unclear, this genus might also be derived from some *Obtusitermes*-like ancestors. *Obtusitermes*, *Parvitermes*, and *Caribitermes* all possess a long mixed segment and a very

long P1. These 2 generally correlated features (Bignell 1994) reach an extreme development in *Antillitermes*. The long and curved enteric valve spines in all samples of *A. subtilis*, and the whole appearance of the enteric valve armature in the Cuban and South Hispaniolan species of this species are remarkably similar to those of *Parvitermes* species. As no such spines have so far been discovered in other nasutitermitine genera world wide (Kovoor 1969; Mathews 1977; Fontes 1987a, b; Roisin and Pasteels 1996), their presence in both *Parvitermes* and *Antillitermes* is likely to be a synapomorphy. Alternatively, *Antillitermes* could be more closely related to the South American monotypic genus *Cortaritermes* Mathews, 1977. In workers of *C. silvestrii* (Holmgren, 1910), P1 is also very long and forms an almost circular loop on the right side of the paunch, as in *A. subtilis* (Fontes 1987a, figures 269–272). However, contrary to *A. subtilis*, P1 lacks a complicated pattern of loops on the left side of the paunch in *C. silvestrii*. According to Mathews (1977), the enteric valve armature of *C. silvestrii* is very similar to that described by Kovoor (1969) for the South African species *Baucaliotermes hainesi* (Fuller, 1922), which consists of extremely elongated swellings garnished with small spines (6 μm long or less). Such a pattern is thus extremely different from that observed in *A. subtilis*. An increase in length and volume of the proctodeum is one of several features characteristic of an evolution toward humivory, although this increase may affect different sections of the proctodeum in different taxa (Noirot 1992, Bignell 1994). Other features of *Antillitermes* indicating humivory include the enlargement of the angle between apical and 1st marginal teeth, the concavity of the molar area and the reduction of the molar ridges. The almost hexalateral symmetry of the enteric valve observed in the North Hispaniolan population of *A. subtilis* is probably a derived trait, common in humivorous genera (Fontes 1987b), but of unknown adaptive value. In any case, *Antillitermes* appears phylogenetically unrelated to the mainland humivorous nasutes of the *Subulitermes* group, characterized by a relatively simple gut anatomy without mixed segment, or with a very short one (Fontes 1987b).

Of particular biogeographic interest is the fact that the number of Greater Antillean nasute genera also present on the neotropical mainland is now reduced from 3 (as mentioned in Scheffrahn et al. 1994) to 2: *Nasutitermes* and *Constrictotermes*. The presence of several species of *Nasutitermes* in the Greater Antilles is easy to explain by the well-known abilities of this genus for dispersal over water gaps, as emphasized by its wide occurrence in the Lesser Antilles (Scheffrahn et al. 1994, Thorne et al. 1994). The single Cuban *Constrictotermes* species shows no notable divergence from its mainland congeners (Křeček et al. 1996) and could derive from a relatively recent colonization. The remainder of the Antillean genera may be—*Antillitermes* still remaining doubtful—the result of radiation from a single ancestral species, related to *Obtusitermes*. Whether this ancestor was a vicariant from the Cretaceous separation of the Greater Antillean island arc or a more recent disperser cannot be ascertained. Ground-dwelling termites often show low dispersal ability compared to wood-dwelling ones, because log rafting seems to be the exclusive means for incipient colonies or self-sustaining colony fragments to cross water gaps more than a few kilometers wide (Ahe 1984). For South American nasutes, their penetration into the Lesser Antilles can serve as an indication of their dispersal abilities. So far, no nasute genus except *Nasutitermes* has penetrated farther north than Tobago, on the continental platform (Scheffrahn et al. 1994). This constitutes evidence for poor dispersal abilities in this group, consistent with our hypothesis of a single ancient inoculation of an *Obtusitermes*-related ancestor into the Greater Antilles.

A remaining question is the total absence of records of smaller nasutes from Jamaica. Jamaica has been less extensively covered by recent surveys than the other Greater Antillean islands. However, thanks to earlier collectors (see Snyder 1956), the number of termite species recorded from Jamaica reaches 19, which seems proportionate to this island's area, by comparison with Puerto Rico (22 species), Cuba (30), or Hispaniola (35) (Scheffrahn et al. 1994). If confirmed by future records, the absence of smaller nasutes from Jamaica could be explained by their low dispersal ability, conjugated with the extensive or complete submersion of this island during the mid-Tertiary. Such a distribution pattern has been described for several other insect taxa (Buskirk 1985).

Acknowledgments

The following persons and institutions provided specimens of interest for this study (see *Appendix 1*): Margaret S. Collins and David A. Nickle (National Museum of Natural History, Washington, DC [NMNH]); Sule Oygur Fischl (American Museum of Natural History, New York [AMNH]); Valter Raineri (Museo Civico di Storia Naturale "Giacomo Doria," Genova, Italy [MCGD]); Janine Casevitz-Weulersse (Muséum National d'Histoire Naturelle, Paris, France [MNHNP]); Patrick Grootaert (Institut Royal des Sciences Naturelles, Brussels, Belgium [IRSNB]); Eliana M. Cancello (Museu de Zoologia, Universidade de São Paulo, Brazil [MZUSP]). Thanks are also due to all collectors who let us benefit from their efforts. We thank J. Tsai and R. W. Howard for critically reviewing the manuscript. The research of Y.R. in Panama and the studies of J.K. on Cuban samples were supported by Smithsonian fellowships. Additional financial support was provided by the Belgian FRFC (grant No. 2.4560.96). This is contribution No. R-05115 of the University of Florida Experiment Station Journal Series.

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Appendix 1: Collection Data of Material Examined and Illustrated

Antilliternes (=*Parvitermes*) *subtilis:* Paratypes, DR451, Las Lavas, Santiago Province, Dominican Republic, 8-VI-1992, collected by R. H. Scheffrahn, J. A. Chase, J. R. Mangold, and J. de la Rosa G. (NMNH and authors' collection) (Figs. 1F, 2E, 3F and 4D)—Playa Siboney, Santiago Province, Cuba, 2-VII-1966, collected by I. Hrdý (Fig. 2F)—DR874, 8 km N of Pedernales, Dominican Republic, 28-X-1993—4 samples from 4 other localities in Dominican Republic and 3 samples from 3 other localities in Cuba (see Scheffrahn and Křeček 1993).

Caribitermes (=*Parvitermes*) *discolor:* Paratypes from type colony, Adjuntas, Puerto Rico, 8–13-VI-1915 (AMNH)—DR500, Los Haitises National Park, Hoto Mayor Province, Dominican Republic, 10-VI-1992, collected by R. H. Scheffrahn, J. A. Chase, J. R. Mangold, and J. de la Rosa G. (Figs. 2G and 3E)—2 samples from La Vega Province, Dominican Republic, 19-VI-1991—PORT1, Maricao, Puerto Rico, 3-VI-1993, collected by Y. Roisin (Figs. 1E and 4C)—62 samples from 19 other localities in Puerto Rico.

Constrictotermes cyphergaster: Syntypes, Coxipò, Mato Grosso, Brazil (MCGD); MZUSP0155, Brasilia, Brazil, X-1961, collected by R. L. Araujo (Fig. 3D).

Constrictotermes guantanamensis: Paratypes from type colony (C236), Loma de Macambo, Guantánamo Province, Cuba, 1-XII-1971, collected by J. de la Cruz.

Obtusitermes panamae: Paratypes from type colony, Quipo, Panama, 18-V-1923, collected by I. Molino and J. Zetek (NMNH)—PANT79, Parque Nacional Soberanía, Rio Agua Salud, Panama, 26-IV-1991, collected by Y. Roisin (Figs. 1C and 2H).

Parvitermes (=*Obtusitermes*) *aequalis:* Holotype soldier, Camagüey, Cuba, 11-VII-1923, collected by B. T. Barreto (NMNH—C26, La Piñuda Buey Cito, Bayamo Oriente, Cuba, 10-XI-1964, collected by J. Křeček (Fig. 2B)—6 samples from 6 localities in Cuba (collected by I. Hrdý or J. Křeček).

Parvitermes (=*Velocitermes*) *antillarum:* Syntypes, Santo Domingo, collected by M. Sallé (MNHNP—Haiti, 22-VI-1976, collected by V. A. Spaeth (Fig. 2C).

Parvitermes brooksi: Paratypes, Soledad, Cienfuegos, Cuba, 1-IV-1924, collected by T. Barbour and W. S. Brooks (NMNH)—C10, Campo Florida, Loma de Coca, Cuba, 21-VI-1972, collected by J. Křeček (Figs. 1A and 3A)—C88/22, 2 km W Ciudad Trinidad, Sancti Spíritus Province, Cuba, 20-VII-1988, collected by J. Křeček (Figs. 2A and 4A)—5 samples from 5 other localities in Cuba, collected by J. Křeček—22 samples from Andros Island, Bahamas, 29-V to 1-VI-1995.

Parvitermes collinsae: Holotype and paratypes from type colony (DR801–802), 2 km W Fondo Negro, Barahona Province, Dominican Republic, 20-III-1993, collected by J. A. Chase, J. de la Rosa G. (NMNH, authors' collections)—Paratypes, 24 series from 10 localities, Dominican Republic (see Scheffrahn and Roisin 1995).

Parvitermes flaveolus: Paratypes from type colony, Manneville, Haiti, XII-1912, collected by W. V. Mann (AMNH)—66 samples from 28 localities in the Dominican Republic.

Parvitermes pallidiceps: Paratypes from type colony, Diquini, Haiti, XI-1912, collected by W. V. Mann (AMNH)—48 samples from 12 localities in the Dominican Republic (see Scheffrahn and Roisin 1995).

Parvitermes (=*Terrenitermes*) *toussainti:* Paratypes from type colony, Millot, N. Haiti, I-1913, collected by W. V. Mann (AMNH)—DR376, Santo Domingo, old amusement park, Dominican Republic, 4-V-1992, collected by R. H. Scheffrahn and J. A. Chase (Figs. 1B, 2D and 3B)—DR927, Bao, Santiago Province, Dominican Republic, 20-VIII-1994, collected by J. A. Chase, J. Křeček, J. de la Rosa G. and R. H. Scheffrahn (Fig. 4B)—50 samples from 27 localities in the Dominican Republic.

Parvitermes wolcotti: Holotype soldier, Boquetón, Puerto Rico, 7-XI-1923, collected by G. N. Wolcott (NMNH)—18 samples from 6 localities in Puerto Rico (see Scheffrahn and Roisin 1995)—4 samples from 3 localities on Guana Island (British Virgin Islands), 18–26-X-1992, collected by J. Křeček.

Parvitermes (=*Velocitermes*?) n. sp. B: DR131–132, Bomba de Gaunuma, Dominican Republic, 16-VI-1991, collected by R. H. Scheffrahn, J. A. Chase, J. R. Mangold, and J. de La Rosa G.—DR495–496, Los Haitises National Park, Hoto Mayor Province, Dominican Republic, 10-VI-1992, collected by R. H. Scheffrahn, J. A. Chase, J. R. Mangold, and J. de la Rosa G.

Tenuirostritermes tenuirostris: Syntypes, Sierra del Nayarit, Jalisco, Mexico, 1990, collected by L. Diguet (IRSNB)—Calabazas Canyon, Santa Cruz County, Arizona, 20-VII-1969, collected by W. L. Nutting and J. M. Pasteels—MEXT6, Road Mazatlán-Culiacán, Mexico, 5-X-1993, collected by Y. Roisin.

Velocitermes barrocoloradensis: Paratypes from type colony, Barro Colorado Island, Panama,

26-II-1924, collected by T. E. Snyder (NMNH)—PANT152, Barro Colorado Island, Panama, 2-VIII-1991, collected by Y. Roisin (Figs. 1D, 2I, and 3C).

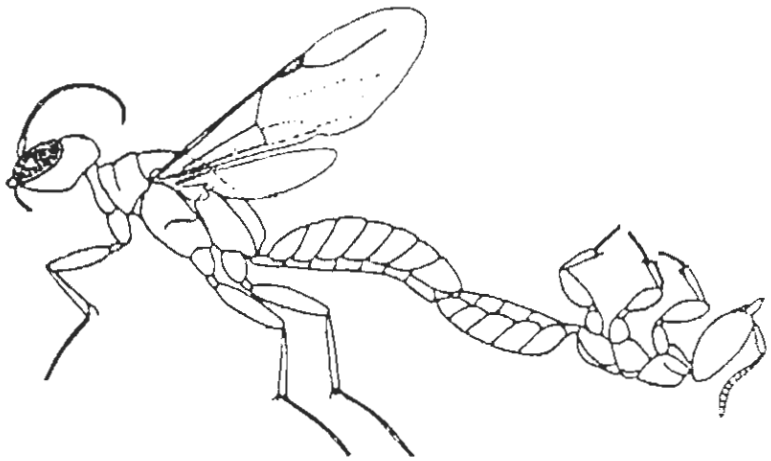
Velocitermes heteropterus: Syntypes, Coxipò, Mato Grosso, Brazil (MCGD).

Velocitermes (=*Parvitermes*) *Laticephalus*: Paratypes from type colony, Covendo, Bolivia, 1921, collected by W. V. Mann (NMNH).

Received for publication 16 August 1995; accepted 30 April 1996.

BETHYLID WASPS

"The Bethylidae attack the larvae of beetles and of moths living in hidden situations, such as in the soil, in burrows in wood, leaf mines and galls, etc. These are minute wasps, the females often wingless and sometimes blind. They are usually much smaller than their hosts, and the female typically lays several eggs on the host and several larvae develop on it....



"Phoretic copulation: the male carries the female about for an hour or more during mating, suspended from his posterior end by the interlocking genitalia."

H. E. Evans and M. J. Eberhard. 1970. The Wasps. University of Michigan Press, Ann Arbor.

1996. *Mem.Ent.Soc.Wash.* 17:194-208

SYSTEMATIC NOTES ON SOME BETHYLIDAE FROM THE VIRGIN ISLANDS AND PUERTO RICO (HYMENOPTERA: CHRYSIDOIDEA)

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Abstract.—Fourteen species in eight genera are treated. New distribution records are given for six previously described species. Eight species are described as new: ***Anisepyris arawak, A. chupah, Epyris jareckii, E. karli, E. guana, Holepyris skip, Rhabdepyris maboya*** (all from the Virgin Islands) and ***Apenesia yu*** (Puerto Rico). A key is provided for females of the known *Epyris* of the Greater Antilles.

Key Words: Hymenoptera, Bethylidae, Bethylinae, Epyrinae, Pristocerinae, taxonomy

Dedication.—I am pleased to dedicate this paper to Karl, a friend and colleague and a Hymenopterist in the truest sense, one whose work and productivity have been an inspiration to many of us.

This paper is the first of at least two that will treat the bethylid fauna of the islands comprising the Puerto Rico Bank: Puerto Rico and its immediately adjacent islands, as well as the Virgin Islands (British and American). Although poorly collected, these islands very likely have an impressive bethylid fauna, as evidenced by the diversity seen in the small collection reported on here. Most of the specimens were collected during three brief trips to Guana Island (at approximately 18°29′N, 64°34′W), situated less than half a mile off the eastern tip of Tortola, the largest of the British Virgin Islands (BVI).

Guana Island is owned by Dr. Henry Jarecki and is a wildlife sanctuary. Only about 340 hectares in size, Guana is a dry island, now mostly regrown secondary forest. During the colonial period it supported a small sugar cane plantation. Currently a small hotel and support structures occupy a portion of the island; most of the island is forested and little disturbed.

In addition to the specimens that I have been able to collect on my trips to the Caribbean, I have been able to study types critical to the current study from other institutions: the Canadian National Collection (CNC), Ottawa; the Museum of Comparative Zoology (MCZ), Cambridge; the National Museum of Natural History (USNM). Additionally, I have been able to examine a small collection of miscellaneous bethylids from the U.S. Virgin Islands, principally St. Croix, from the collection of Montana State University (MSUC). Unless otherwise noted, all specimens are deposited in the collections of the Natural History Museum of Los Angeles County (LACM).

The most important works on New World Bethylidae are those of H. E. Evans; especially important as a starting point is his synopsis (1964) with its keys to the several subfamilies and their constituent genera. Subsequent to that synopsis of the family, there have been several important revisions of genera, especially those of

Pseudisobrachium (1961), *Rhabdepyris* (1965), *Anisepyris* (1966), and *Epyris* (1969). Pertinent smaller papers are listed in the Literature Cited.

Terminology

The terminology, abbreviations and acronyms used in the descriptions below follow those established by Evans in his various publications on the systematics of the American Bethylidae. The only notable exception is that I employ the term "mesosoma" in preference to his use of "thorax." The following explanations for the terms are mostly those of Evans.

HE—Height of eye: maximum height (or length) of eye as measured in lateral view.

LFW—Length of fore wing: measured as a more accurate indication of size (in fully winged forms) than body length, since the latter is much influenced by the position of the head and the amount of extension of the abdomen.

LH—Length of head: measured from median apical margin of clypeus to median point of vertex, expressed in millimeters and/or as a ratio with width of head.

LT—Length of "thorax," i.e. mesosoma: measured in lateral view from the pronotum (excluding the collar) to the apex of the propodeum.

OOL—Ocellocular line: shortest distance from a lateral ocellus to nearest eye margin.

PD—Puncture diameter.

WF—Width of front: minimum width, i.e. at the point of closest approximation of the eyes.

WH—Width of head: maximum width, including eyes, usually expressed as a ratio with length of head and/or maximum width of mesosoma.

WOT—Width of ocellar triangle: distance across and including lateral (posterior) ocelli.

Antennal segments: Antennal segments are numbered from first to last, the scape being the first segment; reference to following segments is abbreviated to A2, A3, A4, etc.

Discal carinae [of propodeum]: the major longitudinal carinae of the propodeal disc, including median but excluding sublateral and lateral carinae.

Lateral carinae [of propodeum]: the longitudinal carinae along the sides of the propodeal disc.

Length of propodeal disc: measured along midline, but exclusive of declivity.

Length of propodeum: measured in full dorsal view including declivity and including the projections which embrace the sides of the mesonotum (in females in which these are present).

Propodeal formula: in wingless females, the width of the propodeum anterior to the constriction as compared to the width at the constriction and as compared to the greatest width posterior to the constriction.

Transverse carina [of propodeum]: the transverse carina margining the propodeal disc behind (i.e. separating it from the declivity).

Width of propodeal disc: the maximum width in full dorsal view.

In order to facilitate comparison, the descriptions of new species follow the same format and style as those of Evans in his various publications on bethylids.

Subfamily Bethylinae

Goniozus crassifemur Evans

Goniozus crassifemur Evans, 1969a: 10–11; ♀.

This species was described from Dominica (type locality) in the Lesser Antilles and from St. Croix. Evans (1970) reported on and characterized a male from Cuba that he tentatively assigned to this species.

Specimen examined: BRITISH VIRGIN ISLANDS, *Guana I.:* 1 ♀, White Beach flats, 24–30 June 1993, Malaise trap (R. R. Snelling).

Parasierola rivularis Evans

Parasierola rivularis Evans, 1969a: 7–8; ♀.

Although originally based on specimens from Dominica, *P. rivularis* was later recorded from Jamaica by Evans (1970).

Specimens examined: BRITISH VIRGIN ISLANDS, *Guana I.:* 2 ♀♀, North Beach woods, 15–20 Apr. 1993, Malaise trap (R. R. Snelling); 1 ♀, same except 20–25 Apr. 1993; 1 ♀, same except 24–30 June 1993; 1 ♀, same except White Beach, 1–7 July 1993.

SUBFAMILY EPYRINAE

Anisepyris aurichalceus (Westwood)

Epyris aurichalceus Westwood, 1874: 160–161, pl. 31, fig. 3; ♀.

Anisepyris aurichalceus: Kieffer, 1905: 96. Kieffer, 1914: 442. Evans, 1964: 99. Evans, 1966a: 7, 21–24; figs. 10–11, 20–22, 29; ♀ ♂.

Anisepyris viridis Kieffer, 1907: 12–13; ♂. Preoccupied.

Anisepyris viridellus Kieffer, 1914: 438. New name. Evans, 1959: 70, 71.

Anisepyris cubensis Fouts, 1928: 125–126; ♀.

Evans (1966a) noted that this species, originally described from Cuba, is widely distributed in the Greater Antilles, including Puerto Rico, St. Thomas and St. Kitts; one specimen was recorded from Miami, Florida. It is probably also present on Hispaniola and Jamaica.

Specimen examined: BRITISH VIRGINS ISLANDS, *Guana I.:* 1 ♂, lower Quail Dove Ghut, 20–25 Apr. 1993, Malaise trap (R. R. Snelling).

Anisepyris arawak Snelling, NEW SPECIES

Figs. 1, 11, 23, 26

Holotype: ♂, BRITISH VIRGIN ISLANDS, *Guana I.:* Long Man's Point trail, 23–30 Oct. 1962, Malaise trap (R. R. Snelling, in LACM).

Paratype: ♂, same data as holotype, in LACM.

Description: Length 3.6–3.8 mm; LFW 2.23–2.32 mm.

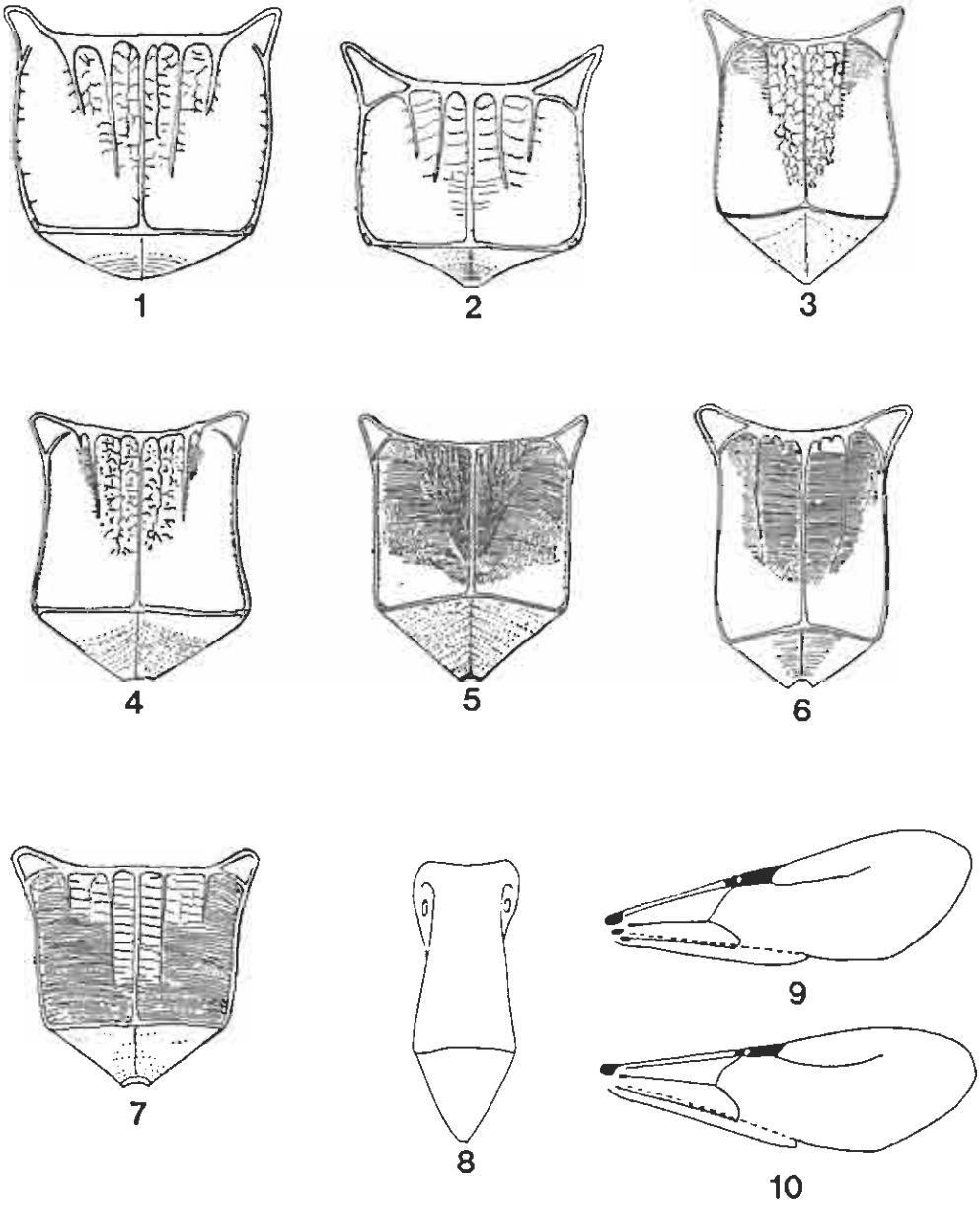
Head and most of mesosoma bright metallic green, with scattered coppery reflections (most conspicuous on lower frons); side of pronotum and side of propodeum blackish green; carinate area of propodeal disc blackish; metasoma black; antennae blackish, but with weak greenish reflections, especially on scape; mandibles and legs testaceous; wings hyaline and slightly brownish.

Margins of head above eyes, in frontal view (Fig. 11), slightly convex and convergent, broadly rounded onto weakly convex vertex. Clypeal apex acute, median carina projecting slightly beyond margin. Mandible 5-dentate, apical and preapical teeth subequal and larger than inner 3 teeth. Antennal scrobes weakly carinate. Median impression of frons elongate, weak. WH 1.0–1.04 × LH; eyes convergent below; WF 0.62–0.63 × WH, 1.23–1.24 × HE; distance from eye summit to summit of vertex 0.48–0.53 × HE. Ocellar triangle compact, OOL 0.92–1.00 × WOT. Antennae elongate, A3 about 0.3× as long as A2, A4 and All each about 4× as long as wide. Frons strongly alutaceous and moderately shiny, lower one-half appearing granulose; punctures minute, separated by 1.5–3.0 PD.

Dorsum of mesosoma slightly shinier and less strongly alutaceous than frons; punctures of pronotum and mesoscutum similar to those of frons but mostly separated by about 3.0 PD. Scutellar groove curved back and slightly enlarged at each end. Propodeal disc (Fig. 1) about 1.4× as wide as long, with five well developed carinae and two much weaker submedian carinae; posterior fovea distinct; sides of disc alutaceous and moderately shiny; side of propodeum very delicately longitudinally striate. Lower mesepisternal fovea broadly open above. Tarsal claws dentate.

Apical margin of subgenital plate distinctly projecting in middle and concave between short submedian teeth (Fig. 23). Dorsal arm of cuspis obliquely narrowed to subacute apex (Fig. 26); apex of paramere truncate in profile (Fig. 26).

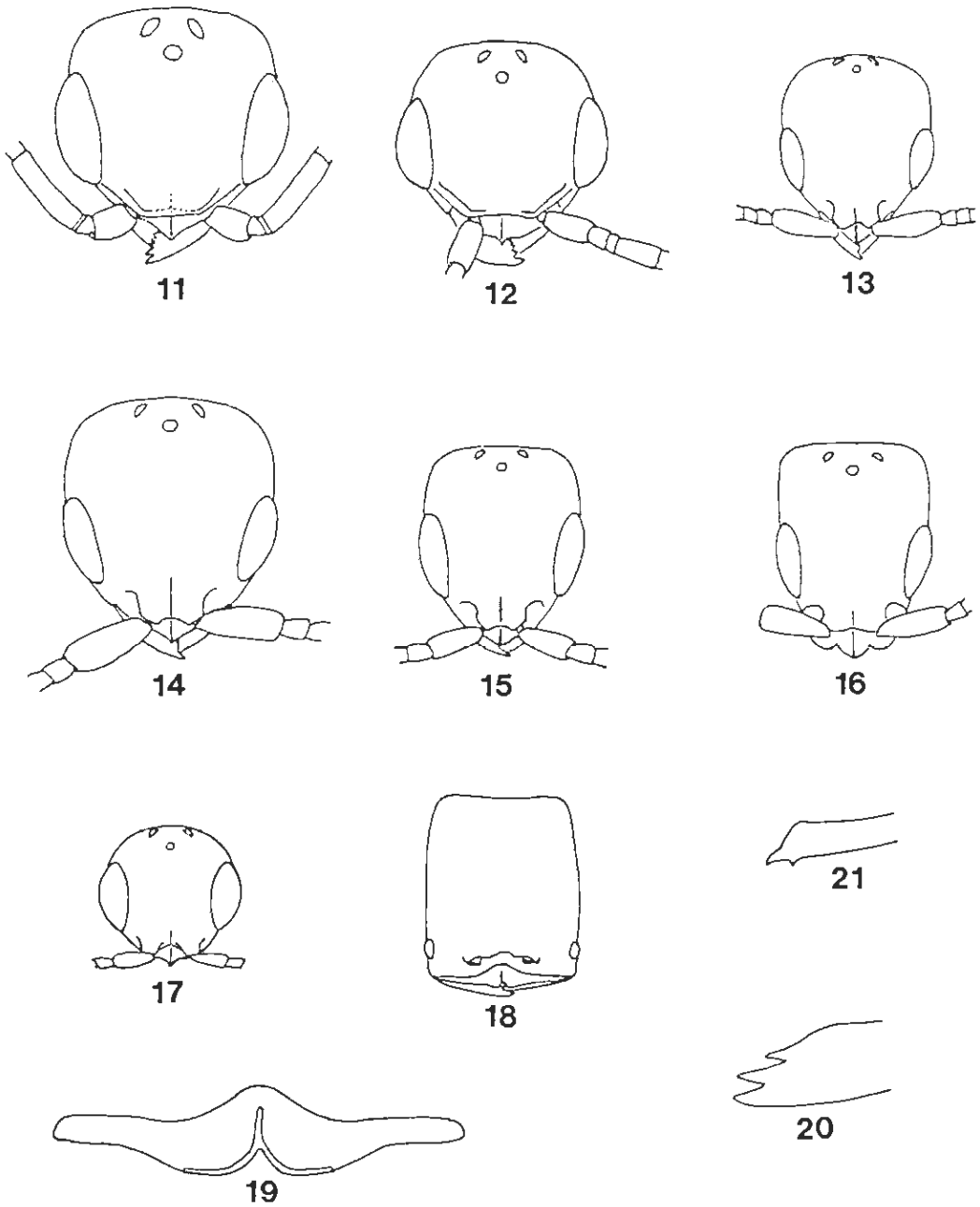
Etymology: Named for the Arawak people, resident in the Virgin Islands prior to



Figs. 1–8. Dorsal view of propodeum: 1, *Anisepyris arawak*; 2, *A. chupah*; 3, *Epyris guana*; 4, *E. jareckii*; 5, *E. karli*; 6, *Holepyris skip*; 7, *Rhabdepyris mahoya*; 8, *Apenesia yu*. Figs. 9–10, forewing: 9, *Epyris guana*; 10, *E. karli*.

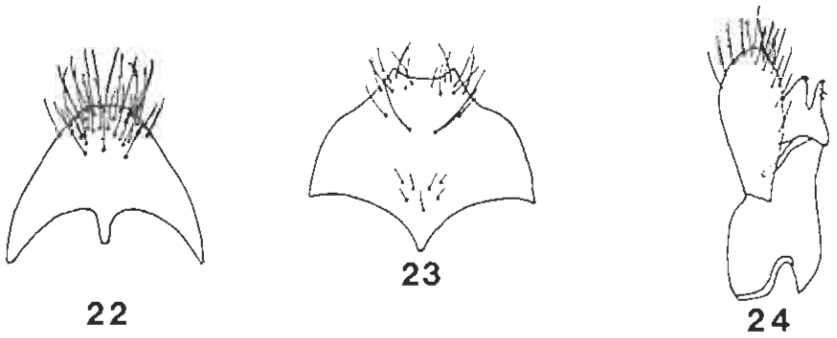
the arrival of Europeans; the name is a noun in apposition.

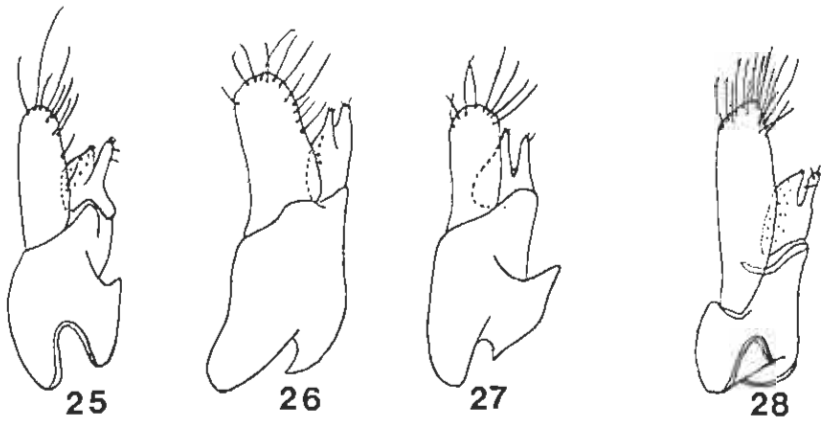
Remarks: This species belongs to the excisus group of Evans (1966a), the males of which are characterized by the very elongate antennae, the distinctly carinate antennal scrobes, and the head and mesosoma metallic greenish or bluish. There are four described species in this group, three of which (*A. excisus* Evans, 1959; *A. darlingtoni* Evans, 1966a; *A. jocundus* Evans, 1966a) are found in the Greater Antilles and



Figs. 11–18. Frontal view of head: 11, *Anisepyris arawak*; 12, *A. chupah*; 13, *Epyris guana*; 14, *E. jareckii*; 15, *E. karli*; 16, *Holepyris skip*; 17, *Rhabdepyris maboya*; 18, *Apenesia yu*. Figs. 19, 20, clypeus and mandible, respectively, of *A. yu*. Fig. 21, mandible of *Epyris manni*.

one (*A. dominicanus* Evans, 1966a) in the Lesser Antilles. Males are known only for *A. darlingtoni* and *A. jocundus*; both differ from *A. arawak* by the coarser, closer punctures of the frons, the well defined carina bordering the antennal scrobe, and the profile of the genitalic capsule (Fig. 25). These are also larger species. Other species of *An-*





Figs. 22, 23. Male subgenital plate: 22, *Anisepyris chupah*; 23, *A. arawak*. Figs. 24–28, lateral view of male genitalia: 24, *Anisepyris ecuadoriunus*; 25, *A. jocundus*; 26, *A. arawak*; 27, *A. chupah*; 28, *A. metallicus*. Figs. 24, 25, 28 redrawn from Evans (1966).

isepyris occur in the Greater Antilles, but males of these, when known, have shorter antennae (A3+A4 no more than 2.8× as long as thick, All no more than 3× as long as thick), many lack scrobal carinae, or differ in various other features.

From *A. chupah*, described below, *A. arawak* differs in the much coarser sculpture of the lower frons, the partially metallic greenish propodeal disc, the presence of seven discal carinae, and the generally greenish color.

Anisepyris chupah Snelling, NEW SPECIES

Figs. 2, 12, 22, 27

Holotype: ♂, BRITISH VIRGIN ISLANDS, *Guana I.:* North Beach woods, 7–12 July 1993, Malaise trap (R. R. Snelling), in LACM.

Paratypes: 1 ♂, same data as holotype (LACM). U.S. VIRGIN ISLANDS, *St. John:* 1 ♂, Estate Caneel Bay, Caneel Hill, 240 ft., 17 Dec. 1991–2 Jan. 1992 ("VIBFP collectors"; MSUC). *St. Croix:* 1 ♂, Estate

North Star, 60 ft., 23 Feb.–23 Mar. 1993 (J. Keularts; MSUC); 1 ♂, same except 23 Mar.–23 Apr. 1993; 1 ♂, same except 19 Jul.–23 Aug. 1993.

Description: Length 3.4–4.5 mm; LFW 2.03–2.55 mm.

Head, pronotal disc, mesoscutum, scutellum, and mesepisternum dark blue-green; side of pronotum, entire propodeum, and metasoma black; antennae blackish brown, scape with very weak metallic reflections; mandibles brown at base, becoming testaceous distad; legs testaceous, except coxae and femora brown; wings hyaline and slightly brownish.

Head (Fig. 12) about as described above for *A. arawak*, but lower frons evenly alutaceous, not appearing granulose, and not contrasting with upper frons. Mandible with 5 prominent teeth, inner 4 approximately equal in size, smaller than apical tooth. Lower margin of clypeus angulate in middle. WH 1.04–1.05 × LH; WF 0.62–0.67 × WH, 1.25–1.50 × HE; distance from eye summit to summit of vertex 0.43 × HE; OOL 1.33 × WOT. A3 about 0.5× length of A2; AII about 2.1× as long as wide.

Mesosoma about as described for *A. arawak*, but scutellar groove distinctly enlarged at each end; propodeal disc (Fig. 2) about 1.4× as wide as long, with five carinae, submedian pair extending about 0.66 of distance to posterior carina and outer pair about one-half length of submedian pair; carinate area shiny, with irregular transverse rugae, remainder of disc alutaceous and duller.

Apical margin of subgenital plate narrowly rounded in middle (Fig. 23). Apex of dorsal arm of cuspis acute (Fig. 27); apex of paramere truncate in profile (Fig. 27).

Etymology: This species is affectionately dedicated to a long-time resident of Guana Island, "Chupah," a large female of *Iguana pinguis*, who has a definite "sweet tooth"; the name is a noun in apposition.

Remarks: This species differs from the preceeding most notably in the stouter antenna, the presence of only five discal carinae and the narrowly rounded subgenital plate.

In Evan's revison (1966) of *Anisepyris*, *A. chupah* keys to couplet 20 separating *A. metallicus* Kieffer, 1905, and *A. ecuadorianus* Evans, 1966. These species belong to the proteus group of Evans, a large complex with about 30 species ranging between the southwestern United States and Brazil and Bolivia; one species, *A. insularis* (Ashmead, 1894) has been described from St. Vincent in the Lesser Antilles. Both *A. ecuadorianus* and *A. insularis* have seven discal carinae and the integument of both head and mesosoma is black (limited "weak" metallic reflections present on frons, pronotum and mesonotum of *A. ecuadorianus*). From both species *A. chupah* further differs in genitalic features (compare figure 27 with 24 and 28). The antennae of *A. metallicus* are more elongate (All 2.5–3.0× as long as thick) and the propodeal disc is transversely striate outside the median carinate area.

Epyris guana Snelling, New Species

Figs. 3, 9, 13

Holotype: ♀, BRITISH VIRGIN ISLANDS, *Guana I.:* Long Man's Point trail, 23–30 Oct. 1992, Malaise trap (R. R. Snelling), in LACM.

Description: Length about 3.5 mm; LFW 1.91 mm.

Front of head and dorsum of mesosoma dark metallic green, except anterior band on mesoscutum and entire propodeal disc blackish; side of mesosoma blackish but mesepisternum with dark greenish tints; metasoma piceous, two apical segments dark reddish brown; antennae and legs testaceous. Wings hyaline and with weak brownish tinge.

Mandible tridentate. Clypeus with narrowly rounded median lobe; lateral lobes much shorter and much more broadly rounded. Antennal sockets about on level of lower margin of eyes (Fig. 13); scape

about 3× as long as wide; ratio of first four antennal segments, 30:7:6:7; A3 slightly wider than long, All slightly longer than wide. Eyes with sparse, long hairs. WH about 0.85 × LH; WF 0.69 × WH, 1.57 × HE; vertex nearly flat in frontal view, distance from summit of eye to summit of vertex about 0.71 × HE. Ocellar triangle compact, well above level of tops of eyes; OOL 1.60 × WOT. Frons shiny, weakly alutaceous, especially between eyes and ocelli, punctures sparse and well defined.

Pronotal contours evenly rounded; pronotal disc about 2× as long as mesoscutum; pronotal disc more sharply alutaceous than frons, similarly punctate; mesoscutum similar, with a few close, fine punctures between notauli; scutellum shinier, with scattered punctures; scutellar pits short and oblique, separated by more than their lengths. Notauli complete. Propodeal disc (Fig. 3) distinctly wider posteriorly, about 1.18× as wide as long; median area shiny, reticulate but without lateral discal carinae other than outermost defining median area; lateral area becoming almost smooth posterolaterad. Profemur stout, about 2× as long as wide; mesotibia strongly spinose; submedian tooth of tarsal claw minute. Transverse median vein of forewing strongly oblique, recurrent posteriorly (Fig. 9). Metasoma polished.

Etymology: Named for Guana Island; the name is a noun in apposition to the generic name.

Remarks: In the revision of *Epyris* (Evans, 1969b), *E. guana* will key to the tricostatus group and, within that group, to *E. hirsutus* Evans, a Brazilian species. As in that species, the head and mesosoma are very sparsely punctate, but *E. guana* has the head more lengthened above the eyes (distance from eye summit to level of vertex margin only about 0.50 × HE in *E. hirsutus*) and the mandibles are tridentate. Separable from other Antillean *Epyris* as indicated in the key below.

Epyris jareckii Snelling, New Species

Figs. 4, 14

Holotype: ♀, BRITISH VIRGIN ISLANDS, *Guana I.:* Long Man's Point trail, 23–30 Oct. 1992, Malaise trap (R. R. Snelling), in LACM.

Paratype: ♀, same except White Beach, 1–7 July 1993.

Description: Length about 5.1 mm; LFW 2.7 mm.

Head, pronotum, mesoscutum, scutellum and mesepisternum dark green; propodeum (including disc) and metasoma black, last two metasomal segments reddish; mandibles, antennae, tegulae and legs testaceous. Wings hyaline, weakly brownish.

Mandible simple, i.e. with no defined teeth based of apical tooth. Median lobe of clypeus narrowly rounded; lateral lobes much shorter and more broadly rounded. Antennal sockets well below level of lower end of eye (Fig. 14); scape almost 3× as long as wide; ratio of first four antennal segments, 28:6:6:7; A3 about as wide as long, All slightly longer than wide. Eyes nearly bare, but with scattered long hairs behind. WH about 0.82 × LH; WF 0.74 × WH, 1.70 × HE; vertex nearly flat in frontal view, distance from summit of eye to level of vertex margin about equal to HE. Ocellar triangle compact, anterior angle nearly 90°, OOL about 1.9 × WOT. Frons slightly shiny and sharply alutaceous between fine, well defined punctures that are 1.5 PD or more apart.

Pronotal contours evenly rounded, disc about 2× as long as mesoscutum; sculpture of disc, mesoscutum and scutellum similar to frons, but mesoscutum anteriorly impunctate and with fine, close punctures on posterior half. Notauli complete. Scutellar pits oblique and separated by slightly more than their lengths. Propodeal disc (Fig. 4) about 1.4× as wide as long; median area shiny, coarsely reticulate, reticulae with more or less longitudinal bias; lateral areas shiny and very weakly roughened. Pro-

femur stout, about 1.8× as long as wide; mesotibia strongly spinose; submedian tooth of tarsal claw minute. Transverse median vein of forewing strongly oblique, recurrent posteriorly. Metasoma polished.

Paratype agrees generally with above description except: length about 4.8 mm; LFW 2.3 mm; WH 0.89 × LH; WF 0.70 × WH, 1.64 × HE; distance from summit of eye to level of vertex margin slightly less than HE; OOL about 1.8 × WOT.

Etymology: This species is dedicated to Dr. Henry Jarecki, in appreciation of his support of my research on Guana Island.

Remarks: This is another member of the tricostatus group and will run to couplet 8 in Evans' key but fails to agree with either lug. This species is separable from other Antillean *Epyris* as noted in the key below.

Three males from Guana Island may belong to this species: 1, North Beach woods, 20–25 Apr. 1993, malaise trap (R. R. Snelling); 1, same except White Beach, 1–7 July 1993; 1, same except Long Man's Point trail, 23–30 Oct. 1992.

The mandibles are slender and possess an oblique, edentate cutting edge. The third antennal segment is not ring-like, and is about 1.5× as long as the second, and about 0.75× as long as the fourth. The frons is moderately shiny, sharply alutaceous and with sparse, minute punctures. WH about equal to LH; WF about 0.8 × WH, about 1.6 × HE; distance from eye summit to level of vertex margin about 0.4 × HE; OOD about 1.1 × WOT.

Epyris karli Snelling, NEW SPECIES

Figs. 5, 10, 15

Holotype: ♀, BRITISH VIRGIN ISLANDS, *Guana I.*: White Beach, 1–7 July 1993, Malaise trap (R. R. Snelling), in LACM.

Description: Length about 4 mm; LFW 1.9 mm.

Head and dorsum of mesosoma dark green, except median area of propodeal disc; median area of propodeal disc, side of mesosoma, metasoma all blackish, but last two metasomal segments somewhat reddish; mandibles, antennae (except blackish dorsum of scape), tegulae, tibiae and tarsi all yellowish brown; femora blackish except at base and apex where they are yellowish brown. Wingly hyaline, faintly brownish.

Mandible tridentate. Clypeus with narrowly rounded median lobe; lateral lobes much shorter and more broadly rounded. Antennal sockets slightly below level of lower eye margin; ratio of first four antennal segments about 27:10:7:10; A3 about as wide as long, All slightly longer than wide. Eyes with sparse short hairs. WH (Fig. 15) about 0.9 × LH; WF 0.65 × WH, 1.26 × HE; vertex margin nearly flat in frontal view; distance from summit of eye to level of vertex margin about 0.71 × HE. Ocelli in compact triangle well above level of eyes, near vertex margin; OOL 1.50 × WOT. Frons shiny and weakly alutaceous, punctures sparse and well defined.

Pronotal contours evenly rounded, disc more than 2× as long as mesoscutum; pronotum, mesoscutum and scutellum texture similar to frons, pronotum with similar, more widely spaced punctures. Notauli complete, but narrower anteriorly. Scutellar pits short and oblique, separated by about 3× their length. Propodeal disc (Fig. 5) about 1.5× as wide as long, median area granulopunctate and with about six short, obscure discal carinae in addition to complete median carina; remainder of disc slightly shiny, sharply and finely alutaceous. Profemur stout, about 1.7× as long as wide; mesotibia conspicuously spinose; median tooth of tarsal claw minute. Transverse median vein of forewing moderately curved, not recurrent behind (Fig. 10). Metasoma polished.

Etymology: It is with pleasure that I dedicate this species to my respected friend and colleague, Karl Krombein, a fellow Hymenopterist (as opposed to myrmecologist, melittologist, sphecidologist, etc.).

Remarks: Unlike the foregoing two

species, *E. karli* is a member of the rufipes group and in the key to members of that group by Evans (1969b) fails at couplet 11 since it does not agree with the combination of features in either lug. Separable from other Antillean *Epyris* as noted in the following key.

KEY TO GREATER ANTILLEAN *EPYRIS*, Females Only

1 Mandibles lacking claw-like apical tooth and never with ventral tooth beneath it; head longer than wide 2
– Apical tooth of mandible curved and claw-like and with small tooth on margin (Fig. 21); head slightly wider than long . . . . . *manni* Evans
2(1) Head and dorsum of mesosoma (except propodeum) dark metallic green; discal carinae not as below; wings uniformly light brown 3
– Head and pronotum (except dark posterior margin) rufotestaceous; mesosoma otherwise black; propodeum with three median discal carinae extending to posterior carina; forewing with 2 brown spots . . *hispaniolae* Evans
3(2) Median area of propodeal disc more or less shiny, not dull and granulopunctate 4
– Median area of propodeal disc dull and granulopunctate, discal carinae very short . . . . . *karli*, n. sp.
4(3) Transverse median vein of forewing strongly oblique, sharply recurrent behind (Fig. 9) 5
– Transverse median vein only moderately oblique, rounded behind but not recurrent (Fig. 10) . . . . . *cubanus* Evans
5(4) Ocellocular area as densely and sharply alutaceous as middle of frons; propodeal disc, adjacent to median area, without transverse striolation; eye about as long as distance from eye summit to level of vertex margin . . . . . *jareckii*, n. sp.
– Ocellocular area conspicuously shinier and less definitely sculptured than middle of frons; propodeal disc, adjacent to median area, with transverse striolation; eye length about 1.25× distance from eye summit to level of vertex margin . . . . . *guana*, n. sp.

Genus *Holepyris* Kieffer

The genus *Holepyris* has not, as noted previously by Evans (1970), been subjected to a modern revision. Fourteen species were reported from the New World by Evans (1964), three from the Caribbean. One additional Caribbean species was described by Evans (1970).

Holepyris incertus (Ashmead)

Epyris incertus Ashmead, 1894: 189; ♂.
Holepyris incertus: Kieffer, 1914: 389. Evans, 1969: 5; 1970: 347–348.

This appears to be a common and widely distributed species. The type locality is St. Vincent; Evans (1969) recorded it first from Dominica and then (1970) from St. Croix, St. John, and St. Thomas, in the U.S. Virgin Islands, and from Cuba.

Specimens examined: BRITISH VIRGIN ISLANDS, *Guana I.:* 2 ♀♀, lower Quail Dove Ghut, 15–20 Apr. 1993, Malaise trap (R. R. Snelling); 2 ♀♀, same except 20–25 Apr. 1993; 1 ♂, same except North Beach woods, 15–20 Apr. 1993; 1 ♀, same except North Beach woods, 24–30 June 1993; 2 ♀♀, same except White Beach flats; 2 ♀♀, same except White Beach, 1–7 July 1993; 2 ♀♀, 4 ♂♂, same except plantation area, 16–20 Oct. 1992; 2 ♀♀, same except Long Man's Point trail, 23–30 Oct. 1992. PUERTO RICO, *Mona I.:* 1 ♀, *Casuarina* plantation, 7–13 Nov. 1992, Malaise trap (R. R. Snelling & J. A. Torres).

***Holepyris skip* Snelling,** NEW SPECIES

Figs. 6, 16

Holotype: ♀, BRITISH VIRGIN ISLANDS, *Guana I.*: White Beach, 7–12 July 1993, Malaise trap (R. R. Snelling), in LACM.

Paratypes: 2 ♀♀, same except 1–7 July 1993; 1 ♀, same except White Beach flats, 24–30 June 1993; 1 ♀, same except plantation area, 16–20 Oct. 1992. Paratypes in LACM and MCZ.

Description: Length about 3.4–3.9 mm; LFW 1.66–2.15 mm.

Head and body blackish; mandibles (mostly), antennae and legs testaceous. Wings hyaline, brown tinged.

Entire front of head with abundant long, coarse, reclinate setae, those of frons directed dorsad, those of vertex exceptionally long and directed mesad; several exceptionally long setae at dorsolateral angles reach down along head margin to slightly below eye summits. Similar setae also present on gula, dorsum and sides of mesosoma; some long setae present also on primary wing veins.

Margins of head (Fig. 16), above level of eyes subparallel and abruptly rounded onto vertex; vertex nearly straight in frontal view. Clypeus large, median lobe extending well below lateral lobes and its apex narrowly rounded; median carina evely arched in profile. Antennae arising well below lower ends of eyes, scrobes ecarinate; ratio of first four segments about 30:9:6.5:9; All about 1.3× as long as wide. WH 0.71–0.72 × LH; WF 0.65–0.68 × WH, 1.26–1.36 × HE; distance from eye summit to summit of vertex 1.06–1.07 × HE. Anterior ocellar angle obtuse; OOL about 1.3 × WOT. Preoccipital carina weakly defined across back of vertex. Frons alutaceous and shiny between numerous minute punctures separated by 1.5–2.5 PD.

Pronotal disc about 1.3× as wide as median length; anterior margin without definite thickened ridge; posterior margin not paralleled by a series of small foveae. Parapsidal furrows complete, but notauli extending only slightly beyond midlength of mesoscutum; scutellar groove about 5× as wide as median length. Propodeal disc (Fig. 6) about as wide as long, margins subparallel in dorsal view for most of length, very slightly convergent distad; median carina complete; two very short and weak submedian carinae present; outer discal carina strong and extending beyond midlength; disc irregularly transversely striate. Profemur about 1.8× as long as deep; mesotibia aspinose.

Etymology: This species is named for James "Skip" Lazell, whose interest and enthusiasm were instrumental in fostering my work on Guana Island.

Remarks: In addition to the above type material I have seen a single female from PUERTO RICO, *Mona I.: Casuarina* plantation, 28–30 Oct. 1991, Malaise trap (R. R. Snelling and J. A. Torres).

Among the species of *Holepyris* presently known from the Greater Antilles, *H. skipi* is easily recognized by virtue of the closely punctate frons with abundant coarse, reclinate setae. Somewhat similar setae are present in *H. incertus*, but they are finer and the surface of the frons is dull, owing to the denser and more sharply defined sculpturing. In that species, too, the dorsolateral angles of the head are more rounded and the median clypeal lobe is short and proportionately broader.

The only other species in our area is *H. vison* Evans, 1970, a smaller and conspicuously more gracile species that lacks the cephalic setae; the WH is only about 0.62 × LH and the distance from the eye summit to the summit of the vertex is about 1.5 × HE. Only the type of *H. vison*, from St. Croix, is known.

Rhabdepyris maboya Snelling, New Species

Figs. 7, 17

Holotype: ♀, BRITISH VIRGIN ISLANDS, *Guana I.:* North Beach woods, 1–7 July 1993, Malaise trap (R. R. Snelling; LACM).

Paratypes: 1 ♀, same data as type; 1 ♀, 2 ♂♂, same except 20–25 Apr. 1993; in LACM. U.S. VIRGIN ISLANDS, *St. John:* 1 ♀, Estate Coral Bay, 14 May 1984 (W. B. Muchmore; MSUC).

Description: ♀: Length about 2.5–2.9 mm; LFW 1.37–1.45 mm.

Head coppery, becoming metallic emerald green on vertex; mandibles and antennae testaceous, scape paler beneath, flagellum darker above. Dorsum of mesosoma metallic emerald green, except propodeal disc darker; side of pronotum emerald green, mesepisternum more or less coppery, side of propodeum darker, more bluish; legs testaceous, except outer face of profemur

mostly blackish, with strong greenish to bluish reflections. Wings hyaline, brown-tinged. Metasoma piceous.

Mandible slightly longer than malar area. Clypeus obtusely angulate in frontal view and with distinct median tooth (Fig. 17); median carina sinuate in profile. Ratio of first four antennal segments about 22:7:6:7; distal segments (except last) slightly wider than long. WH 1.02–1.03 × LH; WF 0.59–0.62 × WH, 1.16–1.18 × HE. Ocelli small, angle at anterior ocellus slightly less than 90°, OOL and WOT subequal. Frons so coarsely alutaceous as to appear granulose, but strongly shiny, scattered minute punctures almost indiscernible.

Pronotum, mesoscutum and scutellum shiny, much more weakly alutaceous than frons and with scattered obscure punctures. Pronotal contours evenly rounded, groove parallel with posterior margin absent across middle one-half. Notauli strongly divergent cephalad, strong behind, but barely perceptible anteriorly. Scutellar groove slightly widened at each end. Propodeal disc (Fig. 7) about 1.4× as wide as long; disc shiny; median area tricarinate and with one obscure, very short carina at lateral margins of median area; entire disc irregularly transversely striate, more coarsely so in median area. Profemur about 2.7× as long as wide.

♂, Length 1.8–2.0 mm; LFW 1.10–1.13 mm.

Color about as in female but frons less strongly coppery and with more greenish tints, mesepisternum emerald green, side of propodeum blackish. Antennae brown, paler beneath, especially scape. Coxae, trochanters and femora blackish and with strong green reflections. Protibia testaceous; meso- and metatibiae brown; tarsal segments testaceous.

Outer two teeth of mandible much stronger than three inner teeth. Ratio of first four antennal segments 14:5:8:7; segments 3 and 11 each about 2× as long as wide. WH 1.08–1.13 × LH; WF 0.62–0.65 × WH, 1.45–1.48 × HE; OOL 0.82–0.88 × WOT.

Etymology: The specific name is a Taino (Arawak) word for a perverse or troublesome spirit.

Remarks: Both this species and the next belong to the nominate subgenus of *Rhabdepyris* as defined by Evans (1965): The eyes are bare and the head, body and principal wing veins are clothed with many coarse, dark setae; the posterior margin of the pronotal disc is paralleled by a groove, usually foveate, although this groove is broadly interrupted in the two species found in the Virgin Islands. In Evans' key to the species of this subgenus *R. maboya* will run to couplet 3 separating *R. huachucae* Evans (Arizona) and *R. muesebecki* Evans (México to Bolivia) and is most similar to *R. muesebecki*. From that species, as described by Evans, *R. maboya* differs by the more robust antennal segments, the different proportions of the head and the characteristic sculpture of the frons.

This species differs from *R. versicolor* Evans by the much coarser sculpturing, especially of the frons, as well as the brighter color. A median clypeal tooth is conspicuous in *R. maboya* but lacking in *R. versicolor*. A third species present in the Greater Antilles, *R. muesebecki* Evans, ranges from the southeastern United States (Missouri and Florida) south to Bolivia, and was reported from Hispaniola (Dominican Republic) by Evans (1979), but is a more typical member of *Rhabdepyris* s. str., in that the head and body are black and without metallic coloration.

Rhabdepyris versicolor Evans

Rhabdepyris (Rhabdepyris) versicolor Evans, 1970:342; fig. 1; ♀.

This species was originally described from a single female collected at East Point, St. Croix. The specimens recorded below agree very closely with the type except that the metallic green color of the mesosoma is more brilliant, especially on the propodeum. In my specimens the mandible, scape and second antennal segment are very pale,

almost white; these areas are only slightly more yellowish in the type.

Specimens examined: BRITISH VIRGIN ISLANDS, *Guana I.:* 1 ♀, North Beach woods, 20–25 Apr. 1993, Malaise trap (R. R. Snelling); 1 ♀, same except 1–7 July 1993; 1 ♀, same except White Beach, 7–12 July 1993.

Scleroderma wilsoni Evans

Scleroderma wilsoni Evans, 1964:179; ♀.

This odd little species was originally described from an apterous female from Pinar del Río, Cuba. The present specimens differ from the type most obviously in their color: the head and body are brown, rather than bright testaceous. But, as in the type the posterior portion of the propodeum is very pale, almost white.

One of the females is fully alate; the others are apterous. Such polymorphism is usual in *Scleroderma,* as pointed out by Evans (1964). Aside from the presence of wings and the mesosomal structure usual to an alate wasp, this specimen closely resembles the apterous specimens, especially in color. However, in addition to the largely pale propodeum, the pronotum is also pale, but the pale color of both areas is tinged with brown.

The following measurements and proportions will augment the description of the type; figures for the alate individual are in parentheses. Length about 2.0–3.5 (2.9) mm; (LFW 1.70 mm); LH 1.14–1.19 (1.14) × WH; WF 0.53–0.55 (0.57) × WH, 1.50–1.62 (1.47) × HE; mesosoma length 0.60–1.00 (0.91) mm.

Specimens examined: BRITISH VIRGIN ISLANDS, *Guana I.:* 1 ♀, North Beach woods, 15–20 Apr. 1993, Malaise trap (R. R. Snelling); 1 ♀, same except 20–25 Apr. 1993; 1 ♀ (alate), same except 1–7 July 1993. PUERTO RICO, *Mona I.:* 1 ♀, near airstrip, 13 Nov. 1992, ex gallery in dead branch of *Leucaena leucocephala* (Fabaceae) (R. R. Snelling and J. A. Torres).

Subfamily Pristocerinae

Genus *Apenesia* Westwood

Apenesia is a large tropicopolitan genus with extensions into temperate regions. The New World fauna was revised by Evans (1963). At that time 63 species were recognized, three of which were known to occur in the Caribbean. Later, Evans (1969a, c) described an additional six Caribbean species. Previously described species from this area are:

A. caribbea Evans, 1969a, ♂ (Dominica); *A. cubensis* Evans, 1963, ♂ (Cuba); *A. delicata* Evans, 1963, ♀ (Jamaica); *A. dominicana* Evans, 1963, ♀ (Dominica); *A. flaviscapus* Evans, 1969a, ♂ (Dominica); *A. insulana* Evans, 1969c, ♂ (Jamaica); *A. jamaica* Evans, 1969c, ♂ (Jamaica); *A. luteola* Evans, 1969c, ♂ (Cuba); *A. vaurieorum* Evans, 1969c, ♂ (Jamaica).

One additional species, represented by a single female, is now available from Puerto Rico, the first *Apenesia* from that island.

***Apenesia yu* Snelling,** NEW SPECIES

Figs. 8, 18–20

Holotype: ♀, PUERTO RICO: El Yunque, 2000 ft elev, 29 July 1950 (W. F. Buren), in LACM.

Description: Length 3.7 mm; LH 0.78 mm; WH 0.64 mm; LT 1.19 mm.

Head and body reddish yellow, appendages paler, tips of mandibles reddish.

Head shiny (Fig. 8), almost polished between dense to subcontiguous, coarse, sharply defined piligerous punctures. Mandible with three distinct teeth, basal angle of dentate margin broadly rounded (Fig. 20). Clypeus (Fig. 19) with deeply V-shaped median emargination, median carina or ridge present along distal 0.60, terminating at apex of emargination. Margins of head, in frontal view, weakly divergent below so that greatest WH is about 1.2× least WH; LH 1.22 × WH; vertex very weakly convex in frontal view. Eye slightly longer than wide, apparently consisting of a single

facet; distance between eyes 6 × HE and eye width slightly greater than length of malar area. Scape curved, about 4× as long as wide; flagellum much widened distad. Pronotal disc 1.2× its posterior width; surface shiny and weakly alutaceous, with some widely scattered punctures laterad. Mesoscutum about 0.53× as long as wide; sculpture similar to pronotum. Maximum length of propodeum (Fig. 8) about 2.0 × maximum width; maximum width of disc 1.30 × minimum width; distance from midpoint of constriction to anterior margin of disc equals about one-half width of constriction; propodeal formula 22:28:30; disc shiny, alutaceous, laterally with coarse, close punctures, delimited posteriorly by transverse carina. Dorsal face of mesepisternum broadly rounded onto lateral face. Mesotibia strongly spinose; metatibia pilose only.

Metasoma petiole short and broad, length about 0.2 × length of metatibia.

Etymology: The name is a Taino (Arawak) word for clear or white, in reference to the prominent, single-faceted eye.

Remarks: Females of Antillean *Apenesia* are known only for *A. delicata* and *A. dominica.* In each of these the punctation of the head is fine and inconspicuous and the propodeal constriction is much more profound. It is possible that *A. yu* is the female of one of the Cuban or Jamaican species presently known only from males. In the *Apenesia* revison by Evans (1963) this species would key to *A. dominica* except that the propodeal disc lacks the longitudinal median impression found in that species.

Acknowledgments

For the opportunity to collect Hymenoptera on Guana Island I am very deeply indebted to James "Skip" Lazell of The Conservation Agency and to Dr. Henry Jarecki. Critical type specimens were made available by Stefan Cover (MCZ), Lubomir Masner (CNC) and Beth Norden (USNM). Specimens from the American Virgin Islands were made available by Mike Ivie (MSUC). A special note of thanks and appreciation must be extended to my friends Juan and Maribel Torres for their generosity and kindness during my visits to Puerto Rico and especially for making possible my visits to Mona Island.

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UNIVERSITY OF MARYLAND AT COLLEGE PARK

COLLEGE OF LIFE SCIENCES • DEPARTMENT OF ENTOMOLOGY

30 October 1996

Dr. James D. Lazell
The Conservation Agency
8 Swinburne Street
Conanicut Island, RI 02835

Dear Skip,

As always, the only problem with my time on Guana Island was that it was too short. Even with the time constraints, however, the stay was productive, and both Bob Denno and I are extremely pleased with the way that our Tropical Ecology Course developed. Each of the students had a valuable experience, and their short but insightful projects will hopefully serve as pilot studies for future work on the fauna of Guana.

Skip, you were an immense help to the course - first in supporting the concept, and throughout our stay in facilitating its success. You went out of your way to make sure that we had what we needed and to acquire it if it was missing (e.g. having the list of plants faxed for Bob's reference). The students learned not only from their individual projects, but also from the field station atmosphere that Scientists' Month generates. You are the central part of that, and we all greatly appreciated your involvement.

With best wishes,

Barbara

Barbara L. Thorne
Assistant Professor

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The Numerical Response of Pit-Building Antlion Larvae to Sustained Movement of Arthropod Prey

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Introduction

Predators often aggregate in areas of high prey density (Price 1984). It has been shown that pit-building antlion larvae (family Myrmeleontidae) aggregate in numbers correlated with prey density within suitable habitats (Boake *et al.* 1984) However, no studies have yet been conducted to ascertain the methods by which antlions identify areas of high arthropod abundance. Because pit-building antlion larvae wait submerged at the bottom of their pits, with only their long mandibles protruding out from the soil (Lucas 1985), it is unlikely that sight is the mechanism used to access prey density. Ants are an antlion's principal prey. Antlion larvae often converge upon nests of ants, where there is a constant source of prey on the soil surface. (Lucas & Brockmann 1981). Non-pit building antlions have been shown to sense vibrations through the dry, particulate soil in which all antlions live (Stange 1970). My field experiments on Guana Island, B.V.I. were designed to examine the aggregation of pit-building antlion larvae in arthropod prey rich areas, and to investigate whether sustained movement of prey on the soil surface stimulates antlion aggregation. My hypotheses were that antlion larvae would preferentially build pits in microhabitats with high prey density, and that

transmitted vibrations of prey movement on the surface of sand would induce antlions to move towards areas of high prey traffic.

Materials and Methods

Areas with dry, loose soil, and dense, low foliage, located on White Bay Beach, Guana Island, BVI were used in all experiments. These habitats contained a large number of antlion pit aggregations.

To determine the relationship of antlions to arthropod prey at various sites in this habitat, 5 cm diameter plastic cups were filled halfway with isopropyl alcohol and buried in the soil so that the lip of the cup was even level with the ground. Of the twenty four cups buried, twelve were in areas that contained no antlion pits; the remaining twelve were buried in areas in which there were at least two pits within 15 cm of the cup. The number of antlions within 15 cm of each cup was then recorded. These cups were left for twenty four hours, collected, and the number of preserved organisms counted.

The submerged alcohol filled cups were used in this experiment gave a more accurate measurement of prey densities than would collection with a light source or other attractant. The collection cups were left out for a complete twenty-four hour span of time to collect insects at all times of day and night, thereby providing a thorough estimate of prey abundance. Different ant and termite species have different daily cycles; some have activity during the day while some are only out at night. Isopods are much more active at night than during the day.

For the portion of experimentation devoted to determining the mechanism by which aggregation is stimulated, twelve sites which contained numerous pits were located. At each of six of these sites, two 5 cm diameter plastic cups were placed inverted on the ground, 30 cm apart, and weighted

down by a medium sized stone. Ten live termites (*Nasutitermes acajutlae* (Holmgren); Nasutitermitinae; Termitidae) were placed under each cup. In trial feedings in this species of termite was readily eaten by antlions in their pits. The number of ant lion pits within a 15 cm radius of each cup was recorded at the time of experimental set up, and again after 24 hours.

Results

The mean number of arthropods caught in the submerged, alcohol-filled cups away from antlion pits was significantly lower than the mean number of arthropods captured in areas with antlions (Graph I; t= 5.637; P= .0000114). Isopods and ants were the most common arthropods found in the collection cups.

There was a positive correlation between the number of antlions located within 15 cm of each alcohol-filled collection cup and the number of arthropods collected (Graph II; $R^2 = 0.141$). The cups placed away from all antlion pits were not included in the calculation of the regression coefficient.

After 24 hours there was a mean increase in the number of antlion pits within 15 cm of inverted cups containing live termites. There was a mean decrease in the number of antlion pits around empty inverted cups (Graph III). The difference between the two treatments was found to be significant (t= 2.40; P= 0.037).

Discussion

This study supported previous work suggesting that antlion larvae aggregate in areas of higher prey densities (Boake *et al.* 1984). The strongest evidence comes from the convincing two fold difference between numbers of potential prey near and away from antlions. This aggregation response to prey

density indicates a classic numerical response of predators to prey (Price 1984).

Different species of antlions have different spatial characteristics reflecting aggregation response (Boake *et al.* 1984). Although the relationship between predator and prey density in my experiments was weak (Graph II), these data suggest trends which should be investigated with further replication, and compared similar relationships in other populations.

One factor contributing to high variation among replicates was a storm that occurred during the night that the alcohol-filled collection cups were in the field. The rain diluted the alcohol, enabling stronger arthropods to escape. Thus, an area with a high ant density but a low isopod density might have appeared as having less prey than an area with a high isopod density and lower ant density. Because changes in weather directly affect the quality of the soil and the quantity of available food, it also affects the activity of pit- building antlion larvae (Rosenberg 1987). Future experiments should be conducted over extended periods of time to sample antlion activity under a variety of environmental conditions.

Although further replication is needed, the preliminary results of the "imprisoned termites" experiment support my hypothesis, and suggest that sustained movement of arthropods on the soil surface stimulates aggregation of antlion larvae. The rain storm during the night of the field experiment may account for a large portion of the variation among replicates. Antlion larvae prefer to build pits in dry sand (Rosenburg 1987). Moistening soil or destruction of a pit by the rain may have prompted antlion movement to sheltered areas. If wet soil inhibits the transmission of vibrations through the soil, then movement of termites trapped on a damp soil surface many not have been perceived by as many antlions as in dry soil.

Finding areas of high prey abundance is unlikely the sole information that antlion larvae gain from sensing vibrations through the loose, dry soil. Antlions are able to appraise the size and other physical aspects (such as physical and chemical defenses) of a potential prey item that nears its pit (Heinrich & Heinrich 1984). Antlion larvae secrete a paralyzing chemical into captured prey (Stange 1970). Anticipating the size of a potential prey might enable the antlion to gauge the amount of chemical needed to sedate it.

More replicates are needed to understand the density and spatial relationships of antlions and their prey, and to verify that prey movement on the soil surface does indeed stimulate aggregation antlion larvae. The preliminary results of these field experiment provide the first suggestion that soil surface vibrations may be a signal that antlion predators use to locate microhabitats with high prey abundance.

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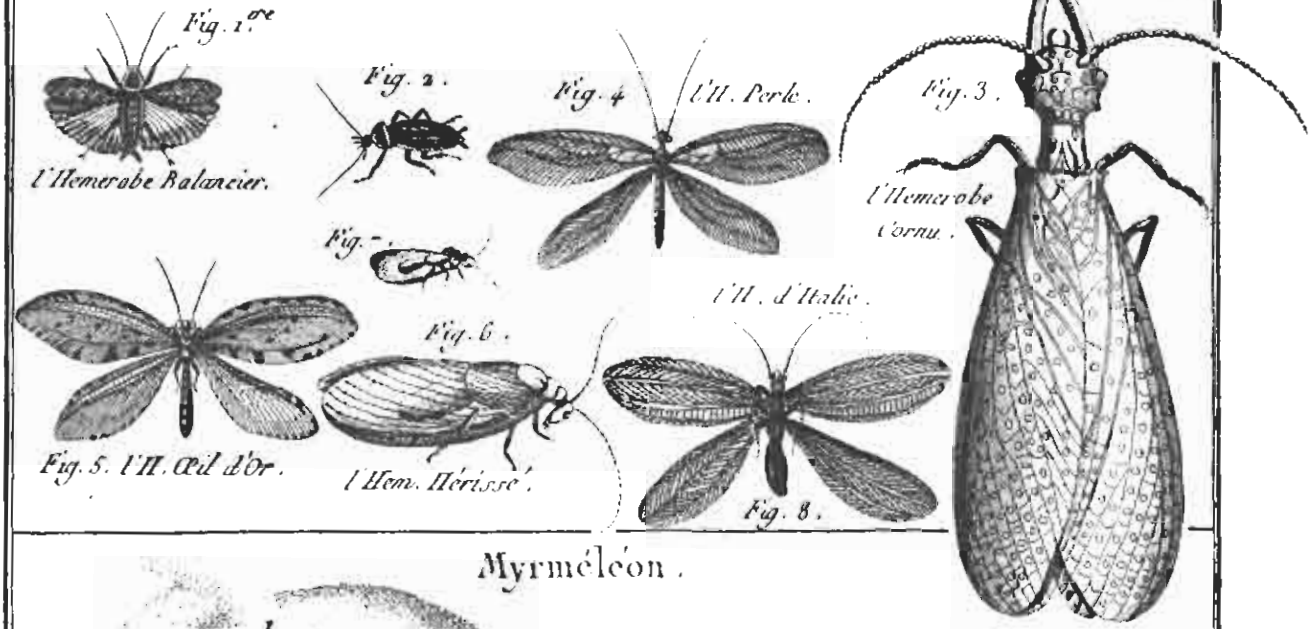
Hemerobe.



Myrméléon.

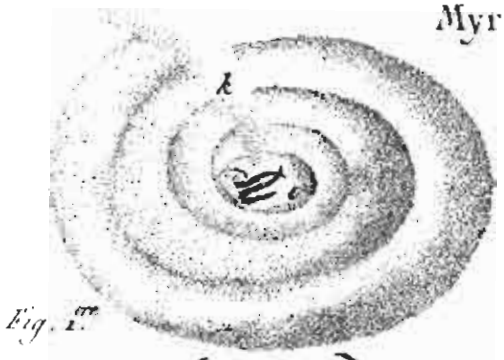
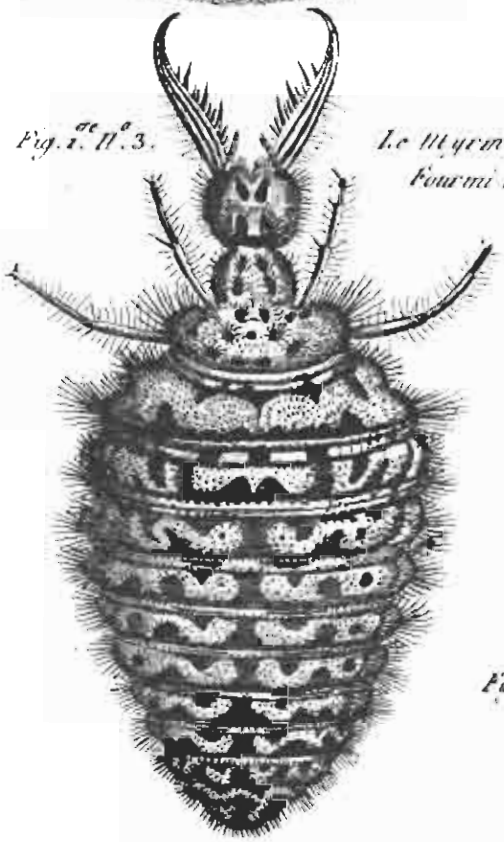


Fig. 1re

Fig. 1re N° 2.

Fig. 1re N° 3.

Le Myrméléon ou Fourmi Lion.



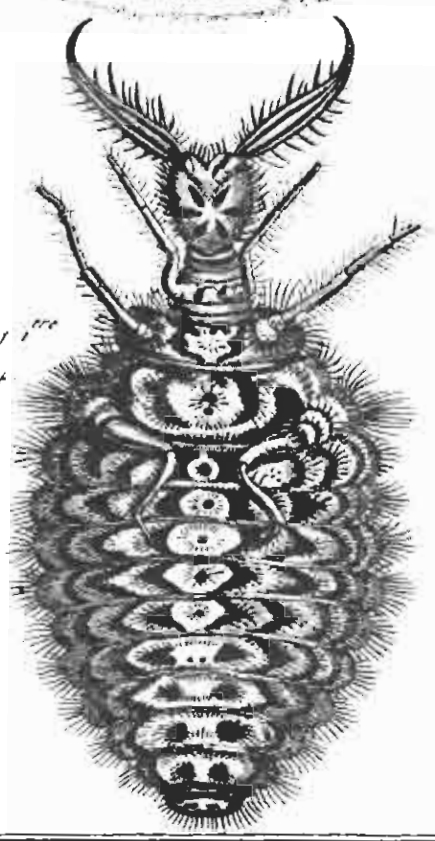


Fig. 1re

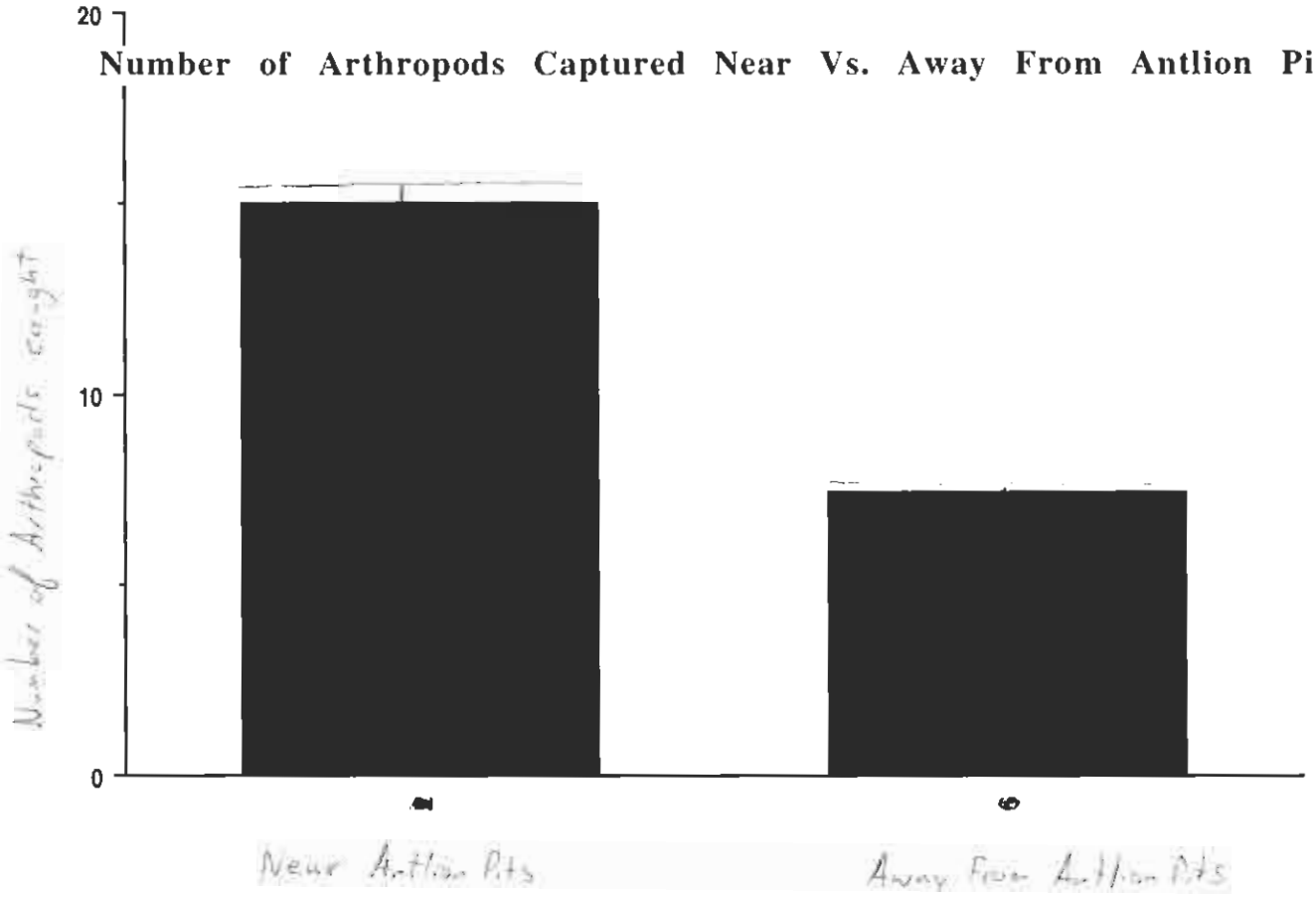
N° 4

Fig. 1re N° 5.

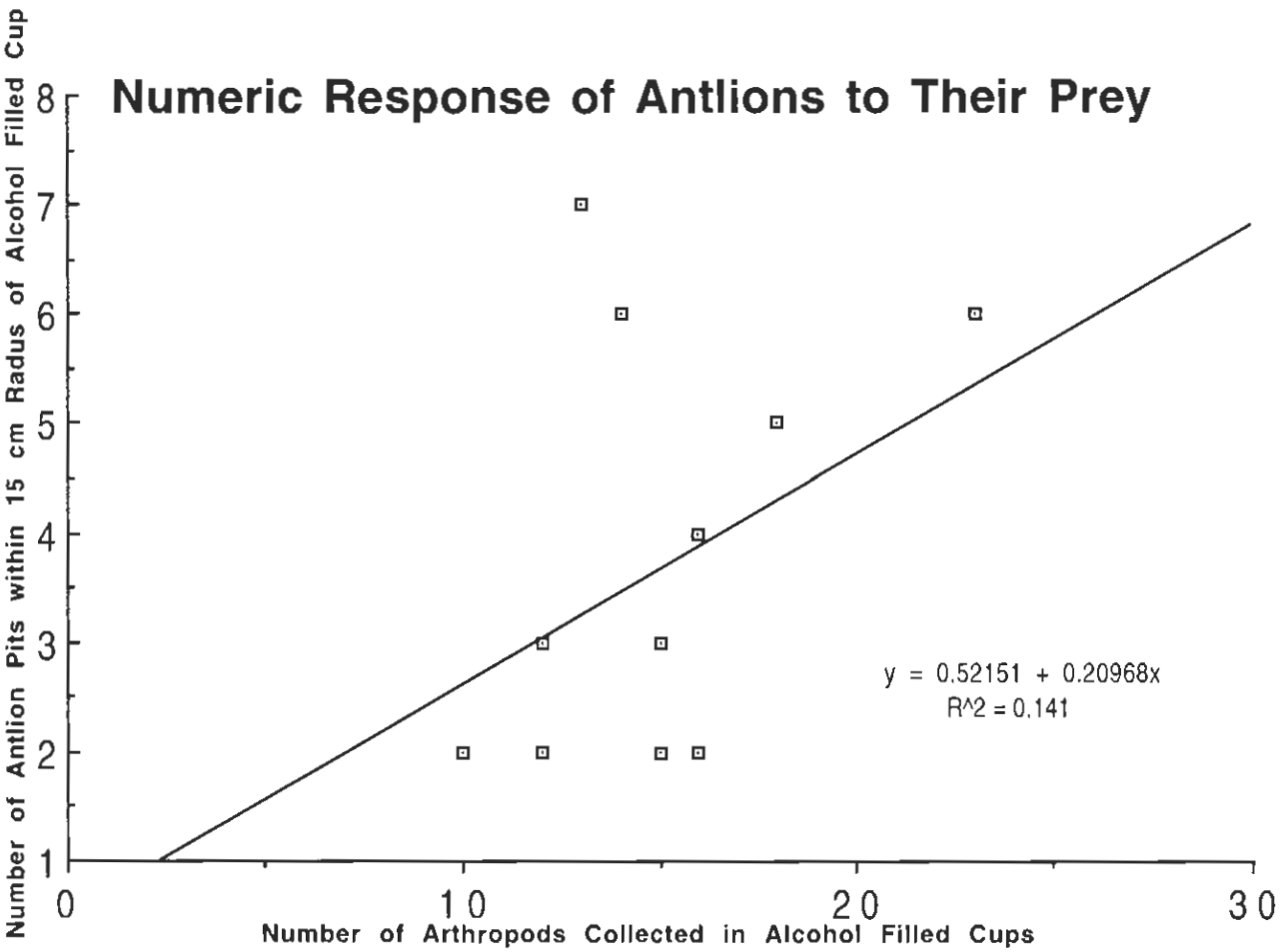
Benard Direxit.

Histoire Naturelle, Insectes.

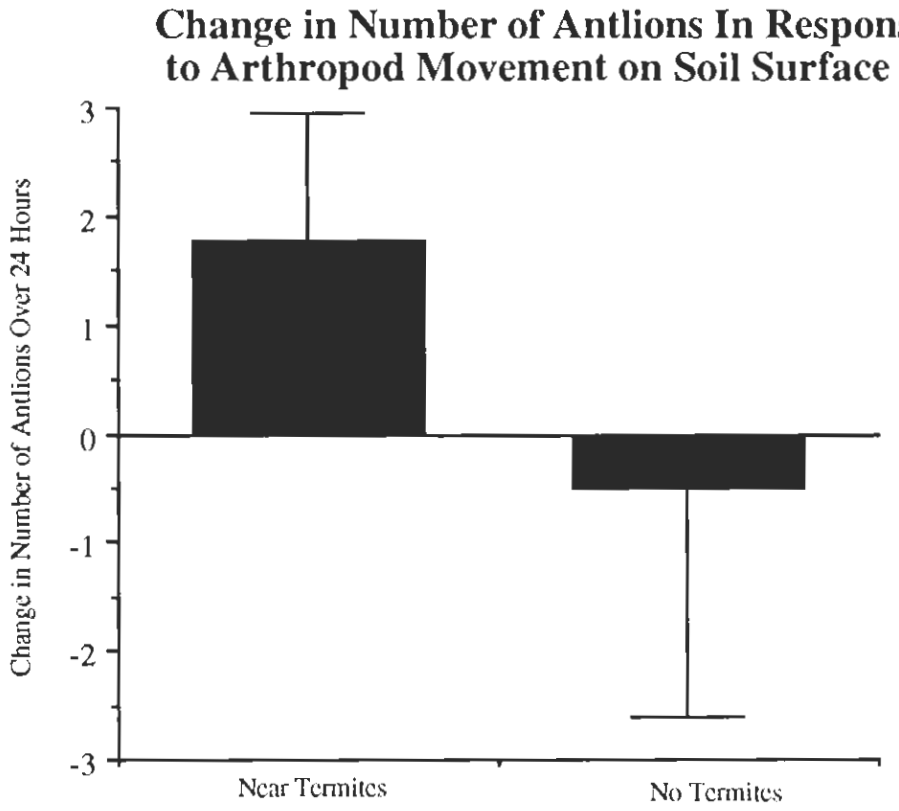
Graph I


Number of Arthropods Captured Near Vs. Away From Antlion Pits
20
10
0
Number of Arthropods caught
a
b
Near Antlion Pits
Away From Antlion Pits

Graph II


Numeric Response of Antlions to Their Prey
Number of Antlion Pits within 15 cm Radus of Alcohol Filled Cup
Number of Arthropods Collected in Alcohol Filled Cups
y = 0.52151 + 0.20968x
R^2 = 0.141
0
10
20
30
1
2
3
4
5
6
7
8

Graph III


Change in Number of Antlions In Response to Arthropod Movement on Soil Surface
Change in Number of Antlions Over 24 Hours
3
2
1
0
-1
-2
-3
Near Termites
No Termites

Insect Community Structure in the Phytotelmata of *Tillandsia fasciculata* on Guana Island, BVI

Sadie Jernigan

ENTM 499H
December 15, 1996

Introduction

Many epiphytes impound water in their leaf axils, providing habitats for aquatic organisms. Aquatic impoundments, or phytotelmata, can be useful systems for asking community-level questions because the limits of the phytotelmata community are relatively discrete (Maguire 1971; Cotgreave et al. 1993). Furthermore, natural phytotelmata have the advantage of retaining environmental heterogeneity in a well defined community. Thus, questions concerning the effects of habitat ephemerality and harshness on community structure can be addressed.

For example, epiphytes may be subject to extremes of temperature, wind, sunlight, and rain resulting in fluctuating or ephemeral conditions for the organisms inhabiting them (Madison 1977). Insect species residing in ephemeral, aquatic habitats are expected to be opportunists with life history traits such as rapid development, small body size, high fecundity, and resistance to desiccation (MacArthur 1962; Stearns 1976). In addition, such ephemeral habitats should support relatively few species due to the difficulties of colonizing and exploiting such short-lived resources (Connell 1978).

Tillandsia fasciculata occurs both attached to the ground and to trees on Guana Island, BVI (Fig. 1). Being farther from the ground, arboreal bromeliads may provide a more variable or ephemeral habitat than terrestrial bromeliads due to the greater extremes of temperature, light, and moisture in the canopy. Thus, I hypothesized that arboreal phytotelmata of *Tillandsia fasciculata* would support a lower species diversity and density of aquatic insects than terrestrial phytotelmata. In addition, the life histories of the inhabitants of phytotelmata should be characterized by rapid development and small body size (Stearns 1976). I tested these hypotheses by comparing the structure (species diversity) of the aquatic community and the life history characteristics (body size) of the fauna in arboreal and terrestrial phytotelmata.

Materials and Methods

Distribution of the Bromeliad on Guana Island

Tillandsia fasciculata grows on many trees, shrubs, and cacti, as well as on the ground along the trail to Long Man's Point on Guana Island, British Virgin Islands. Furthermore, this bromeliad also occurs on the ground (Fig. 1). To determine the distribution and abundance of *T. fasciculata* in trees and on the ground along Long Man's Point trail, I conducted a survey and scored the location of all bromeliads relative to their height (arboreal versus terrestrial) and host tree affiliation. The survey was conducted by scoring all bromeliads growing along a 65m swath within 5 and 10m of the trail on the south-facing slope.

Sampling Phytotelmata and Determining the Aquatic Community Structure

Using a glass pipet, I transferred the aquatic layer from three randomly selected leaf

axils on each plant to 2 ounce plastic cups with lids. I sampled two terrestrial and two arboreal plants on October 15 and five of each on October 16, 1996. Care was taken to select bromeliads of similar size for this assessment. All arboreal bromeliads were anchored between 1 and 2 m from the ground. I measured the total volume of each sample and then sorted and counted the inhabitants in each family using a dissecting microscope.

In order to determine the maximum capacity of each leaf axil, I flooded each plant with water and remeasured the volumes of the sample leaves after the plants had drained for 24 hours. I compared the terrestrial and arboreal bromeliads with respect to actual sample volume, maximum capacity, insect diversity, and insect abundance using t-tests. In order to determine if there was a relationship between mosquito abundance and volume of water in a bract, I conducted linear regressions on terrestrial and arboreal bromeliads separately.

Results

Distribution of the Bromeliad on Guana Island

The bromeliad, *Tillandsia fasciculata*, was about twice as abundant in trees as it was on the ground (Fig. 2). Furthermore, in the arboreal habitat, *T. fasciculata* occurred most abundantly in the tree *Tabebuia heterophylla* followed by *Pisonia subcordata*, *Plumaria alba*, *Jacquinia berteri*, *Randea aculeata*, *Capparis cynophallophora*, and *Morisonia americana* (Fig. 2).

Water Volume in Terrestrial and Arboreal Phytotelmata

The mean water volumes from terrestrial and arboreal phytotelmata were not significantly different ($t=1.659$, $P=0.122$), although there was a trend toward terrestrial phytotelmata containing more water (Fig. 3). The mean maximum capacity per leaf axil, however, was significantly higher for the terrestrial phytotelmata compared to the arboreal ones ($t=2.434$, $P=0.041$; Fig. 3). Thus, there is a tendency for the water volume to be less in arboreal compared to terrestrial phytotelmata, perhaps suggesting that this microhabitat is more ephemeral for bromeliads growing in trees.

Structure of the Insect Community Associated with Terrestrial and Arboreal Phytotelmata

All of the insects present in the aquatic samples were small (<4mm in body length) dipteran larvae or pupae. Mosquitos (Culicidae: *Culex*) were most the most abundant taxon, followed distantly by Ceratopogonidae, Thaumaleidae, and Psychodidae (Fig. 4). The density of individuals (no. per phytotelmata) in each family did not differ significantly between terrestrial and arboreal bromeliads (Culicidae: $t=1.686$, $P=0.118$; Ceratopogonidae: $t=0.835$, $P=0.420$; Thaumaleidae: $t=0.942$, $P=0.365$; Psychodidae: $t=1.087$, $P=0.298$) (Fig. 4). The mean number of insect families present was also not significantly different for terrestrial and arboreal bromeliads ($t=0.234$, $P=0.819$). However, if one outlying observation is removed, the Culicidae became more abundant in terrestrial phytotelmata ($t=3.076$, $P=0.011$; Fig. 4). Thus, there was a slight tendency for terrestrial bromeliads to

house more mosquitoes than arboreal ones.

Linear regression of mosquito abundance per phytotelmata against the actual volume of water in the phytotelmata indicated that there was a positive relationship between mosquito abundance and the volume of water in the microhabitat, with arboreal bromeliads having a steeper slope (slope = 1.2, R^2=.71; Fig. 5) and tighter fit than terrestrial bromeliads (slope=0.44, R^2=.12; Fig. 6).

Discussion

The insects found inhabiting both terrestrial and arboreal phytotelmata were all small larvae and pupae within the Diptera, an order with many aquatic larvae adapted to life in ephemeral or fluctuating environments (Istock 1981). Some dipteran larvae live in very wet soil or water that is saturated with organic or inorganic matter (Teskey 1984). Barrera and Medialdea (1996) found that mosquitos in phytotelmata had slower development times and increased resistance to starvation compared to mosquitos inhabiting water sources on the ground. In light of their results, one would expect that bromeliads provide a more persistent aquatic habitat than is available in water sources on the ground, but that food availability is the stressor. This is consistent with other researchers' findings which suggest that tank bromeliads are efficient collectors and holders of water, an adaptation that enables the plant to obtain water without being rooted in soil (Madison 1970; Maguire 1971; Benzing 1990). These authors point out that although vascular epiphytes tend to exhibit xeromorphic adaptations for retaining water and the ability to impound water in some species, such as the tank bromeliads studied here, they also tend to live in very wet areas. Considering the predominance of succulents in the area where I sampled, I would expect to find more water stress than is encountered by species living in wet areas. I would also expect the organisms inhabiting the phytotelmata to exhibit adaptations to living in ephemeral habitats.

A trend towards mosquitos being more abundant in terrestrial bromeliads than in arboreal bromeliads is suggested by the significant difference in mosquito abundance obtained when one outlier is removed (Fig. 4). This is the only significant difference in abundance of an insect family, but the sample sizes were small and the results should probably be interpreted as suggestive. Nadkarni and Longino (1990) found a lower density of most invertebrates sampled from canopy organic matter as compared to ground organic matter. They suggest that differences in microclimate leading to greater desiccation in the canopy, as well as dispersal difficulties and possible substrate differences could explain the difference in abundance. Although their study did not focus on aquatic communities, they did find differences in abundance of invertebrates at different levels of forest.

Harsher conditions for mosquitos in arboreal phytotelmata are suggested by the greater slope and higher R^2 value for the linear regression of mosquito abundance on liquid volume in arboreal bromeliads compared to terrestrial bromeliads (Figs. 5 and 6). Phytotelmata that are experiencing more severe desiccation would be expected to exhibit mortality due to increasing density of mosquitos as the liquid volume decreases. Less severe desiccation would not be expected to result in high mortality and a steep regression slope unless the carrying capacity had been exceeded prior to desiccation.

The results of this research provide mild support for the hypothesis that the

Figure 1. The bromeliad *Tillandsia fasciculata* occurs both attached to the ground (seen here) and to trees on Guana Island, BVI. The phytotelmata (leaf axils) contain water which supports a community of aquatic insects.

community of insects inhabiting the arboreal phytotelmata of *Tillandsia fasciculata* is less structured (mosquitoes are less abundant) than the community residing in terrestrial phytotelmata. The phytotelmata community in general reflects the harsh and ephemeral nature of the phytotelmata environment (frequent evaporation) because it is characterized by low species diversity (4-5 taxa) and small opportunistic species (dipterans) which undergo rapid development.

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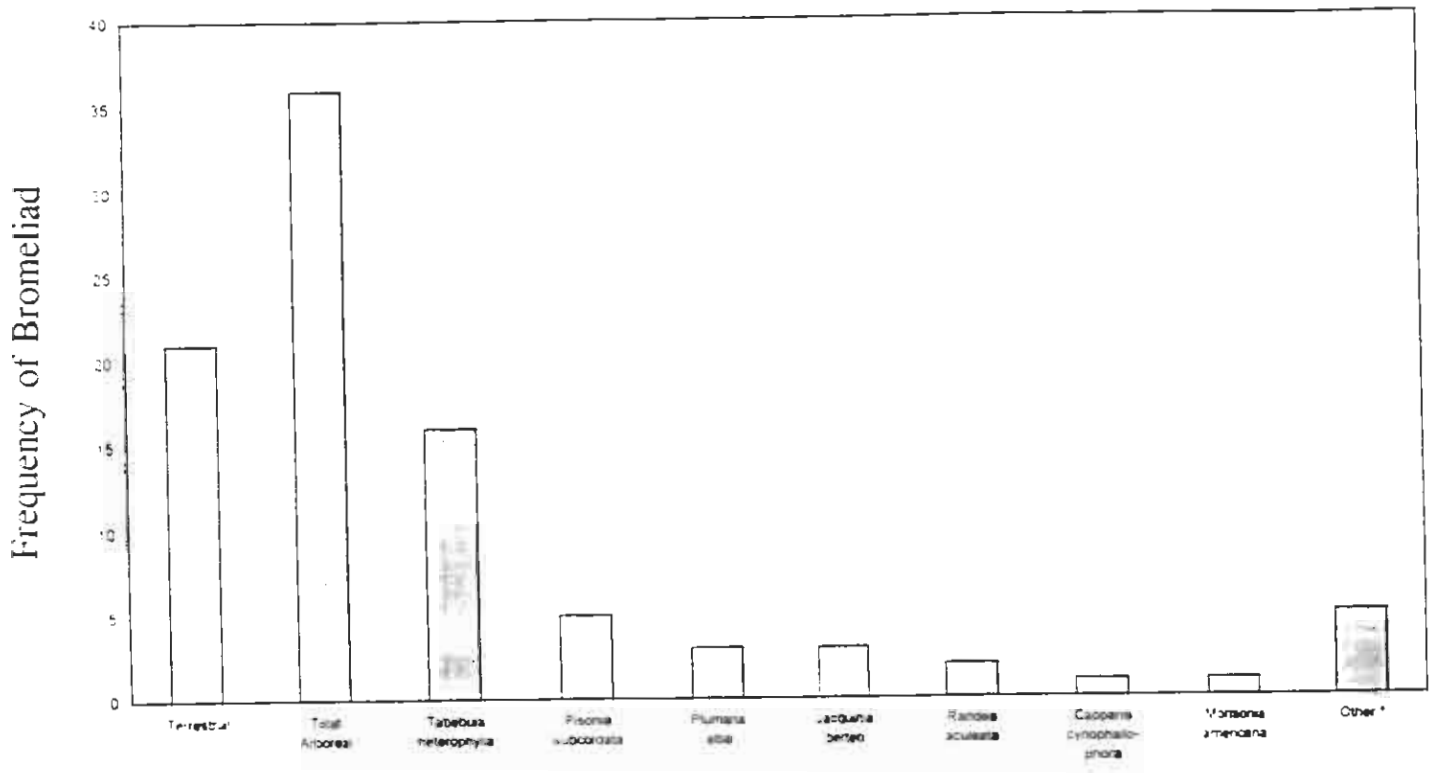

Figure 2. Frequency of the bromeliad, *Tillandsia fasciculata*, in arboreal and terrestrial habitats and on tree taxa on Guana Island, BVI.

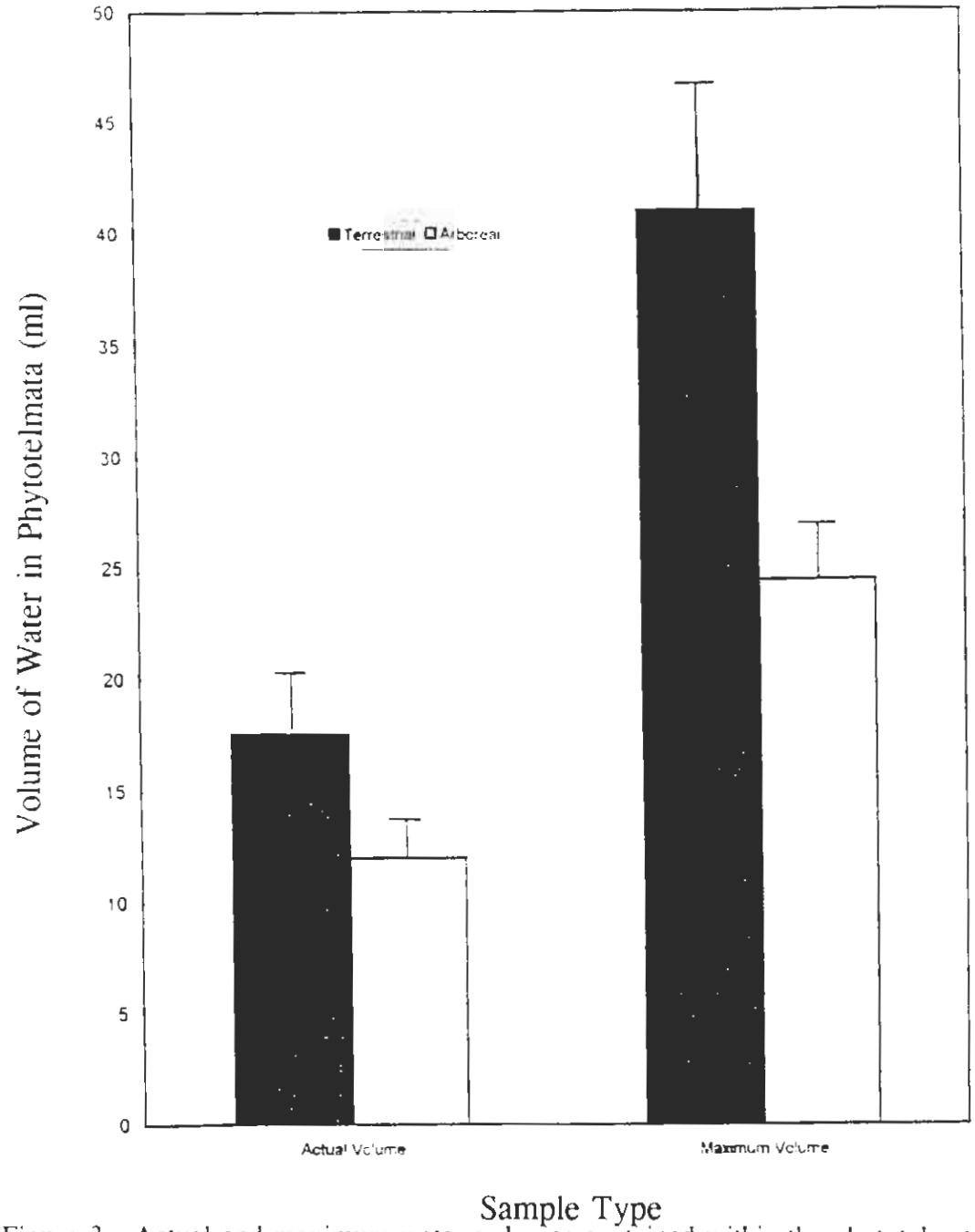

Figure 3. Actual and maximum water volumes contained within the phytotelmata of *Tillandsia fasciculata* growing in terrestrial and arboreal habitats. Actual water volumes were not significantly different ($t=1.659$, $P=0.122$); the maximum water volume of phytotelmata was greater for terrestrial bromeliads ($t=2.434$, $P=0.041$).

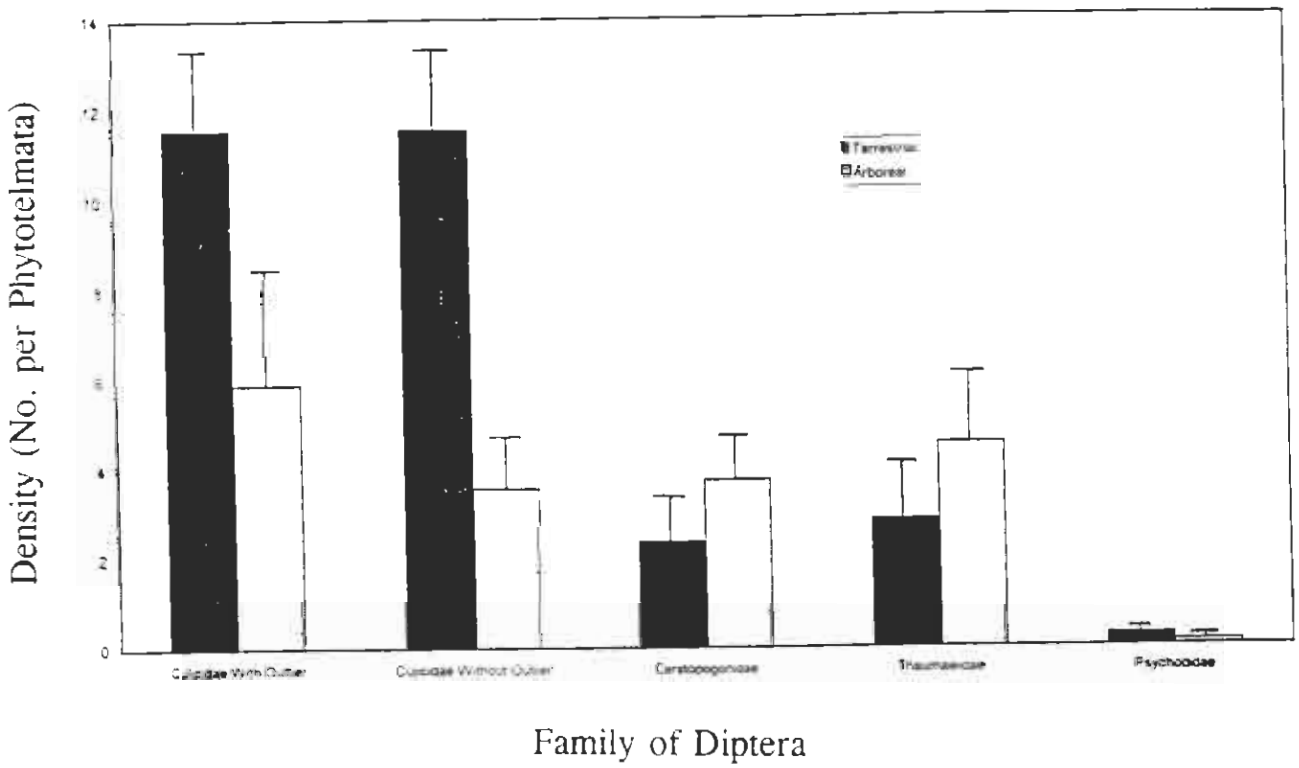

Figure 4. Density (No. per phytotelmata) of dipterans (Culicidae, Ceratopogonidae, Thaumaleidae, and Psychodidae) in the phytotelmata of *Tillandsia fasciculata* growing in terrestrial and arboreal habitats. Only the density of Culicidae (without outlying observation) were significantly more abundant in the phytotelmata of terrestrial bromeliads ($t=3.076$, $P=0.011$).

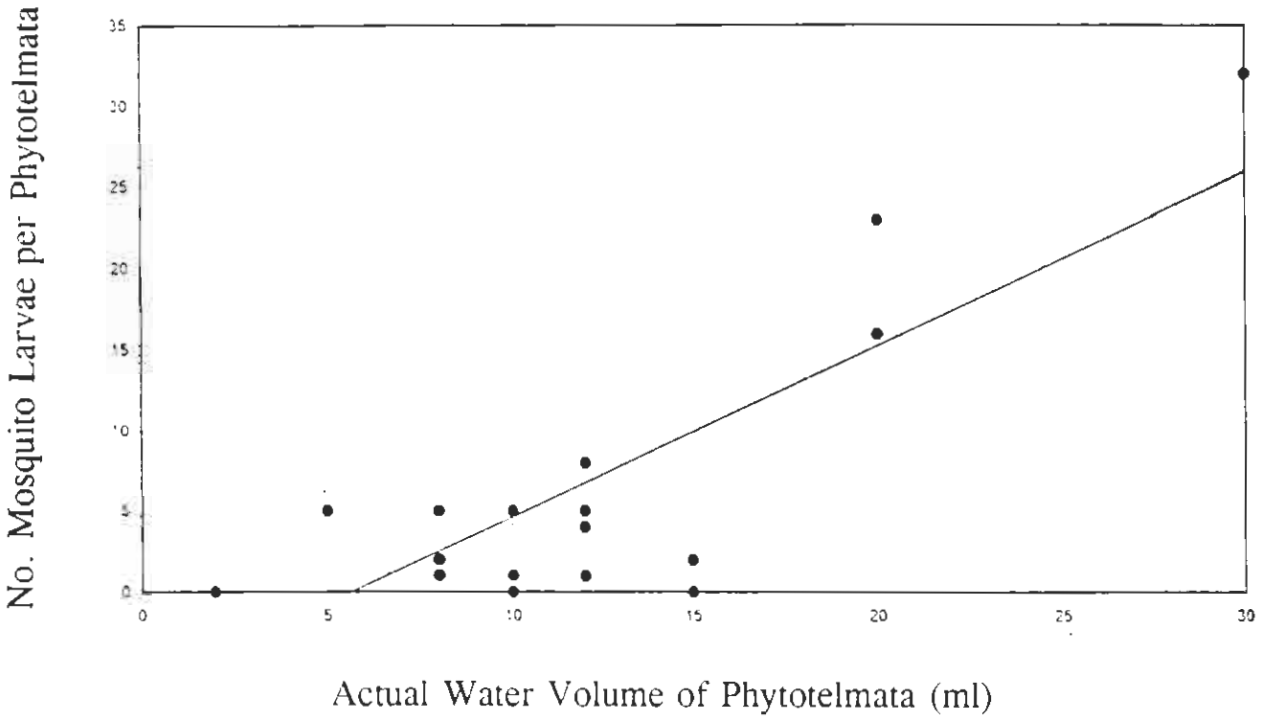

Figure 5. Relationship between mosquito abundance and the volume of water in the phytotelmata of *Tillandsia fasciculata* growing in arboreal habitats on Guana Island, BVI. (slope = 1.2, R^2=0.71).

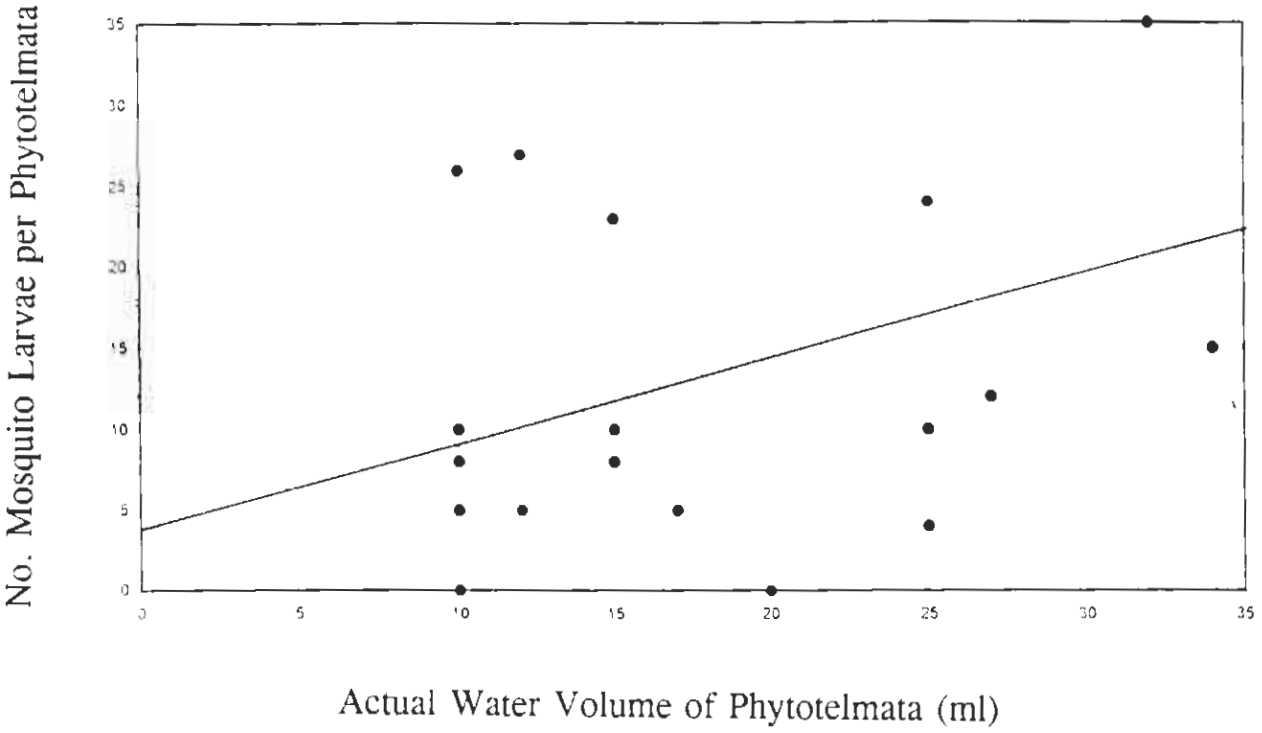

Figure 6. Relationship between mosquito abundance and the volume of water in the phytotelmata of *Tillandsia fasciculata* growing in terrestrial habitats on Guana Island, BVI. (slope=0.44, R^2=0.12).

Habitat persistence and its effect on the incidence of dispersal in a Guana Island planthopper, *Toya vinilia* (Hemiptera: Delphacidae).

Adam Marx

ENTM 499H
December 15, 1996

Introduction

Migration is essential to the success of insects that exploit temporary habitats, otherwise local extinctions would result (Southwood 1977, Denno et al. 1991, 1996). Migration can have a strong stabilizing influence on population fluctuations and the outcome of species interactions, and it directly determines gene flow and the genetic structure of populations (Denno et al. 1991). Finally, migration is considered to be as important as reproductive components of fitness by those who study insect life history strategies (Dingle 1985; Roff 1986). Consequently, those factors that determine the frequency, timing, and success of migration events are generally important in population biology, and of particular interest in the management of highly mobile agricultural pests (Denno et al. 1991).

Here I investigate how habitat persistence has influenced the migration strategy of a the wing-dimorphic planthopper, *Toya vinilia* (Hemiptera: Delphacidae), a phloem-feeding insect which occurs throughout the British Virgin Islands on Salt Grass, *Sprobolus virginicus*. Wing-dimorphic insects such as planthoppers are ideal organisms for investigating the advantages of wings and the evolution of dispersal because flightless and dispersive forms are so easily recognized. Brachypterous adults have reduced wings and cannot fly, whereas macropterous adults possess fully developed wings and can disperse long distances (Fig. 1). The advantage of macroptery (dispersal) is that it allows for escape from deteriorating resources and for the colonization of new habitats (Southwood 1977). The advantage of flightlessness in female planthoppers is that brachypters are both more fecund and reproduce earlier in life compared to their long-winged counterparts (reviewed by Denno et al. 1989).

Wing form in planthoppers is determined by a developmental switch which responds to environmental cues (Denno et al. 1991). Various environmental cues such as crowding and host plant condition; the sensitivity of the switch, however, is under polygenic control (Denno et al. 1991).

Here I test the general hypothesis that habitat persistence determines the incidence of dispersal in populations of *Toya vinilia*. A model exists for planthoppers documenting a negative relationship between habitat persistence and the fraction of the migratory form in a population (% macroptery) (see Fig. 2; Denno et al. 1991). Thus, planthoppers in temporary habitats should have a high incidence of flight-capable forms. I used this model to predict the incidence of dispersal in BVI populations of *T. vinilia*. By aging patches of Salt Grass and by determining the proportion of migratory forms in planthopper populations on Guana, Beef, and Great Camanoe Islands, I was able to assess the effect of habitat age on the incidence of dispersal in this planthopper.

Methods

Distribution of Salt Grass and Planthoppers

Sporobolus virginicus (Salt Grass), the host plant of *Toya vinilia*, grows abundantly throughout the British Virgin Islands (Chase 1971). This grass is restricted to and dominates the upper fringe of salt flats on the intertidal marsh or around isolated salt ponds where it grows in expansive pure stands (Marx, Denno, and Thorne personal observation). The

populations of Salt Grass I sampled were located on Guana Island (periphery of Salt Pond), Beef Island (1km west of the airport), and Great Camanoe (just W of Lee Bay). The planthopper was very common on all patches of Salt Grass I sampled.

Aging of Salt Grass Populations

I was able to obtain minimum estimates of habitat age by asking a long-term resident of the area, Mr. Oscar Chalwell, how long Salt Grass had existed at the three sites. Mr. Chalwell estimated the age of *Sporobolus* habitats on Guana Island, Beef Island, and Great Camanoe at 64, 50, and 50 years respectively.

Sampling Planthopper Populations and the Incidence of Macroptery

The incidence of flight-capable wing forms (macropters) was determined by sweep-net sampling the three Salt Grass patches. One sample transect consisted of 10, 180-degree passes of the sweep net across the surface of the grass taken while walking through the habitat. Five transect samples were taken in each patch at each site (Guana Island, Beef Island, and Great Camanoe). Samples were bagged, returned to the Club Area, and the number of macropters and brachypters in each sample was counted under a stereomicroscope. The proportion of macropterous forms in each populations was determined by dividing the number of macropters by the total number of adults (macropters + brachypters) in cach sample.

Relationship between Habitat Persistence and Incidence of Dispersal

For an insect, habitat persistence (maximum number of generations that can occur) can be estimated from the product of habitat age (years) and the number of generations an insect undergoes each year (Southwood 1977; Denno et al. 1991). Most tropical planthoppers pass through six generations per year. Thus, habitat persistence for *Toya vinilia* was estimated at 384 generations for Guana (64 years x 6 generations), and 300 generations (50 years x 6 generations) for both Beef and Great Camanoe.

To determine if the incidence of dispersal in the three populations of *Toya vinilia* fit the expectations of the model, I plotted the relationship between macroptery (%) and habitat persistence onto the existing model (Fig. 2; Denno et al. 1991). I used a T-test to assess if macroptery (%) in BVI populations of *T. vinilia* differed from those predicted by the model for the same range of habitat persistence (100-500 maximum generations).

Results

The incidence of migratory forms (% macroptery) was very low in all three BVI populations of *Toya vinilia*. For Guana, Beef, and Great Camanoe, % macroptery was 2%, 0%, and 7% respectively. These results provide strong support for the hypothesis that the incidence of dispersal is low in habitats that have persisted for a very long period of time. When these data were compared against data for other planthopper species (Denno et al.

1991), there was remarkable agreement (Fig. 3). Moreover, there was no significant difference between the incidence of macroptery in the BVI populations (3.0 ± 2.1 %) and that for all planthoppers species occupying habitats which have persisted for a similar period of time (1.5 ± 1.1 %) ($t = -0.704$, $P = 0.504$). Theses results provide strong support for the notion that habitat persistence dictates the dispersal strategy of insects. Most individuals of *Toya vinilia* are flightless, because there is apparently little advantage to dispersal in the permanent Salt Grass habitats throughout the BVI.

Discussion

The results that I obtained for *Toya vinilia* in patches of *Sporobolus virginicus* in the BVI clearly show that flightlessness is advantageous in persistent habitats (Fig. 3). Furthermore, my findings are entirely consistent with the predictions of a general model based on dispersal and habitat persistence data for a wide variety of planthopper species sampled across a great diversity of habitats varying in their durational stability (Figs. 2 and 3; Denno et al. 1991). The advantage of flightlessness in permanent habitats stems from the higher reproductive potential of the flightless brachypter compared to the flight-capable macropter in planthoppers (Denno et al. 1989). Brachypterous females are both more fecund and reproduce at an earlier age than their macropterous counterparts. High fecundity (brachyptery) may be favored to offset the high mortality inflicted on planthopper populations by either natural enemies (I observed a large number on spiders in my sweep samples) or physical factors such hurricanes which occasionally devastate these sensitive marsh habitats. My results provide strong support for the view that habitat persistence, rather than habitat insularity, is the primary factor influencing the dispersal strategies of wing-dimorphic insects (Southwood 1977; Denno et al. 1991, 1996; Roff 1986).

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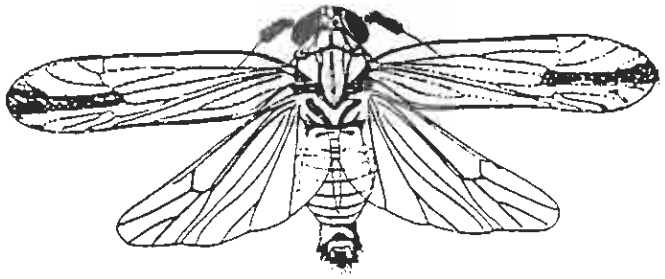
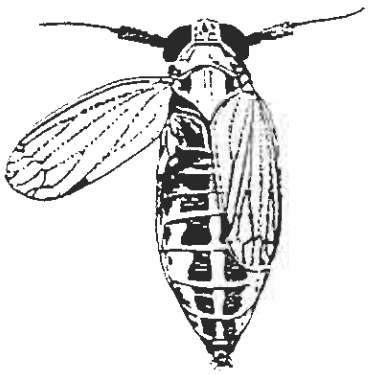


Macropter



Brachypter

Figure 1. Macropter (flight-capable wing form) and brachypter (flightless wing form) of the planthopper *Toya vinilia*.

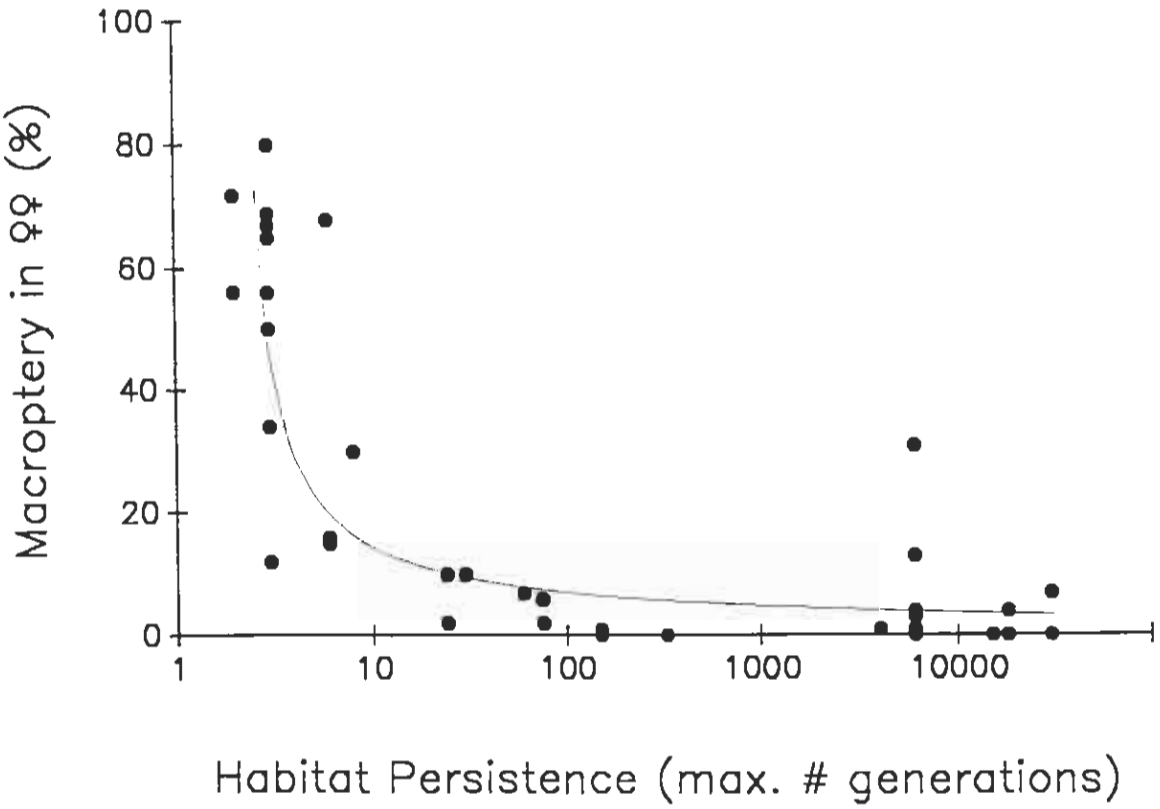


Figure 2. Relationship between macroptery (%) and habitat persistence (the maximum number of generations attainable) for the females of 35 species (41 populations) of planthoppers. Habitat persistence was estimated by multiplying habitat age (yrs) by the number of generations attainable/habitat/yr for each species. Some overlapping points occur. Fitted curves are of the form $y=a+1/(x^b+c)$ where y = macroptery (%); x = log habitat persistence; a = -4.812, b = 0.045, c = -0.948 (From Denno et al. 1991).

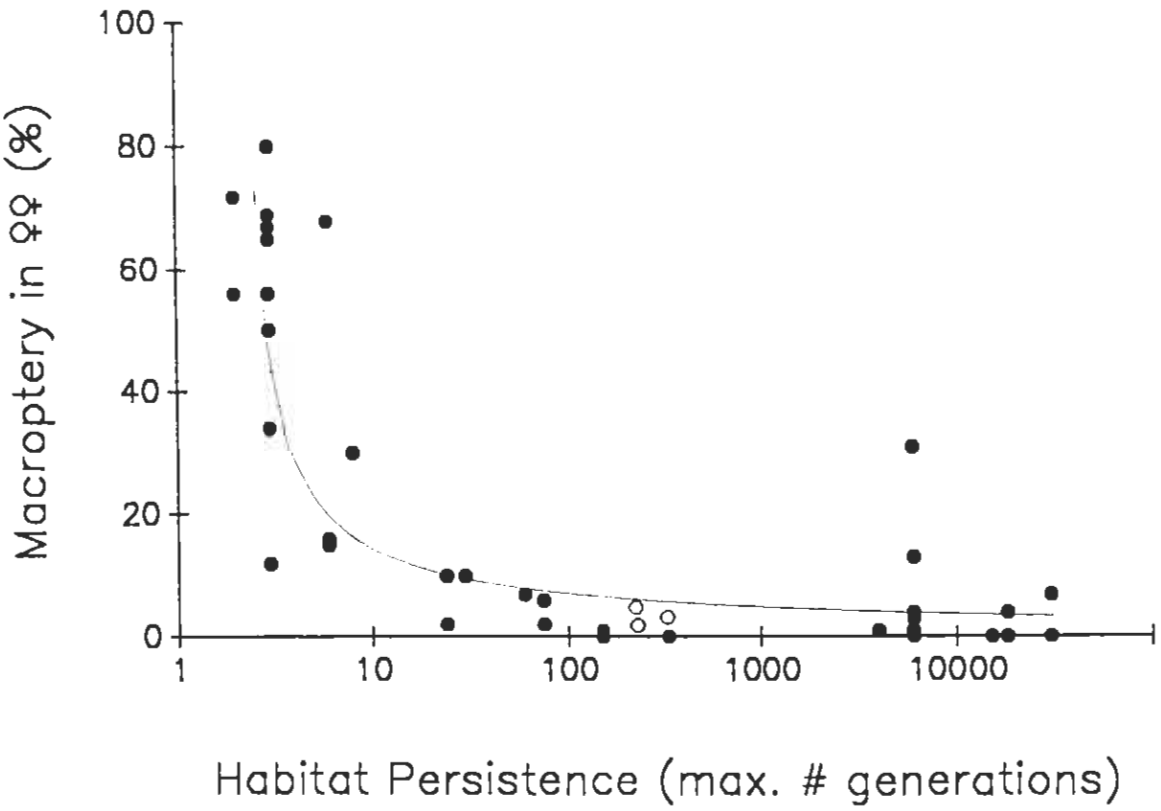


Figure 3. Relationship between macroptery (%) and habitat persistence (the maximum number of generations attainable) for the females of 35 species (41 populations) of planthoppers (dark circles). Habitat persistence was estimated by multiplying habitat age (yrs) by the number of generations attainable/habitat/yr for each species. Some overlapping points occur. Fitted curves are of the form $y=a+1/(x^b+c)$ where y=macroptery (%); x=log habitat persistence; a=-4.812, b=0.045, c=-0.948 (From Denno et al. 1991). Points for *Toya vinilia* (open circles) from Guana Island, Beef Island, and Great Camanoe, BVI are plotted on the general model.

Larval Survivorship of the Shelter-Building Skipper *Polygonus leo* (Lepidoptera: Hesperiidae) on Guana Island, BVI.

Mark Fox

ENTM 499H
December 15, 1996

Introduction

Most species of Lepidoptera are free living as larvae and feed on their host plants in an exposed position (Scott 1986; DeVries 1987). Quite a number of species, however, construct shelters as larvae by tying leaves together with silken strands (Damman 1987; Loeffler 1996). Such larvae either feed within these shelters or conceal themselves within these structures during periods of nonfeeding (Damman 1987; Loeffler 1996). Shelter building provides several potential benefits for caterpillars which include protection from natural enemies or desiccation (Damman 1987; Loeffler 1996). Furthermore, larvae which are unable to form their own shelters have increased survival when placed into artificial leaf-shelters (Cappuccino 1993). Despite these advantages, shelter-building does not occur without a cost to the larva often resulting in delayed development (Loeffler 1996).

Most free-living Lepidoptera species incur a tremendous amount of mortality during the early larval stages (Price 1984). However, due to the protection shelters provide their makers, I hypothesized that shelter-building should reduce larval mortality, particularly during the early instars which are so vulnerable to both invertebrate and vertebrate predation (see Damman 1987).

Shelter-building provides a unique opportunity to determine survivorship schedules of these caterpillars in the field, because most shelter makers vacate their old shelter and construct a new one as they molt to a larger instar (Loeffler 1996). Thus, individual larvae leave a record of their development and survival behind which is evidenced by a sequence of increasing shelter sizes. I was able to make use of this behavior to develop a life table (survivorship and mortality record) for the larvae of the shelter-forming Hammock Skipper, *Polygonus leo* (Lepidoptera: Hesperiidae) which occurs abundantly on Guana Island, BVI. These life table statistics were used to test the hypothesis that shelter formation reduces larval mortality, particularly during the early instar stages.

Methods

Study Site and Life History of the Hammock Skipper

This study was conducted on Guana Island, a privately owned resort and nature preserve in the British Virgin Islands. Larvae of the Hammock Skipper, *Polygonus leo*, fed and built protective leaf shelters on the leguminous tree *Piscidia carthagenensis*. This tree is an abundant species occurring throughout the lowlands of Guana Island where it frequently grows along the edges of paths and clearings between White Bay Beach and North Beach.

Females of the Hammock Skipper lay their eggs on the leaf undersurfaces of their host tree. After hatching, first instar larvae begin to construct a leaf shelter by chewing a rectangular trench around three sides of the lamina leaving a leaf flap. The flap is then folded over from within and the larva secures the flap to the laminar surface with silken strands. The completed shelter looks much like a purse with the larva concealed within. First instar larvae build two shelters as they grow, and each of the remaining four larval instars (2-5) constructs a single shelter. With the exception of the first instar larva, all larvae leave their old shelter after molting and thereafter construct a new, but larger, shelter. With

few exceptions, larvae remain within the shelters during the day and leave the shelter to feed at night. Last instar larvae (5th) leave their larval shelter and build a new one within which they pupate. The pupal shelter, however is of a different construction than the larval shelters. The pupal shelter is made by attaching two whole leaves loosely together with what are essentially silken guy-wires, whereas the larval shelters are made by folding a rectangular piece of leaf over and securing it tightly to the remainder of the leaf with silk.

Larval Survivorship and Mortality

Because individual larvae usually remained on the same or neighboring leaves as they grew, and because there was rarely more than one larva per leaf, it was easy to observe the larval developmental history and its survivorship simply by measuring the distribution of shelter sizes on the leaf. Because the shelters persist on the leaves, taking a large random sample of shelter sizes of this skipper allowed for the calculation of survivorship and mortality schedules (Price 1984).

I gathered larvae of every instar, as well as the leaf-shelter from which each was taken. I then measured the width of the head capsule of each larva, and the area (length X width) of its shelter. These data were analyzed by linear regression to establish the relationship between larval instar and shelter size.

Knowing this relationship allowed me to determine life table statistics for larvae from the distribution of shelter sizes in the field. Subsequently, I took a large random sample of shelters in the field. Altogether, I sampled 360 shelters and measured their sizes. From these data, I was able to calculate survivorship (l_x = the number of larvae alive to begin each larval instar), mortality (d_x = the number of larvae which died within each larval instar), and stage-specific mortality (d_x/l_x) (see Price 1984). Because first instar larvae build two shelters, I calculated life table statistics separately for these two stage classes. During the course of the sampling schedule, I recorded any observed acts of predation on skipper larvae and noted the identity of the predator.

Results

Relationship between Larval Instar and Shelter Area

Linear regression found a significant positive relationship between the head capsule width of a larva (instar) and the area of its shelter ($R^2 = 0.952$, $P<).0001$; Fig. 1). As a larva grew and molted, it made a larger shelter. Furthermore, there was virtually no overlap in shelter size among the different larval instars. This relationship enabled me to predict which larval instar made each shelter by simply measuring shelter area. From this relationship, I was also able to determine that first instar larvae relocate and build a new shelter once before they molt. Thus there were six shelter-area classes (Fig. 1).

Larval Survivorship and Mortality

The distribution of larval shelter sizes in the field allowed me to determine the

survivorship (l_x), mortality (d_x), and age specific mortality (q_x) for the five larval instars of *P. leo*. My data indicate that most larvae died between the first and second instar (Figs. 2-4). Stage specific mortality was highest during the second instar (0.58), followed by the third (0.50) and fourth instars (0.40) (Fig. 4). Spiders were the most frequently encountered predators near and within the larval shelters, and these invertebrate predators were the most likely source of mortality for *P. leo*. Stage specific mortality was least during the first larval instar (0.15 and 0.36 for the two stages of first instars). These data provide support for the hypothesis that shelter building behavior results in increased larval survival during the early, vulnerable instars.

Discussion

Survivorship data for many free-living lepidopterans indicates that there is a tremendous amount of mortality during the first instar stage (Price 1984). My data for the shelter-building skipper, *Polygonus leo*, contrast with this pattern for free-living species. For this skipper, shelter-building was associated with reduced mortality during the early larval instars.

One possible reason for the reduced mortality in the early instars is that shelters protect these sensitive larvae from desiccation during the dry season on Guana. A more likely alternative is that shelters provide some protection from natural enemies (see Damman 1987). It is likely that the larger instars of *P. leo* spend more time outside of their shelters feeding to meet their greater metabolic requirements. This behavior in combination with their greater size and visibility may make them much more vulnerable to predators. I observed several acts of spider predation on larvae of *P. leo*. I found caterpillar remains in a few spider webs, and actually observed a newly emergent larva crawl into a spider web. I also observed the presence of spiders in a large number of empty *P. leo* shelters, although it was difficult to determine whether the spiders killed the larva or simply colonized it after the caterpillar vacated the shelter. Nevertheless, spiders appear to be a very likely mortality source for the larvae of *P. leo*.

The results of the life-table analysis for larvae of *P. leo* indicate that this shelter-building species survives better during the early larval stages than many free-living species of Lepidoptera (see Price 1984). The advantage of shelter-building behavior in the predator-rich tropics may be evidenced in part by the increased diversity of shelter-building skipper species at tropical latitudes (Scott 1986).

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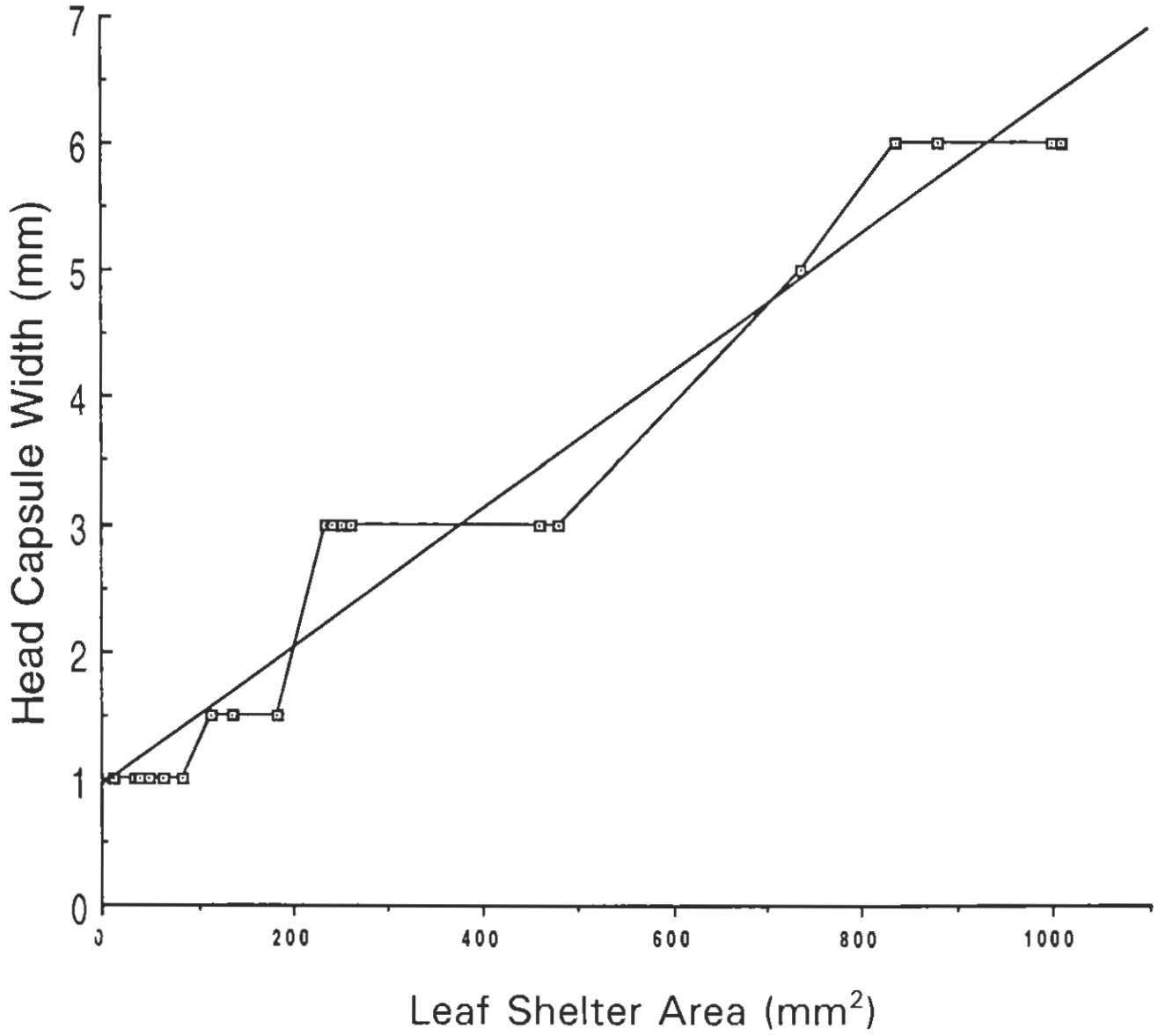

Figure 1. Relationship between the head capsule width (mm) of a larva of *Polygonus leo* and the area (mm^2) of its leaf shelter: $y = 0.947 + 5.418e\text{-}3x$, $R^2 = 0.92$, $P < 0.001$.

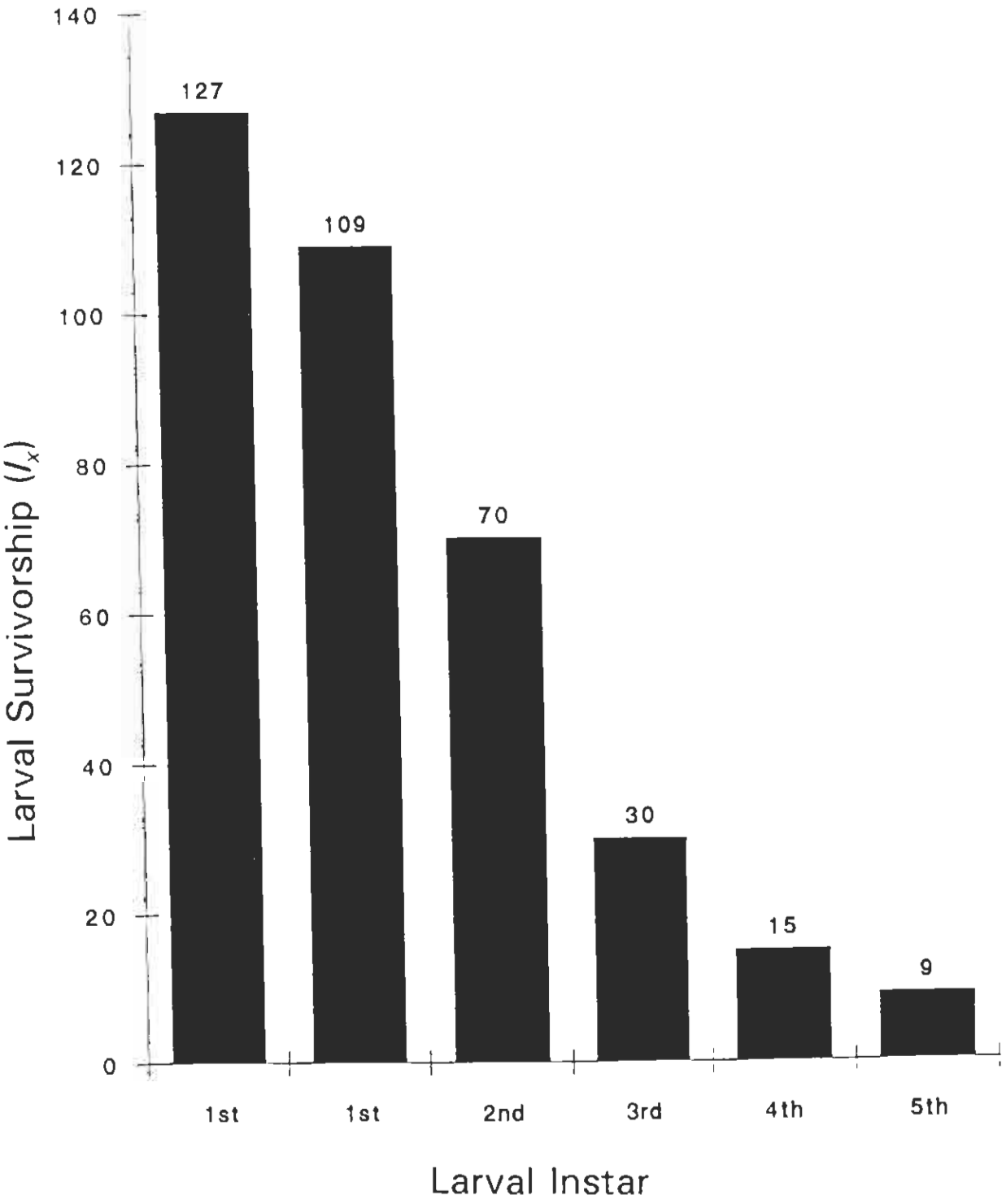

Figure 2. Larval survivorship (l_x) of the five instars of *Polygonus leo*. Note that the first larval instar is divided into two stage classes based on its behavior of constructing two leaf shelters one after the other.

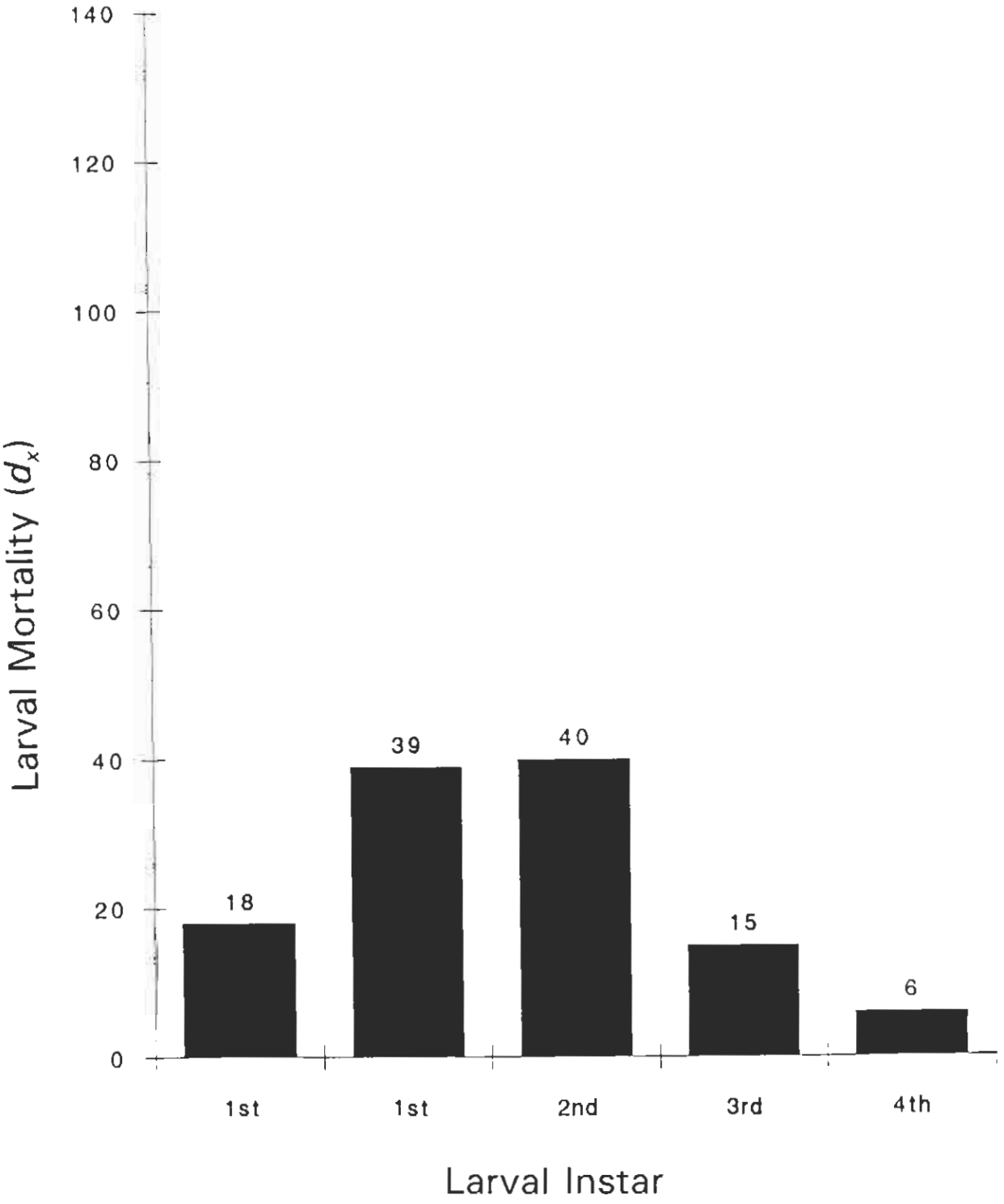

Figure 3. Larval mortality (d_x) of the five instars of *Polygonus leo*. Note that the first larval instar is divided into two stage classes based on its behavior of constructing two leaf shelters one after the other.

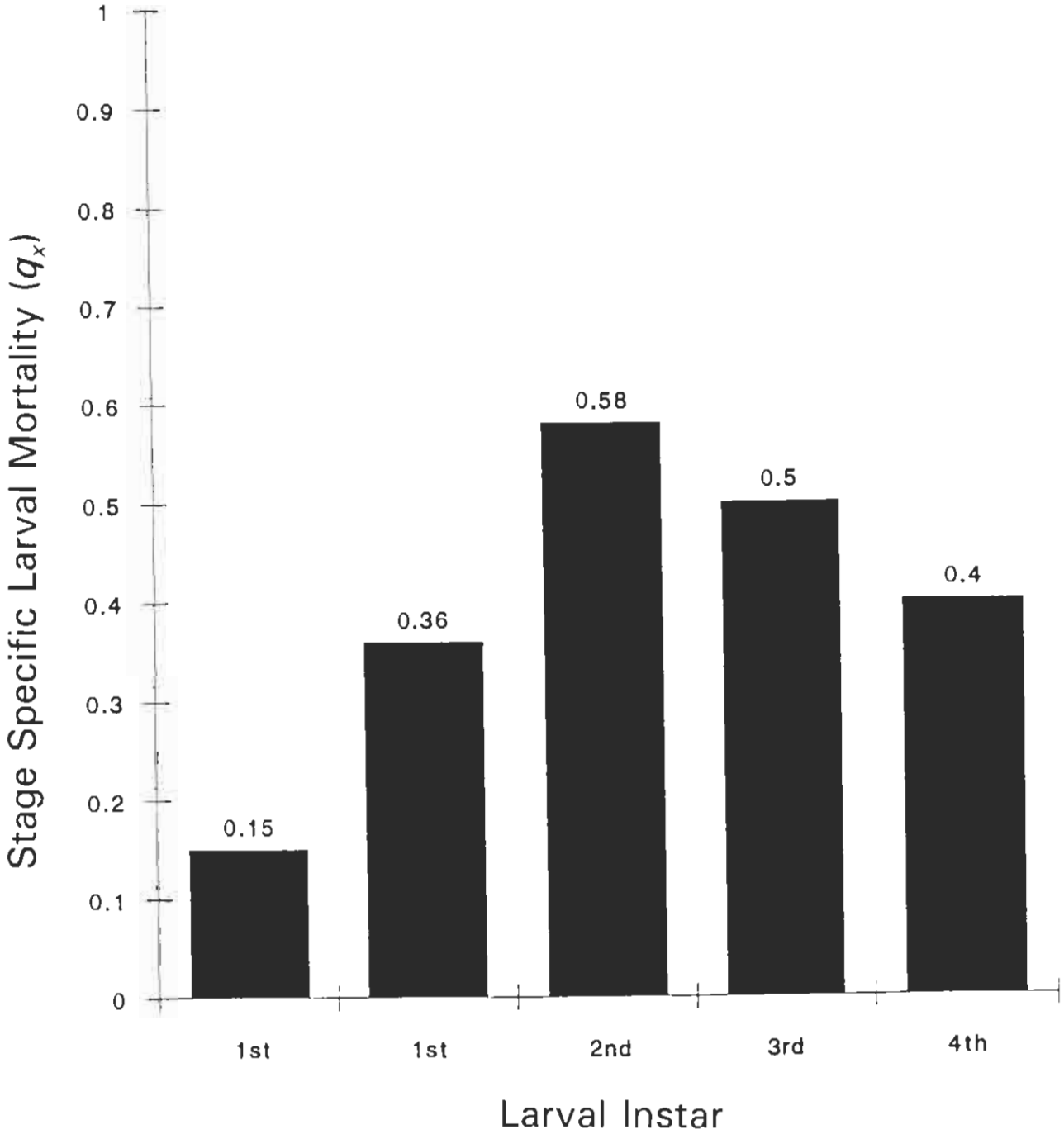

Figure 4. Stage specific larval mortality (q_x) of the five instars of *Polygonus leo*. Note that the first larval instar is divided into two stage classes based on its behavior of constructing two leaf shelters one after the other.

UNIVERSITY OF MARYLAND AT COLLEGE PARK

COLLEGE OF LIFE SCIENCES • DEPARTMENT OF ENTOMOLOGY

February 3, 1997

Dr. James D. Lazell
The Conservation Agency
6 Swinburne Street
Jamestown, Conanicut Island
RI 02835

Dear Skip:

We have finally gotten together a research proposal for this next summer and perhaps future summers. We would like to get your input and any ideas that you might have. I sent a copy to Liana for her input, but I haven't heard back yet. In any case, any thoughts you might have would be greatly appreciated. A copy of the student research reports will follow sometime in spring, certainly by April.

We just moved into a new building, I'm teaching, and I'm on an NSF panel next week, so there doesn't seem to be alot of extra time right now. In any case this proposal is off my desk. Hope all goes well with you and your work.

Sincerely,

Bob

Robert F. Denno, Professor

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Proposed Collaborative Research Project
The Conservation Agency, Guana, Island, BVI.

The Influence of Habitat Isolation and Dispersal Capability on the Genetic Diversity and Population Biology of Salt Marsh/Mangrove Insects: Implications for Conservation Genetics

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ABSTRACT

Habitat isolation/fragmentation can have profound effects on the genetics of local populations by disrupting gene flow and permitting genetic drift to eliminate variation. As a result, local populations may be faced with an increased probability of extinction due to both inbreeding depression and an inability to adapt to environmental changes. Thus, the persistence of such local populations may be dependent on recruitment from neighboring populations. In making complex decisions concerning habitat conservation, we need to take into consideration these population genetic and metapopulation dynamic factors, but currently such efforts are hampered by a lack of data on the population genetic and dynamic effects of fragmentation.

We propose to survey allozyme variation and population density of six salt marsh/mangrove insect species occupying both isolated and contiguous habitats in the British Virgin Islands (BVI) and US Virgin Islands (USVI) to determine the effects of habitat isolation on gene flow, genetic diversity, and metapopulation dynamics. Insects are ideal for examining such issues because of their abundance and diversity. Specifically, we will sample pairs of populations both within islands (contiguous) and between islands (isolated across open water) that are separated by similar distances. By comparing gene flow between the populations in isolated marshes with that found among populations in contiguous marshes, we will determine the effect of habitat isolation on gene flow in each species. Furthermore, we will discover whether populations within the same island are indeed less genetically differentiated than their counterparts on different islands. Additionally, we will determine the effects of habitat fragmentation on population dynamics by comparing population size, the dynamical synchrony of populations, and predator/prey ratios within (contiguous habitats) and between islands (fragmented habitats).

Furthermore, because the insects we have selected for study are found in the same salt marsh/mangrove habitats, but differ markedly in dispersal ability, we can examine the role of mobility in determining the population genetic and population dynamic consequences of habitat isolation. Two flightless species, a cricket and a scale insect, are predictably sedentary, and we expect that of our six focal species they will be most influenced by habitat isolation. We hypothesize that two more mobile species, a termite and a planthopper, will show a weak response to the fragmentation of their habitats. Finally, we predict that two highly mobile species, a katydid and a leafhopper will be least affected by disjunctions in their habitat.

INTRODUCTION

The preponderance of research on the effects of habitat fragmentation has focused on short-term ecological responses, including declines in species diversity, altered predator-prey interactions, and shifts in nutrient dynamics (Saunders et al. 1991). Recently, researchers have begun to emphasize that the population genetic and evolutionary consequences of habitat fragmentation may be equally important to the long-term persistence of populations (Boecklen 1986; Gilpin 1987, 1991; Lande and Barrowclough 1987; Falk and Holsinger 1991; Hanski 1989). Of particular importance is understanding whether populations in fragmented habitats maintain sufficient genetic variation to limit inbreeding depression and to allow future adaptation, thus reducing chances of extinction. In theory, levels of genetic variation within a population will be shaped by both gene flow from neighboring populations and genetic drift within the population itself, and these factors in turn may be influenced by rates of dispersal, population size, and the degree of habitat fragmentation and isolation (Boecklen 1986; Gilpin 1991; Peterson and Denno in press). Unfortunately, few empirical studies have examined these issues.

Of the three studies to date that have examined the hypothesis that habitat fragmentation reduces gene flow among populations, two found no evidence that this is the case (Foré et al. 1992; King 1987). This should come as no surprise, however, because one of the studies was of wind-pollinated sugar maples (Foré et al. 1992), and the other involved beetle populations separated by very small distances (< 1km) (King 1987). The third study involved a comparison of cave cricket species in habitats of varying fragmentation (Caccone and Sbordoni 1987). In these crickets, a surface-dwelling species exhibited much greater levels of gene flow than species which were restricted to caves. Furthermore, gene flow levels in the cave-dwelling species were correlated with the contiguity of their caves. To our knowledge, no other studies have explicitly examined the hypothesis that genetic variation is reduced in insular/fragmented habitats, so we remain relatively uninformed regarding how habitat fragmentation influences the genetics of natural populations.

Underlying the effects of habitat fragmentation on the genetic structure of populations are its effects on metapopulation dynamics and local population persistence (Hanski 1987, 1989; Kareiva 1987; Hanski and Gilpin 1991; Saunders et al. 1991; Thomas and Harrison 1992). Isolated habitat fragments may harbor small populations due to high rates of emigration (Kareiva 1983, 1985; Hanski 1987, 1989; Hanski and Gilpin 1991; Thomas and Harrison 1992). Furthermore, fragmented populations may experience reductions in natural enemies and thus fluctuate more dramatically (Hanski 1987; Kareiva 1987). Thus, isolated habitat fragments may house smaller populations which fluctuate more chaotically and experience frequent extinctions (Hanski 1987, 1989; Hanski and Gilpin 1991; Thomas and Harrison 1992). The rescue of such populations is dependent on the arrival of dispersers from other habitat patches, a phenomenon which is less likely if all sub-populations fluctuate synchronously (Harrison and Quinn 1989). Because dispersal is often density dependent and associated with deteriorating resources (Denno and Peterson 1995), the probability of successful rescue will be less if all sub-populations simultaneously experience poor resources and emigrate (Harrison and Quinn 1989). Thus, small populations fluctuating in concert should be associated with extinction (Harrison and Quinn 1989), the loss of genetic

variability within population fragments, and discrepancies in genetic structure among sub-populations (Boecklen 1986; Gilpin 1991; Peterson and Denno in press). No study of any organism has successfully linked population dynamics with the loss of genetic variability.

The British Virgin Islands (BVI) and United States Virgin Islands (USVI) offer an ideal opportunity for studying the effects of habitat fragmentation and isolation on the population ecology and genetics of phytophagous insects. The Puerto Rico Bank includes all the islands (BVI and USVI excluding St. Croix) from Puerto Rico itself to Anegada, the northeasternmost island in this geologic unit (Lazell 1989, 1996; Fig. 1). During the last glacial maxima (17,000 yr ago), the entire Puerto Rico bank was emergent and what are now oceanic islands became hills or ranges on a lowland plateau (Donnelly 1988; Lazell 1989; Thorne et al. 1994). During such times, lowland habitats expand and coalesce (Lazell 1989), and gene flow is likely much more extensive. In contrast, during interglacial periods the sea level rises resulting in the fragmentation, constriction, and oceanic isolation of once widespread lowland habitats (Donnelly 1988; Lazell 1989). Thus, insects inhabiting lowland habitats likely experience population bottlenecks, the loss of genetic diversity, or extinction.

Using six species of insects that occur in the salt marsh/mangrove habitats of the BVI and USVI and that vary in their dispersal capability, we will determine the effects of habitat fragmentation and isolation on gene flow, genetic diversity, and population dynamics. Specifically, we will sample pairs of populations both within islands (contiguous) and between islands (isolated across open water) that are separated by similar distances. By comparing gene flow (estimated from allozyme surveys) between the populations in isolated marshes with that found among populations in contiguous marshes, we will determine the effect of habitat fragmentation on gene flow in each species. Furthermore, we will elucidate whether populations within the same island are less genetically differentiated than they are from their counterparts on different islands. Also, we will determine if populations on small isolated islands (fragmented habitats) compared to populations on large islands are smaller in size on average, fluctuate more chaotically, or incur an imbalance in trophic structure created by reduced densities of natural enemies.

The insects we have selected for study can be found in the same salt marsh/mangrove habitats, and yet they differ markedly in dispersal ability (Thorne et al. 1994; Denno and Thorne unpublished data). Two species, a cricket (Gryllidae), and a scale insect (Diaspididae) are very sedentary, and we hypothesize that their genetic structure and population dynamics will be the most influenced by habitat fragmentation. We further expect that two somewhat mobile species, the termite *Nasutitermes acajutlae* (Temitidae), and the planthopper *Toya venilia* (Delphacidae), will show a weak response to the fragmentation of their habitats. Finally, we predict that two highly mobile species, a katydid (Tettigoniidae) and the leafhopper *Tideltellus marinus* (Cicadellidae) will be less affected by disjunctions in their habitat. Importantly, to control for phylogenetic nonindependence we have selected insects belonging to two distinct insect lineages. The cricket, termite, and katydid belong to the orthopteroid lineage, whereas the scale insect, planthopper, and leafhopper are hemipteroids. Thus, within each lineage, each of the three dispersal categories (low, moderate, and high) is represented allowing for a non-phylogenetically confounded assessment of how fragmentation interacts with dispersal capability to affect the genetic structure and population dynamics of populations.

OBJECTIVES

In the proposed research, we will assess the influence of habitat fragmentation on the population genetics and dynamics of a suite of herbivorous insects occurring in the marshes/mangroves of the BVI and USVI. Because these insects vary in mobility, we expect them to respond differently to habitat fragmentation. Our work will document how fragmentation influences both gene flow among populations and levels of genetic diversity within populations. It will further elucidate how the genetic effects of fragmentation are shaped by species-specific mobility. Furthermore, the work will examine the effects of fragmentation on population dynamics. This information will aid in the design and maintenance of habitat refuges that maximize the long-term preservation of genetic diversity within populations occupying the marshes of the BVI and USVI, a critical step in ensuring the persistence of these vitally important habitats.

Objective 1:

To determine the effect of habitat fragmentation on levels of gene flow among populations.

Objective 2:

To assess whether the influence of habitat fragmentation on gene flow is correlated with species-specific mobility.

Objective 3:

To determine the effect of habitat fragmentation on levels of genetic diversity in local populations.

Objective 4:

To determine the effect of habitat fragmentation on population size, population fluctuation, and trophic structure of the insect community.

RESEARCH PLAN

The six insect species we have selected for this work all occur in the marsh/mangrove habitats of the BVI and USVI. Three of the species (the hemipteroid insects) are specialist sap-feeders on Salt Grass (*Sporobolus viginicus*). This grass is restricted to and dominates the upper fringe of salt flats on the intertidal marsh or around isolated salt ponds where it grows in expansive pure stands (Chase 1971; Denno and Thorne personal observation). The remaining three insect species (the orthopteroid insects) are associated with mangroves, adjacent to which Salt Grass frequently grows (Thorne et al. 1994; Thorne and Denno personal observation. Because these six insects are more or less restricted to these lowland habitats, we can be fairly certain that they all face a similar degree of habitat fragmentation both within and between islands. In addition, because these six species are all common (Thorne et al. 1994; Denno and Thorne unpublished data), it will be easy to obtain a sufficient number of insects for genetic and population analysis. Finally, although these

species occur in the same habitat, they differ dramatically in mobility. This key life history difference allows us to make predictions about the relative effects of habitat fragmentation on the population genetics and dynamics of the different species.

Study Organisms

Salt Grass and mangroves occur extensively throughout the BVI and USVI and the West Indies in general (Chase 1971; Lazell 1996; Jarecki and Denno personal observation). The three hemipteroid insects feed exclusively on Salt Grass. One undetermined scale species (Diaspididae) is small (2mm in length) and very immobile. As with many scale insects, dispersal is primarily performed by crawling nymphs or males with poor flight capability (Hanks and Denno 1993). Because areas of unsuitable habitat or open water undoubtedly form significant barriers to dispersal for nymphs of this species, we predict that gene flow will be much greater among populations of this scale species in contiguous habitats (within islands) than in fragmented habitats (between islands). As a result of these low levels of gene flow, we also predict that genetic variation (heterozygosity) will be greater in populations occupying contiguous habitats.

Intermediate in mobility is the wing-dimorphic planthopper, *Toya venilia* (Delphacidae) (2mm in length). Most individuals of this planthopper are short-winged and flightless (>95%), but there is a low incidence of migratory forms in all populations we sampled (Denno and Marx, unpublished data from Guana, Beef, and Great Camanoe Islands). Nevertheless, the production of migratory forms in planthoppers is density dependent and is associated with population outbreaks (Denno et al. 1991; Denno 1994). Thus, this species has moderate dispersal capability as the result of sporadic bouts of migrant production (see Denno et al. 1996). Also restricted to Salt Grass is the highly mobile leafhopper *Tideltellus marinus* (Cicadellidae) (Kramer 1971; Denno personal observation). We predict that due to its limited mobility, this planthopper will show slightly reduced gene flow and heterozygosity in fragmented habitats (between islands). In contrast, we expect that habitat fragmentation will have little or no effect on the population genetics of the highly mobile leafhopper (all adults are macropterous and potentially highly mobile).

Similarly, the orthopteroid species also encompass a spectrum of dispersal capabilities ranging from a flightless cricket, through the moderately mobile termite, to the more mobile katydid, all of which are associated with lowland marsh/mangrove habitats. The cricket is brachypterous with reduced wings (C. Bartlett, S. Miller personal observation), and as a consequence we expect this species to be most affected by habitat fragmentation. The termite, *Nasutitermes acajutlae* occurs throughout the BVI and USVI including many of the small islands (e.g. Guana, Great Thatch, Little Camanoe, George Dog, and Necker) (Thorne et al. 1994). This termite constructs arboreal carton nests of variable size on the trunks of mangroves and other trees (Thorne et al. 1994). Colonies consist primarily of wingless reproductives, workers, and soldiers which have very limited dispersal capability (Thorne et al. 1994). Mature colonies release an annual brood of winged reproductives which have moderate dispersal capability (Nutting 1969). Thus, we expect populations of this termite to exhibit some evidence of genetic differentiation in relation to habitat fragmentation, but only at the largest spatial scale (between the furthest islands). In contrast, we predict that the

fully-winged katydid will exhibit high levels of gene flow among islands and that its genetic structure will be relatively uninfluenced by fragmentation.

For practical reasons, we restrict our assessment of metapopulation dynamics to the guild of multivoltine hemipterans on salt grass. The long-lived orthopteroid insects would require a long-term commitment to adequately assess their population dynamics, not to mention the greater sampling difficulties in the mangrove habitat. Nevertheless, for the hemipterans, we predict a low mean population size with high variance for the sap-feeders on small isolated islands where population bottlenecks seem frequent and rescue effects are more unpredictable. In contrast, we expect a larger population size with less variance on the more contiguous habitats on large islands where rescue effects are more likely (Harrison and Quinn 1989). In the metapopulation context, we predict that population size will be smaller on small isolated islands if populations fluctuate synchronously among islands. Synchronous population fluctuations across small islands will deminish the probability that one island will act as a donor of colonists (usually when population outbreaks occur) at a time when rescue by colonists on another island population is needed (when population size is critically low) (see Harrison and Quinn 1989). We also envision the effects of fragmentation on population size to be stronger as dispersal ability decreases. Last, we predict that the trophic structure will differ between contiguous and fragmented situations such that predator/prey ratios will be higher in contiguous compared to fragmented habitats. Low predator/prey ratios on small islands should promote herbivore escape, foster population instability, and increase the probability for extinction.

Population Genetic Surveys (Objectives 1-3)

To estimate levels of gene flow among populations and genetic diversity within populations of each species, we will employ starch gel electrophoresis, following the methods of May (1992) and Peterson (1995, 1996). The advantages of allozyme electrophoresis over the more recently developed DNA-based techniques include low cost and rapid results, both of which are critical for large surveys of variation within and among populations (Amos and Hoelzel 1992; May 1992). In addition, it typically offers numerous independent measures of genetic variation, a feature that is desirable for estimating levels of gene flow among populations (Slatkin and Barton 1989).

For this study, we will collect 50 individuals per population of each species (termite excepted, see below) from six selected island pairs in the BVI and USVI, with each pair consisting of one large island (e.g. Tortola) and one small island (e.g. Guana) (see Fig. 1). Within each island pair, we will sample (sweep-net or hand collect, see Denno 1977) two populations from the large island and one population from the offshore small island. Because the termite is a social insect which forms large arboreal colonies, only 10 colonies per island (10 workers per colony) will be sampled for allozyme analysis.

Thus, a comparison of gene flow can be made between contiguous habitats (populations within the large island) and fragmented habitats (populations on the large and small island). For each island pair, populations both within and between islands will be separated by a similar geographic distance ($\sim$5-15 km), depending on the configuration of the island pair. Because two populations from large islands will be sampled, the population

nearest in distance to the small island population will be chosen for comparison. All species will be collected at the same locations to control for distance in comparison among different species. The six island pairs along with populations to be sampled and distances separating these populations are listed in Table 1 (refer to Fig. 1 for island locations). All field-collected samples will be placed immediately in liquid nitrogen prior to allozyme analysis.

Following an initial survey of allozyme variation in each species, we will select 5-10 polymorphic loci per species, a simple accomplishment for most insect taxa (May 1992; Peterson and Denno in press). We have already performed this survey with *Prokelisia* planthoppers, revealing 7 polymorphic loci in *P. marginata* and 6 in *P. dolus* (Peterson and Denno, unpublished data). After selecting the loci for each species, we will determine the genotype at each locus for each individual collected from the 18 populations.

Genetic Analyses (Objectives 1-3)

From the allele frequencies in each of the populations, we will estimate levels of gene flow between the populations in fragmented (between island) and contiguous habitat (within island) situations using both Weir and Cockerham's (1984) theta and Nei's (1973) G_{ST}, both of which are good estimators of Wright's (1951) F_{ST} (Slatkin and Barton 1989; Cockerham and Weir 1993). F_{ST} is a measure of the degree to which populations are genetically subdivided, and can be used to estimate gene flow among populations by the equation: $F_{ST} = 1/(4Nm + 1)$, where Nm is the average number of individuals exchanged between populations per generation (Wright 1951).

To assess the influence of habitat fragmentation on levels of gene flow in each species (Objective 1), we will compare the gene flow estimates in contiguous and fragmented habitats using ANOVA with habitat configuration (fragmented or contiguous), lineage (hemipteroid or orthopteroid), and dispersal capability (low, moderate, or high) as sources of variation in the model (Sokal and Rohlf 1981; SAS 1990). We hypothesize that gene flow will be greater in contiguous habitats, due to the presence of a corridor of suitable habitat along which genes can flow. We further hypothesize that this difference in gene flow in the two habitat types will be greatest in the sedentary species due to their inability to cross areas of unsuitable habitat (Objective 2). A significant effect of dispersal capability on gene flow will support this hypothesis.

To assess the influence of habitat fragmentation on local genetic diversity (Objective 3), we will calculate average levels of heterozygosity (across all allozyme loci) in each population using the program "Genes in Populations" (written by May, Krueger and Eng, 1992). We will use ANOVA (Sokal and Rohlf 1981; SAS 1990) of the heterozygosity measures of each species to test our hypothesis that genetic diversity is lower in the populations occupying fragmented habitats. The estimates of heterozygosity will be artificially high for all populations since we will only have data from polymorphic loci. However, since all populations of a species will be surveyed for the same loci, these estimates will nonetheless provide a good measure of the relative differences in heterozygosity in the two habitat types.

Populations Dynamics and Trophic Structure (Objective 4)

Using another subset of islands (Fig. 2), we will determine the effects of habitat fragmentation on the population size, population dynamics (synchronous or asynchronous fluctuations among island populations), and trophic structure of the hemipteroid insects (scale, planthopper, and leafhopper) associated with Salt Grass (Objective 4). For this objective, we have selected Tortola, Virgin Gorda and Anegada (large islands) and an archipelago of smaller satellite islands including Guana, Great Camanoe, Scrub, Great Thatch Ginger, Peter, and Norman (Fig. 2). Each population will be sampled tri-monthly for two years to determine (1) average population size, (2) temporal and spatial variance in population size, and (3) predator/prey ratios. Three samples per location, each consisting of 10 sweeps with a net, will be taken on each date in predefined patches of Salt Grass. The effect of habitat fragmentation on the population and community parameters will be assessed using ANOVA (Sokal and Rohlf 1981; SAS 1990). The major predators of hemipteroid insects in the BVI appear to be spiders and ants (Denno unpublished data from Guana, Beef, and Great Camanoe Islands). We expect populations to be smaller, temporal population fluctuations to be greater (higher within-island variance), spatial population fluctuations to be more asynchronous (higher between-island variance) and predator/prey ratios to be lower on the more highly fragmented small islands than the more contiguous large ones.

PROSPECTUS

The marsh and mangrove habitats of the BVI and USVI have undergone severe fragmentation over the last 20,000 years as a result of changes in sea level (Lazell 1989). Human intervention and developing land use have exacerbated the effects of constricting habitats (Lazell personal communication). These habitats are vital to the natural resources and economy of the region, so we must make every effort to ensure the long-term persistence of the remaining marshes and mangroves (Jarecki REPORT). To reduce the likelihood of population extinctions, it is important that we design preserves and retain marshes that will allow the maintenance of genetic variation in populations of organisms associated with these sensitive habitats. To do so, we must understand how habitat fragmentation has influenced the population genetics of marsh/mangrove inhabitants. We propose to examine the effects that habitat fragmentation has had on gene flow, genetic diversity, and population dynamics in six dominant herbivores of the marsh/mangrove community of the BVI and USVI. Because these insects differ significantly in mobility, comparisons between species will provide important data on the role of mobility in determining the population genetic consequences of habitat fragmentation. This work will provide important background data for future studies of the population genetic response of individual marsh species to varying levels of habitat fragmentation. With information such as this, we will be able to make specific recommendations for the design of wetlands preserves that take into account the need to maintain genetic diversity and critical population size.

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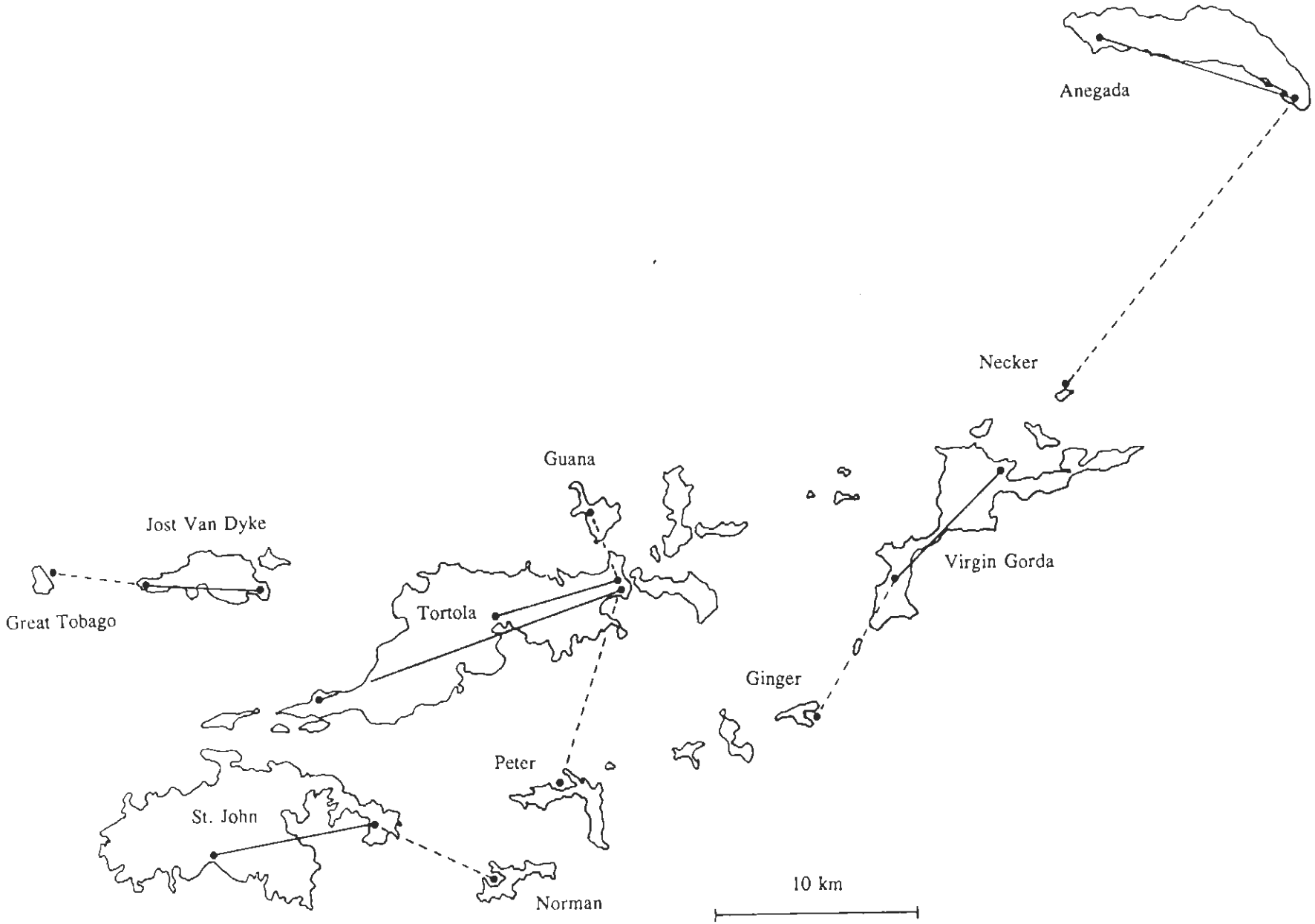
Table 1. Island pairs and sampling locations used to assess the effects of habitat fragmentation (between island comparison) versus habitat contiguity (within island comparison) on gene flow between populations of salt marsh/mangrove insects. The large island in each pair is listed first.

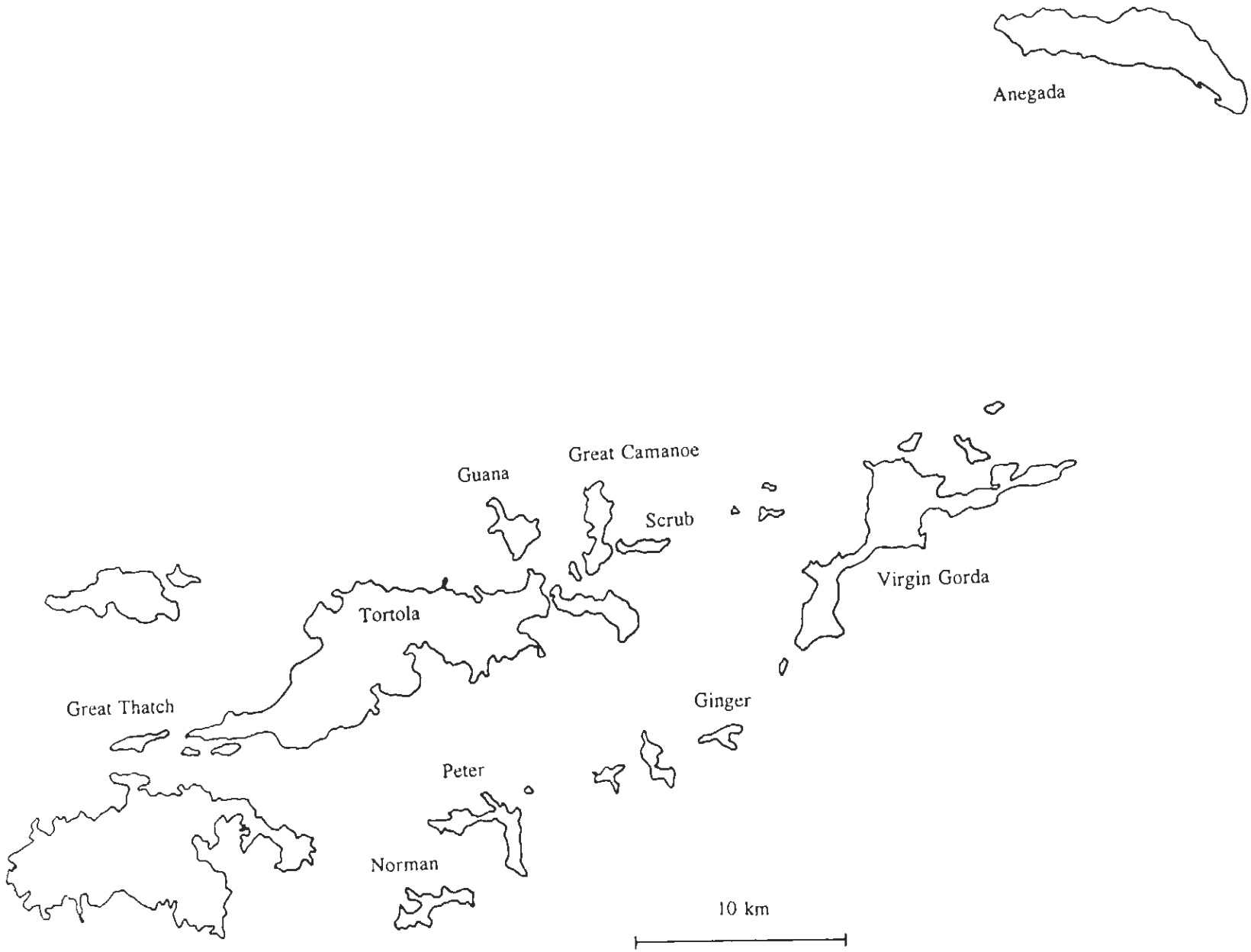
Island Pair	Within Island Comparison (Locations, Distance in km)	Between Island Comparison (Locations, Distance in km)
Tortola/Guana	East End, Road Town, 5km	East End, White Bay, 5km
Tortola/Peter	East End, Belmont Pond, 12km	East End, Great Harbor, 10km
Virgin Gorda/ Ginger	Spanish Town, Gun Point, 7km	Spanish Town, White Horse, 8km
Anegada/Necker	East End, Flamingo Pond, 10km	East End, Necker, 16km
Jost Van Dyke/ Great Tobago	West End, Little Harbor, 7km	West End, Man O War Bay, 5km
St. John/Norman	Reef Bay, Round Bay, 8km	Round Bay, The Bight, 7km

FIGURE DESCRIPTIONS

Figure 1. Island pairs and sampling locations in the British Virgin Islands and US Virgin Islands used to assess the effects of habitat fragmentation (between island comparison, dashed line) versus habitat contiguity (within island comparison, solid line) on gene flow between populations of salt marsh/mangrove insects. For each island pair, populations within and between islands are separated by a similar geographic distance (~5-15 km), depending on the configuration of the island pair. See Table 1 for names of specific sample locations.

Figure 2. Islands in the British Virgin Islands and US Virgin Islands used to compare the effects of habitat fragmentation (small island versus large island) on the population size, population dynamics (synchronous or asynchronous fluctuations among island populations), and trophic structure of the hemipteroid insects associated with Salt Grass. Tortola, Virgin Gorda and Anegada (large islands) and an archipelago of smaller satellite islands including Guana, Great Camanoe, Scrub, Great Thatch Ginger, Peter, and Norman are designated. Each population will be sampled tri-monthly for two years to determine (1) average population size, (2) temporal and spatial variance in population size, and (3) predator/prey ratios. We expect populations to be smaller, temporal population fluctuations to be greater (higher within-island variance), spatial population fluctuations to be more asynchronous (higher between-island variance) and predator/prey ratios to be lower on the more highly fragmented small islands than the more contiguous large ones.


Anegada
Necker
Guana
Jost Van Dyke
Great Tobago
Tortola
Virgin Gorda
Ginger
Peter
St. John
Norman
10 km


Anegada
Guana
Great Camanoe
Scrub
Virgin Gorda
Tortola
Great Thatch
Ginger
Peter
Norman
10 km

Guana Island Microcrustacea and Associated Invertebrates
Preliminary Report of Activities, October 6-13, 1996

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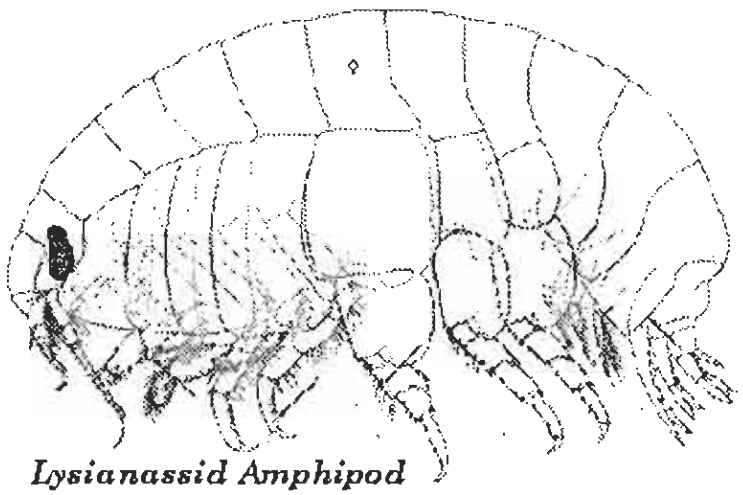
Introduction

The taxonomic status of most microcrustaceans and their associated infaunal invertebrates is relatively unknown for most regions of the Caribbean at this time. Only a few groups have been documented in any detail, and most of this work has been concentrated at the few Caribbean marine biological laboratories such as those at Discovery Bay, Jamaica, and Carrie Bow Key, Belize; the latter a facility established relatively recently by scientists associated with the United States National Museum. In the case of microcrustaceans, i.e. amphipods (scuds, beach hoppers), and



Lysianassid Amphipod

isopods (sea roaches, sowbugs, pillbugs), tanaids, cumaceans, and leptostracans, only the isopods have been

Isopod Crustacean

documented in faunal reviews encompassing the entire Caribbean region (Kensley, 1989). For the amphipod and tanaid crustaceans, most investigators of the Caribbean fauna still rely heavily on the outdated monographs of B.W. Kunkel (1910) and H. Richardson (1902) focusing primarily on the presumably related, but distant, Bermuda fauna. Most subsequent work has focused on single species descriptions of new taxa based upon material from a variety of Bermuda and Caribbean localities; in the case of the latter these have focused almost exclusively upon the fauna associated with Carrie Bow Key, Belize, Florida, and Jamaica.

With respect to microinvertebrates, which must be targeted specifically, the fauna of the British Virgin Islands is almost unknown. In light of this fact, my goal was to begin developing a collection that would help contribute to a much needed accounting of the microinvertebrate fauna of this region. From a faunal collecting viewpoint,

the BVI is ideally situated between the northern Bahamian and southern tropical South American regions.

Field Work and Methods

In October, 1996, I visited Guana Island to make a preliminary assessment of the microfauna associated primarily with the near-shore shallow intertidal and subtidal zones. Collecting was concentrated in areas of high algal diversity or an abundance of coral debris (rubble); both areas typically harbor large numbers of microinvertebrates rarely seen by specialists focusing on the conspicuous macrofauna. The collections were made utilizing the *formalin-wash technique*, whereby small samples of algae encrusted rock and rubble are rinsed off in a bucket of seawater laced with a small amount of formalin (formaldehyde). This rinsing stimulates microcrustaceans and other invertebrates to abandon their nestling habitat where they can be easily collected by washing through a small (0.5 mm mesh) screen. This sort of technique has proven invaluable at targeting the smallest nestling organisms that can not be collected by casual "sight picking" with forceps. Typically, this latter method only targets the larger, easily seen, crustaceans (and other invertebrates) and entirely misses the hidden nestling, or cryptic, fauna. Furthermore, the number of specimens obtained by the wash technique is quite high, increasing the likelihood of finding the rarer forms.

Because of the difficulty of reaching the remote parts of Guana Island, only twenty five lots (each lot containing from a few to several hundred individuals) were obtained during the week of my stay. Six of these samples were made utilizing SCUBA in the shallow subtidal region proximal to the main boat launch area. Many of the more

remote locations were sampled only once and more than half of the potential sites were sampled only once.

When the collections were returned to my laboratory at the Peabody Museum, Yale University, I and various students working under my direction, and that of my research collaborator, Dr. Michael Gable of Eastern Connecticut State University, have been sorting the specimens to their respective taxonomic groups. Once the entire collection has been sorted and cataloged (target date, end of May 1997), I will send specimens to various specialists, nationally and internationally, for final taxonomic workup. Finally, as specimens are catalogued the data records will placed on the Peabody Museum World Wide Web server (URL address: http://www.peabody.yale.edu), so that the specimen data are of immediate use to interested scientists.

Presently, the list of specialists (and their area of interest) involved in the Guana Island micro-invertebrate survey are as follows:

Ms. Tara Casanova, Southern Connecticut State University, New Haven, CT - gammaridean amphipods (with E.A. Lazo-Wasem)

Dr. Michael F. Gable, Eastern Connecticut State University, Willimantic, CT - Willimantic, CT - melitid amphipods and leptostracans

Dr. Leslie H. Harris, Polychaete Section, Los Angeles County Museum of Natural History, Los Angeles, California - polychaete annelids

Dr. Franz Krapp, Museum Alexander Koenig, Bonn, Germany - pycnogonids

Dr. Traudl Krapp-Schiekel, Museum Alexander Koenig, Bonn, Germany - stenothoid and leucothoid amphipods

Mr. Eric A. Lazo-Wasem, Peabody Museum, Yale University, gammaridean amphipods (excluding groups mentioned above)

Mr. Thomas Sawicki, Eastern Connecticut State University - leptostracans

Synopsis of Preliminary Results

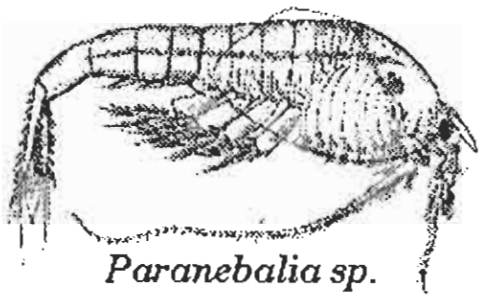
Although the sorting process is far from done, the faunal diversity of the near shore habitats investigated at Guana Island is certainly very great, and will reveal many new Caribbean records for the groups collected. Furthermore, microcrustaceans such as amphipods and isopods do not have planktonic larvae and most forms do not migrate far from their nestling habitat. Because of this, a relatively high degree of endemism can be expected for the BVI microcrustacea once the fauna is carefully surveyed.

Predictions made by Dr. Lazell, specifically, that the fauna of the island will vary considerably between localities due to habitat variability, have been correct. Some organisms were found in great abundance at only a single collecting site. For example,

Polyopthalmus pictus
bristle worm

the marine bristle worm *Polyopthalmus pictus*, often cited as a common, cosmopolitan species, was found only in a few wash samples of

algal tufts at North Beach. The leptostracan cf. *Paranebalia* sp. was very



Paranebalia sp.

abundant in a single algal sample at the southern end of White Bay Beach; repeated collections at adjacent sites did not yield many additional specimens. Many amphipod crustaceans have been found which are definitely not well studied for the Caribbean region. For example, lysiannassid amphipods, generally not abundant in tropical habitats, were represented by only a few individuals, and more specimens are needed before an identification can be made, because the present material does not include both sexes of this group.

This documentation of the Guana Island microcrustacean fauna is preliminary, as many crustaceans, mainly amphipod species, are represented by only a few specimens in the collection I made. Furthermore, some entire groups, such as the tanaid crustaceans, are represented by only a handful of individuals.. This scenario is typical of tropical/subtropical shallow-water collecting. For example, E.A. Lazo-Wasem and M.F. Gable have been conducting surveys of the Bermuda amphipod fauna for over ten years, amassing a total of over 10,000 specimens, and yet, many species are still only represented by a few individuals. Certainly, repetitive collecting "en masse", over a period of years, is necessary to achieve complete coverage and include the rarer species.

It should be noted that some algal nestling invertebrates, such as the polychaete annelids, will not be well known without further, separately targeted collecting, even though the relative biomass and diversity of these organisms is extremely high in tropical reef habitats. Unfortunately, the method utilized (formalin wash) to secure microcrustaceans and other arthropods (pycnogonids, or sea spiders), does not favor the collection of soft bodied forms such as annelids because these techniques typically cause these organisms to tightly adhere to their surrounding algal/rubble habitats, rather than abandon their hold. As a result, only the larger forms will be documented unless more specialized collecting methods (hand picking of bulk samples) are employed. Even though only a few annelid specimens were collected along with the microcrustaceans, the material has yielded some unusual forms. Dr. Leslie Harris (Los Angeles County Museum) believes that one specimen may represent an entirely new genus, and several other forms have distinctive, and possibly unique characters. We hope that further sorting of the collection will result in our finding more specimens of these interesting worms.

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BIOTROPICA 29(1): 111–116 1997

Evaporative Water Loss in Nine Insular Populations of the Lizard *Anolis cristatellus* Group in the British Virgin Islands[1]

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ABSTRACT

We studied water loss in eight insular populations of the lizard *Anolis cristatellus wileyae* and in one population of *A. ernestwilliamsi* in the British Virgin Islands. We found a strong negative correlation between habitat aridity and total and cutaneous water loss rate (ranging from 1.5–10.3 mg g^{-1} h^{-1}) and a strong positive correlation between habitat aridity and integument resistance to water loss (28.5–199.0 s cm^{-1}). Water loss and integumentary resistance of *A. ernestwilliamsi* were similar to what would be predicted for *A. cristatellus* living in the same habitat. The Guana Island population of *A. cristatellus* was significantly different from all other populations. We believe two processes are responsible; phenotypic plasticity explains most of the observed variability, but genetic differentiation may be responsible for the distinction of lizards from Guana.

Key words: Anolis cristatellus; A. ernestwilliamsi; *evaporative water loss; habitat aridity; Sauria.*

MAINTAINING WATER BALANCE is critical for survival in all terrestrial organisms. Evaporative water loss (EWL) is a major avenue of water loss to the environment. In lizards and snakes, evaporation mainly occurs at the skin surface (Ec), rather than through the respiratory tract (Er). It is likely, therefore, that physiological adaptations leading to minimization of EWL (and especially Ec) might be important to survival in terrestrial biotopes. It is not surprising, therefore, that there is a documented correlation between habitat aridity and EWL in many species of reptiles (Bentley & Schmidt-Nielsen 1966, Mautz 1982). Often, scale size is also correlated to habitat aridity (reviewed in Malhotra & Thorpe 1991).

Little information exists regarding intraspecific variation in EWL. Previous studies (Hillman & Gorman 1977, Hertz *et al.* 1979, Hillman *et al.* 1979, Kobayashi *et al.* 1983, Kattan & Lillywhite 1989, Eynan & Dmi'el 1993) have often allowed lizards to acclimate to uniform conditions before testing them and only utilized a few populations in each species studied. Until recently (Kattan & Lillywhite 1989, Eynan & Dmi'el 1993), the resistance of the integument to water vapor, R, was not reported in intraspecific comparisons. This is especially important because R is relatively insensitive to short-term environmental variation (Eynan & Dmi'el 1993). To avoid confounding phylogenetic effects (Harvey & Pagel 1991) and to increase our understanding of the importance of within-species variation in physiological parameters, we chose to study different populations within the same species. Interspecific comparisons led us to hypothesize that environmental aridity would be reflected in lizard EWL. Specifically, we predicted that, as the environment becomes more arid, population-specific EWL will drop to compensate for the lower availability of water. Unlike previous studies, we wanted to test unacclimatized lizards, so that their EWL would reflect that in nature as closely as possible. Thus, we were interested in the total level of acclimatization of a lizard to its environment, rather than separating phenotypic plasticity from genetic factors.

Anolis cristatellus is an arboreal lizard common on many of the Greater Puerto Rico Bank islands. Hillman and Gorman (1977) studied two populations of *A. c. cristatellus* in Puerto Rico, and Hertz *et al.* (1979) examined an additional Puerto Rican population. There is a large amount of variation in environmental conditions on these islands, providing an ideal natural setup for our study. Because

[1] Received 23 January 1995; revision accepted 25 September 1995.

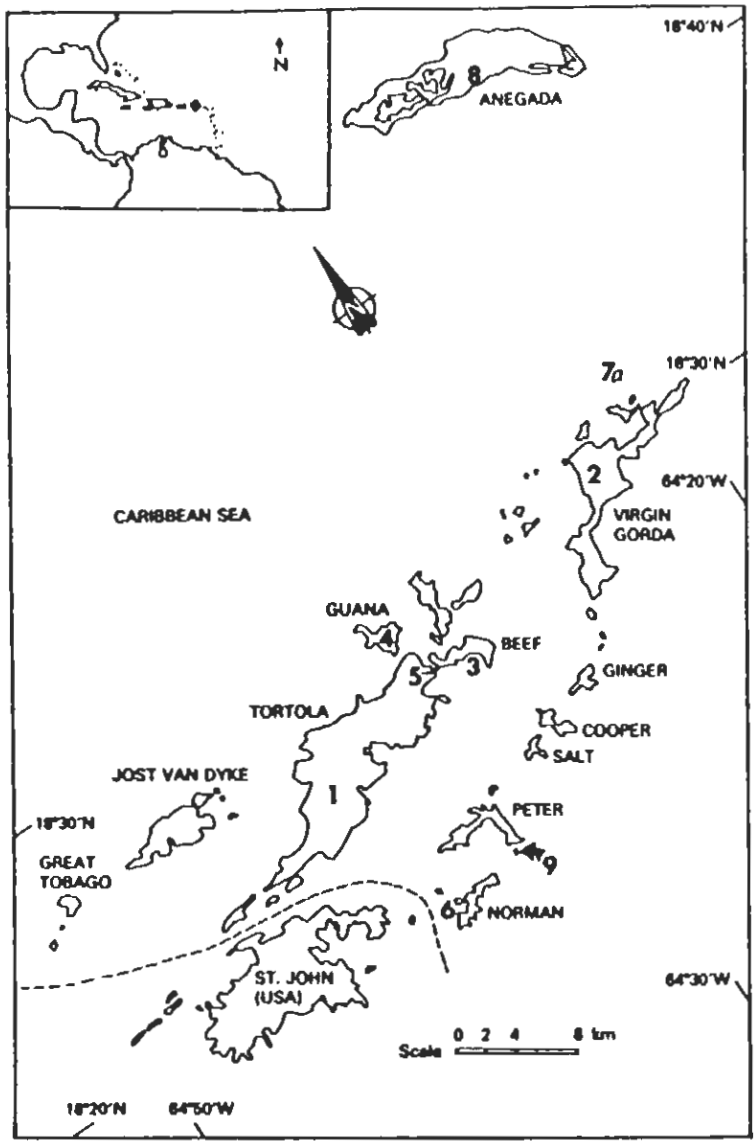

FIGURE 1. Map of the British Virgin Islands. Study populations of *Anolis cristatellus* and *A. ernestwilliamsi*. 1—Sage Mt., Tortola; 2—Virgin Gorda; 3—Beef Island; 4—Guana Island; 5—Bridge, Tortola; 6—Norman Island; 7—Necker Island; 8—Anegada; 9—Carrot Rock. Insert shows the position of this island group in the Caribbean.

the larger *A. ernestwilliamsi*, which is derived from and closely related to *A. cristatellus*, has considerably smaller scales (Lazell 1983), we thought a comparison of EWL between these two species would also be valuable. We therefore studied eight disjunct populations of *A. cristatellus* from seven islands in the British Virgin Islands (BVI; Fig. 1). Additionally, we studied *A. ernestwilliamsi*.

MATERIALS AND METHODS

We noosed lizards in the field during October 1993 and transported them to the laboratory on the same day. *A. cristatellus* was found in habitats ranging from the subtropical moist forest of Sage Mountain of Tortola (see Ewel & Whitmore 1973, for full description of this habitat type) to the

TABLE 1. *Locations, sample sizes (N), relative humidity at collection site (RH), and body dimensions of* Anolis cristatellus *from eight insular populations. Data from Carrot Rock are for A. ernestwilliamsi only. SD is one standard deviation of the mean.*

Location	N	RH (%)	Mass (g) Mean	Mass (g) SD	Body surface (cm^2) Mean	Body surface (cm^2) SD
Sage Mt., Tortola	6	79	5.94	0.46	40.2	2.5
Virgin Gorda	6	67	4.11	0.30	31.4	4.9
Bridge, Beef Isl.	6	70	7.67	1.14	47.9	4.0
Bridge, Tortola	6	70	7.85	1.77	45.8	6.6
Guana Isl.	12	63	7.63	1.96	49.3	4.0
Norman Isl.	6	67	5.27	0.80	42.5	4.9
Necker Isl.	6	67	8.27	1.46	55.3	6.5
Anegada	6	62	6.40	1.66	44.9	5.8
Carrot Rock	5	67	13.19	2.08	58.4	6.2

scrubby ridges typical of Guana Island. However, it was never encountered under a closed canopy. Our perception is that *A. cristatellus* usually takes shaded perches in all habitats but occasionally moves into the sun, especially in early morning or following rain showers. Patches of both sun and shade are ubiquitously available in all habitats in which we observed this species, and temperatures at collection sites varied from 27–33°C.

Average rainfall at Tortola ranged in the years 1960–1991 from 790–1840 mm/year and the average was 1200 mm/yr (A. Swain, pers. com.). As there are no rainfall records for most of the islands in the BVI, we used several methods to represent habitat aridity. Following Malhotra and Thorpe (1994), we repeatedly measured relative humidity (RH) at each site during the collection period (which coincided with animal activity times and the duration of laboratory experiments; see below). We used a portable electrical psychrometer (Psychro-Dyne, Environmental Tectonics Corporation) at the actual collection sites, thus measuring the conditions experienced by the animals at their chosen perches. Average RH values are given in Table 1. However, repeated measurements taken at Guana over the entire study period yielded daily RH values ranging from 60–90 percent, reflecting the differences between dry and rainy days; this is enough to place Guana at both the highest and lowest ends of the spectrum (Table 1). Similar fluctuations were noted at all sites. Unless they are taken over long periods, RH measurements thus represent highly transitory conditions and are misleading.

At both extreme habitats (Guana Island and Sage Mountain), we quantified microhabitat insolation (availability of sunlight at the perch site) at sites where lizards were perched. On each of 39 Sage Mountain and 46 Guana Island perch sites, we centered a 14 × 21.5 cm piece of paper. Shaded areas were marked on the paper and later cut out and weighed to determine the percentage of insolation in the immediate microhabitat of each lizard. At both sites, lizards had perched in total shade, full sun, and intermediate conditions. At Sage Mountain, insolation was 25 ± 34.6 percent (mean ± SD) and on Guana Island it was 17 ± 30.2 percent. The difference between the sites was not statistically significant ($t = 1.16$, $P = 0.25$, 2-sided t-test). Thus, we do not consider canopy cover a useful measure for determining habitat aridity.

In the absence of a quantitative measure of aridity we attempted to produce a qualitative one. Following Hillman *et al.* (1979), we mostly based our assessment on vegetation type and cover. Two of us (GP and JL) who have been to all the study sites independently ranked them on a scale of 1 (wet) to 10 (dry). The two ranking systems were highly similar. We then asked three additional biologists (all of whom have worked in the BVI for several years at least) to rank the same sites using the same scale; they were asked to use their total experience and take into account different seasons and times of day. We averaged all five values to produce a single index, which was also very similar to our original estimates. The averaged value of this aridity index for each site is presented in Table 2. We prefer this system because it represents a long-term estimate of habitat aridity, rather than the transitory one presented by RH measurements.

In the laboratory, animals were individually housed in plastic boxes (inner dimensions: 20 × 9 × 7 cm) covered with a small-mesh wire net that permitted free air exchange between the box and room atmosphere. Lizards were provided with moistened paper and were kept in the boxes without food for 8–12 h before the experiments commenced (Claussen 1967). A ceiling fan was used to continuously circulate room air and prevent the formation of temperatures and humidity gradients in and near lizard boxes. Data on sample size, body size and mass for each population are provided in Table 1.

On each lizard we performed two experiments that were carried out on two successive days. Before commencing experiments we moved lizards to dry boxes. During the first day, we measured total evaporative water loss (EWL). We used a Precisa balance (model 800M) to measure the mass change of the lizards to the nearest mg over a period of 6–8 hours.

TABLE 2. *Specific evaporative water loss (EWL, mg g^{-1} h^{-1}), cutaneous water loss (Ec, mg cm^{-2} h^{-1}) and integument resistance (R, s cm^{-1}) of* Anolis cristatellus *and* A. ernestwilliamsi. *Results of correlations between* A. cristatellus *physiological characteristics and island aridity indices are provided at the bottom, with 2-sided* P*-values given in parentheses. The aridity index goes from 1 (=wet) to 10 (=dry).*

Location	Aridity index	EWL		Ec		R	
		Mean	SD	Mean	SD	Mean	SD
Sage Mt., Tortola	1.0	5	1.2	0.5	0.1	59	14.3
Virgin Gorda	2.3	10	4.7	1	0.5	29	14.6
Beef Isl.	5.0	4	1.1	0.5	0.1	54	11.4
Bridge, Tortola	5.0	3	1.2	0.4	0.1	55	22.6
Guana Isl.	5.0	1	0.3	0.2	0.1	199	73.4
Norman Isl.	6.3	3	0.4	0.3	0.03	114	15.4
Necker Isl.	6.9	2	0.2	0.2	0.04	155	24.8
Anegada	8.3	3	0.2	0.2	0.05	128	27.5
Carrot Rock	9.3	1	0.4	0.2	0.07	168	71.3
r (Guana included)		−0.66	(0.04)	−0.71	(0.02)	0.57	(0.1)
r (without Guana)		−0.71	(0.04)	−0.77	(0.02)	0.78	(0.02)

At the same time we also measured room relative humidity (using a Psychro-Dyne psychrometer, Environmental Tectonics Corporation) and skin and room temperature (using 36-gauge copper-constantan thermocouples connected to a Wescor TH-65 electronic thermometer). All measurements were taken at 30 min intervals. Ambient RH in the immediate vicinity of experimental boxes was nearly constant at 75.8 ± 2.20 percent (average and SD); the temperature inside the box and in the room were identical (29.8 ± 0.61°C), and that of the skin was 29.7 ± 0.46°C. Animals which defecated during the study were re-weighed and the study restarted.

Measurements of respiratory (Er) and cutaneous (Ec) water loss were conducted on the following day. Using a polyethylene bag, we enclosed each lizard in a box with its head extruded. We assumed that any change in box mass (measured every 30 min for 5–6 hours) was due to loss (respiratory and cutaneous) from the head. By subtracting these results from those obtained from the same animal on the previous day we could isolate body Ec; dividing body Ec by surface area (see below) provided us with EWL per unit surface. We then multiplied this value by the surface area of the head and obtained head Ec. Er was then calculated as total loss from the head minus head Ec. R, the integument resistance, incorporates the resistances of the skin and of the air boundary layer surrounding it; we calculated it using the equation given by Lillywhite and Sanmartino (1993).

Because of the elasticity of reptile skin, removal and direct measurement of skin area (Claussen 1967) was deemed inadvisable. Unlike many other *Anolis*, however, *A. cristatellus* closely approximates an isosceles triangle in cross section, with the venter at its base and the sides of the body forming the two sides. We therefore calculated lizard body surface by pressing each lizard onto graph paper and tracing its ventral side and its flank. These tracings (which included both tail and limbs) were then cut out and weighed; finally, we used the mass of a 100 cm^2 piece of the same graph paper to calculate the surface area of each lizard.

Whenever possible, individuals were released at the end of the study at the site of capture. Other specimens were deposited in the Texas Memorial Museum at the University of Texas at Austin and in the Museum of Comparative Zoology at Harvard University.

RESULTS

We found statistically significant correlations between aridity and all physiological characteristics measured (Table 2). Lizards from Guana were consistently more resistant to water loss than expected from the island's aridity index. Because Guana values were well outside the 99 percent confidence limits, we present two sets of correlations in Table 2, one with Guana data included, the other with those data excluded. The correlations were statistically significant for all physiological indices ($P < 0.05$ in all cases) but not for mass ($P = 0.3$), and correlation coefficients were higher when Guana data were excluded. Values for *A. ernestwilliamsi* could be predicted with great accuracy from the relationship between aridity and water loss in *A. cristatellus* (Table 2, Fig. 2).

The relationship between surface area (S, cm^2)

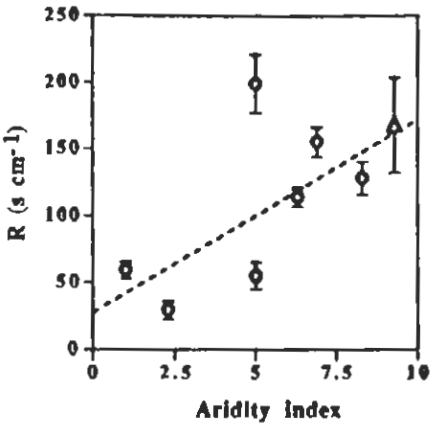

FIGURE 2. The relationship between habitat aridity and integument resistance in *Anolis cristatellus* (circles) and *A. ernestwilliamsi* (triangle). Guana is represented by an empty square. The regression line is based on data for *A. cristatellus* only.

and body mass (M, g) could be described by the equation $S = 16.59\ M^{0.53}$ ($r^2 = 0.69$). Cutaneous water loss comprised on average 75.3 percent (range 71.0–78.5 percent) of EWL. Differences between populations in the importance of Ec relative to that of Er were not statistically significant ($P > 0.05$, Kruskal-Wallis non-parametric ANOVA), nor were they correlated with habitat aridity.

DISCUSSION

Due to methodological differences, our EWL results may not be comparable to those of previous researchers. Our findings on *Anolis cristatellus* EWL and Ec are nonetheless similar to those reported by Claussen (1967) for *A. carolinensis* from Louisiana. *A. ernestwilliamsi* were nearly twice as large as the largest *A. cristatellus* we collected, and their total water loss values were the highest. Once corrected for size, however, *A. ernestwilliamsi* conforms in terms of water loss to the trend indicated by its parent species; its EWL values were very similar to what we would predict for *A. cristatellus* in the same environment.

The constancy of experimental conditions makes intraspecific comparisons within our sample highly informative. With the exception of lizards from Guana, *A. cristatellus* EWL was strongly negatively correlated, and R values were strongly positively correlated, with environmental aridity. This supports our initial prediction and agrees with the findings of studies on lizards in other locations (Hillman *et al.* 1979, Kattan & Lillywhite 1989, Eynan & Dmi'el 1993).

Our findings could be due to genetic differentiation between populations, but are also consistent with a phenotypic plasticity explanation (Hillman *et al.* 1979, Kobayashi *et al.* 1983). In the first case, we may be seeing the beginning of a multiple speciation event; if the second explanation is true, however, then no such event is indicated. Hertz *et al.* (1979) reported an EWL value of 8.3 mg/g/h from a Puerto Rican population of *A. cristatellus*, and our data suggest this is a reasonable value for a population dwelling in a moist habitat. Hillman and Gorman (1977), however, reported considerably lower values (1.9 and 1.8 mg/g/hr) in two other Puerto Rican populations, one of them geographically close to that studied by Hertz *et al.* (1979). Because lizards from virtually the same site were so different but lizards from two distant and climatically different sites were very similar, we suspect the disparity might be due to differences in methodology. This, and the findings of Hillman *et al.* (1979) and Kobayashi *et al.* (1983), support the phenotypic plasticity explanation for the differences we found between populations. The lack of significant differences in mass between islands also supports this interpretation.

The consistent deviation of Guana lizards from predicted values is difficult to explain. Though our evaluation of the island's aridity may have been erroneous, Guana was the only island for which all evaluators assigned the same rank, suggesting that this is unlikely. Nor is a biogeographic explanation plausible: no relation between geography and EWL was evident (Fig. 1). Why then are Guana lizards so different? We suspect that the Guana population may represent a diverging lineage, and that the basis for these differences might be genetic. If so, special attention should be paid to its conservation. With an underlying phenotypic plasticity enabling rapid adjustment to local conditions, selection is only likely to induce genetic differentiation under extreme conditions. This is not the case at Guana. Genetic drift appears to be a more likely explanation, especially when the small size of Guana (340 ha) is taken into account.

MacLean (1985) found scale size to be inversely correlated with water loss rates in the gekkonid lizard genus *Sphaerodactylus* in the BVI. Malhotra and Thorpe (1991) similarly found that *Anolis oculatus* inhabiting the wettest habitats in Dominica had the largest scales, whereas the subspecies that inhabits arid areas had the smallest. Lazell (1994) also found a good correspondence between scale size and habitat aridity in the *Sphaerodactylus vincenti* group in the Windward Islands, Lesser Antilles.

Thus, the very small scales of *A. ernestwilliamsi*, which inhabits the most arid islet in the group, relative to that of *A. cristatellus* (Lazell 1983) were highly suggestive. This led us to hypothesize that scale size might be related to EWL in the *A. cristatellus* complex. However, no significant differences in scale size have been found among populations of *A. cristatellus wileyae* in the BVI (Lazell 1983); this, and the similarity in EWL between *A. cristatellus* and *A. ernestwilliamsi*, suggest that scale size is not a good predictor of the resistance of lizard integument to water loss. Indeed, Malhotra and Thorpe (1991) noted some cases in which the correlation between scale size and aridity was opposite to their own findings. Kattan and Lillywhite (1989) have shown that lipids in the skin are responsible for reducing cutaneous EWL, and this remains the likely mechanism for the differences we observed between these populations. Sheer mass may provide all the additional resistance to water loss *A. ernestwilliamsi* requires for survival.

Though *A. cristatellus* is clearly able to modify its EWL to local environmental conditions, the exact mechanism remains unclear. Cross-fostering experiments, in which animals of different populations are maintained under similar conditions for prolonged periods, are required. However, we believe that two mechanisms are involved: genetic differentiation appears to be occurring on Guana Island, and phenotypic plasticity is apparent in the differences between all other populations.

ACKNOWLEDGMENTS

This project was funded by The Conservation Agency through a grant from the Falconwood Foundation. GP was financially assisted by a grant from the Institute of Latin American Studies at the University of Texas and by Sigma Xi. L. Jarecki, J. Overing and N. Goodyear assisted in producing the aridity index and K. LeVering and Ken Nagy critically read the manuscript. We thank H. Jarecki for access to Guana Island, R. Branson for permission to work on Necker Island, J. Conyers for assistance in collecting *A. ernestwilliamsi*, and R. Rusher and the entire Guana staff for technical assistance with various aspects of this study.

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[no p. 200!]

LIZARD NOTES IN PRESS, HERPETOLOGICAL REVIEW

ANOLIS CRISTATELLUS WILEYAE (Virgin Islands Crested Anole). FRUGIVORY. Anoles have long been known to eat fruit (Lazell 1972. Bull. Mus. Comp. Zool. 143: 22-74) but the frequency and enthusiasm with which they do so have not often been documented. On X October 1996 we watched an adult male crested anole consuming the sweet, magenta fruits of Melocactus intortus (Cactaceae)....

[Perry data in here]

On 11 October 1996 a pair of crested anoles worked a patch of Trichostigma octandra (Phytolaccaceae) profusely in fruit with crimson berries. Access seemed to be a problem; although the anoles could easily have ridden these small, semi-vining herbs down, this apparently did not occur to them. The fruits were about 50cm above the ground in clusters. The female anole climbed down a small vine to consume as many berries as she could reach. The male (now Museum of Comparative Zoology 182075) attacked the berries from the edge of a large boulder. He ate all the berries he could reach, even to the extent of lunging out, loosing his forefeet, and sprawling forward, retaining a grip on the rock only with his rear feet. When captured, this individual defecated Trichostigma seeds and skins in a red matrix. On dissection, his entire gut was packed with fruit. We are indebted to Dr. George Proctor, Puerto Rico Department of Natural Resources, and Dr. Fred Kraus, Hawaii Division of Forestry and Wildlife, for identifying the plants.

Submitted by JAMES LAZELL and GAD PERRY, The Conservation Agency, 6 Swinburne St., Jamestown, RI 02835

ANOLIS STRATULUS (Saddled Anole). NECTIVORY. Nectar feeding has been recorded in anoles (Liner 1996. Herpetol. Rev. 27: 78), but we believe it is far more commonplace and widespread than published records indicate. On X October and adult male saddled anole.....

[Perry data in here]

Nectar droplets form on the top of the coral-red flowers of Pedilanthus tithymaloides (Euphorbiaceae) and are sweet to the taste. We are indebted to Dr. Richard Howard, Gray Herbarium, Harvard, for confirmation of the plant's identity.

Submitted by GAD PERRY and JAMES LAZELL, The Conservation Agency, 6 Swinburne St., Jamestown, RI 02835, U.S.A.

16.xi.96

One more, based on 1995 data, in press in Biotropica.

Subj: Re: 1996 data
Date: 96-11-14 10:34:22 EST
From: 72370.1210@CompuServe.COM (Gad Perry)
To: razdmiel@post.tau.ac.il ("razi dmi'el")
CC: jcinjtown@aol.com (Skip)

Gents,

I've now graphed all the R data for all 1996 lizards. As you said, there is no change in average population values with time. That is consistent with mortality being the main factor in the cahnges we saw before. On the other hand, there is a lot of individual variation. The same individuals have huge changes, and that is consistent with phenotypic responses. As in the past, Guana's line is highest, and Sage Mt's is lowest. I haven't run any stats yet, but from the graphs it looks like Sage and Necker ought to be different, whereas Necker and Guana have a lot of overlap and may not.

Variation is very low in Sage Mt lizards, very high in Guana, and intermediate in Necker lizards. That makes me think of Razi's note on the feeding habits of the beasts, and whether that had anything to do with it. I will try to plot delta-R versus delta-mass to see if there is a pattern there.

I am a bit concerned that R may not be as good at removing effects of driving force (especially RH) as it is supposed to be. This comes from a note from Skip saying you saw that R was higher on dry days. I do not have RH data on the sheets I got, and would appreciate getting those data so I could try and see if there is something to this.

As for Table 3 for the previous MS - Razi, I'd appreciate it as soon as you can get it to me, because I am anxious to get that MS out of the way ASAP. Obviously the GIF report comes first, but please do this as soon after as you can.

A general comment: this is probably premature - data analysis is still far from over, after all - but I am thinking we may need to repeat some of this work next year - if there is a next year that is.

Gad

TERRITORIAL INTERACTIONS BETWEEN RESIDENT AND NONRESIDENT MALE *Anolis cristatellus* LIZARDS ON GUANA ISLAND, B.V.I.

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INTRODUCTION

Intraspecific territoriality in reptiles is often based upon competition for food and mates (Stamps 1977, Myers 1981, Schoener 1982). A territory is defined as an exclusive area that is defended to keep out rivals (Krebs & Davies 1978). Previous research has demonstrated that female *Anolis* lizards typically defend territories to protect food resources while males protect mating territories (Stamps 1977). In October, 1966, I studied intraspecific competition for territories among *Anolis cristatellus* males on Guana Island, B.V.I.

Anolis cristatellus lizards are sexually dimorphic, both in body size (males are larger than females) and in the presence of a male tail crest that is held permanently erect and extends the entire length of the tail. The tail may grow back if it is lost, but the crest will never regenerate. It is therefore possible for a large male to have a reduced or absent tail crest. Experienced herpetologists working on related species on Guana Island predicted that crest size would not correlate with competitive superiority in territorial interactions between male lizards (personal communication, G. Perry, J. Lazell). In other species of *Anolis*, the incumbent male is commonly the winner in a forced territorial interaction (Stamps 1977). My hypothesis was that resident males would dominate interactions with an intruding male, with outcomes independent of the relative length of their tail crest.

MATERIALS AND METHODS

Dr. Gad Perry shared with me his detailed maps of the territories of marked *Anolis cristatellus* male lizards near the hotel compound on Guana Island. These maps had been updated in the two weeks prior to my arrival. The interaction experiments that I conducted involved introducing a tethered, nonresident male lizard into the territory of an undisturbed resident *A. cristatellus*. Dr. Perry's data provided identifiable territories associated with previously measured, marked (by toe clips and nail polish) male lizards. I designated those marked lizards as the "residents". Outside of the study area I collected unmarked male lizards to be the introduced "nonresidents". All nonresident lizards were measured for body size and tail crest length. The lizards were held in

individual mesh bags until the time of the interaction experiment. Individual nonresident and resident lizards were used in only a single interaction.

I used a fishing pole-like apparatus to lower each nonresident lizard into a resident's territory. The pole was a long stick that had a monofilament line threaded through a series of tape loops down its length, with the line wound onto a spool at the base. The loose end of the line was tied in a slip knot around a lizard's torso. After the lizard was placed into the territory it was given sufficient line slack to move about at will. Resident lizards were left free and undisturbed.

Immediately after the introduced lizard entered the territory I observed and recorded behaviors of both the resident and nonresident males, noting the time at which each behavior occurred. The introduced lizard was removed as soon as a clear "winner" was determined. I defined the "winner" of an interaction as the individual that demonstrated dominance in one of the following ways. If the lizards fought with physical contact and biting, the winner either chased away the loser, or the loser voluntarily retreated. If there was no physical fight, dominance was associated with behaviors such as an erect stance, the appearance of coloration contrasting with the substrate, or raised erectile tissue along the neck and back of the animal. Submissive behaviors associated with losers included a prone posture flat against the substrate, shut eyes, cryptic coloration, and lowered erectile tissue.

RESULTS

The data obtained from twelve interactions demonstrate that resident males do not always dominate when a nonresident lizard is placed in their territory. In four cases the resident lizard "won", in four cases the introduced lizard "won", and in three cases there was no clear winner. Based on these observations, the hypothesis was incorrect.

Crest length of resident and nonresident individuals, however, did correlate with outcome of territorial interactions (Figure 1). Crest length of the introduced male was always larger than that of the losing resident in all cases in which the nonresident won. When the resident won, it had the longer crest in three of four cases. In the ambiguous interactions, both individuals had crest

lengths of approximately equal size. The diagonal line in Figure 1 represents the 1 : 1 ratio of crest lengths between introduced and resident male lizards. Points above the line indicate a larger crest on the nonresident male; data points that fall below the line represent cases in which residents had larger crests. A residual analysis of these data demonstrates that points representing the introduced winners fall significantly above the 1:1 line, while the group of resident winners fall along the 1:1 line. This suggests that introduced lizards must have large tail crests to win in territorial disputes with residents.

Another parameter measured in these experiments was the elapsed time before the interaction was resolved (Figure 2). When a resident lizard won an interaction, it always took more than twenty minutes to resolve. There was extensive posturing and pausing before a fight. However, when an introduced lizard won the interaction, resolution was prompt. In the longest such case it took six minutes until a clear winner could be determined; in the others resolution occurred within two minutes (compared with over an hour for three of the trials in which the resident dominated.

DISCUSSION

The results of my 12 experiments show that residency alone is not an accurate predictor of dominance in territorial interactions between resident and introduced male *Anolis cristatellus* on Guana Island. If the tail crest length of nonresidents is greater than that of the resident male then the introduced male has a high chance of dominating in the encounter. Interactions between two lizards that have long and relatively equal tail crest lengths often result in ambiguous outcomes. The elapsed time until resolution of the interaction was consistently shot (6 minutes or less) when the nonresident lizard won; and significantly longer (11 - >60 minutes) when the resident won.

Similar patterns correlating behavioral dominance and body / crest size have been shown in other species of *Anolis*. For example, aggressive responses of *A. aeneus* males are greatest when the body size ratio of the two fighting lizards are approximately equal (Stamps 1977). Fox *et al.* (1981) suggests that dominance need not involve aggressive action, but may be relayed through the intimidation of body size alone.

During the course of my field experiments there were occasional confounding elements to the trials. A third male appeared in two cases. Occasionally a smaller subordinate male occupies the same territory as a larger, dominate male, but the smaller males typically have little reproductive success (Stamps & Crews 1976). Females also appeared during the experiments, creating distractions for both lizards. Sometimes the resident would turn away from the introduced male to follow the female, and occasionally her presence seemed to catalyze the fight. Females also postured at introduced males. Females of other species of *Anolis* "multibob", a display indicative of fear , performed when she is courted by a new, intruding male (Stamps 1977).

It is important to state that although this type of interaction does occur in nature, any manipulation of an animal may affect its behavior. The process of catching the nonresident males, then keeping them in a bag for several hours may have been traumatic, and affected their behavior when placed into the territory of an undisturbed resident. Further replicates are needed to better understand factors influencing the resolution of territorial interactions between male *A. cristatellus* in the field.

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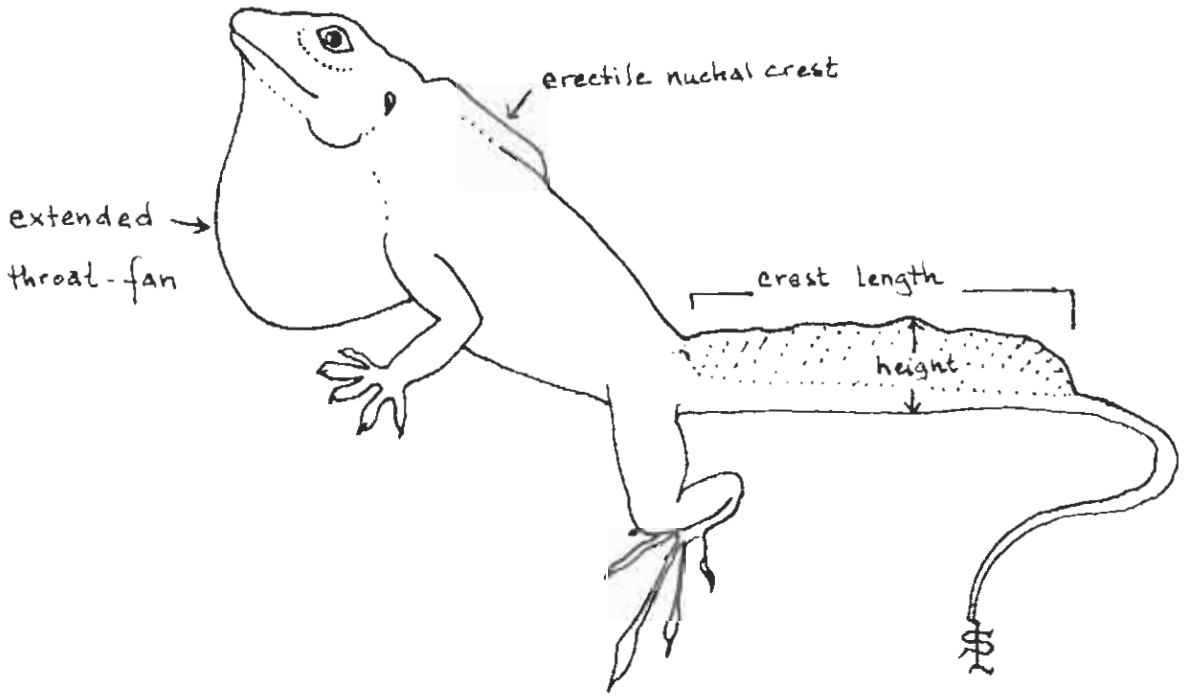


Adult male Anolis cristatellus in full display

Table 1

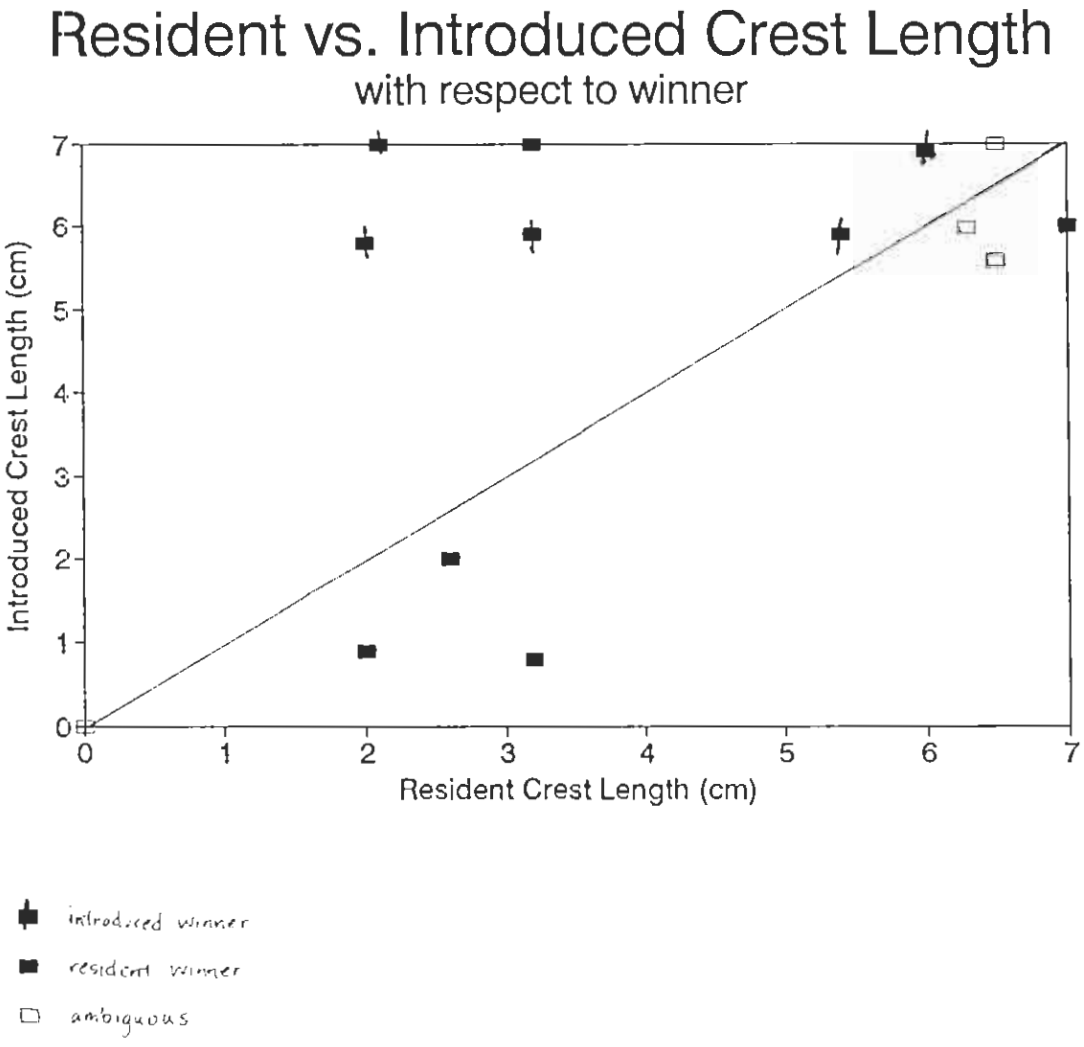
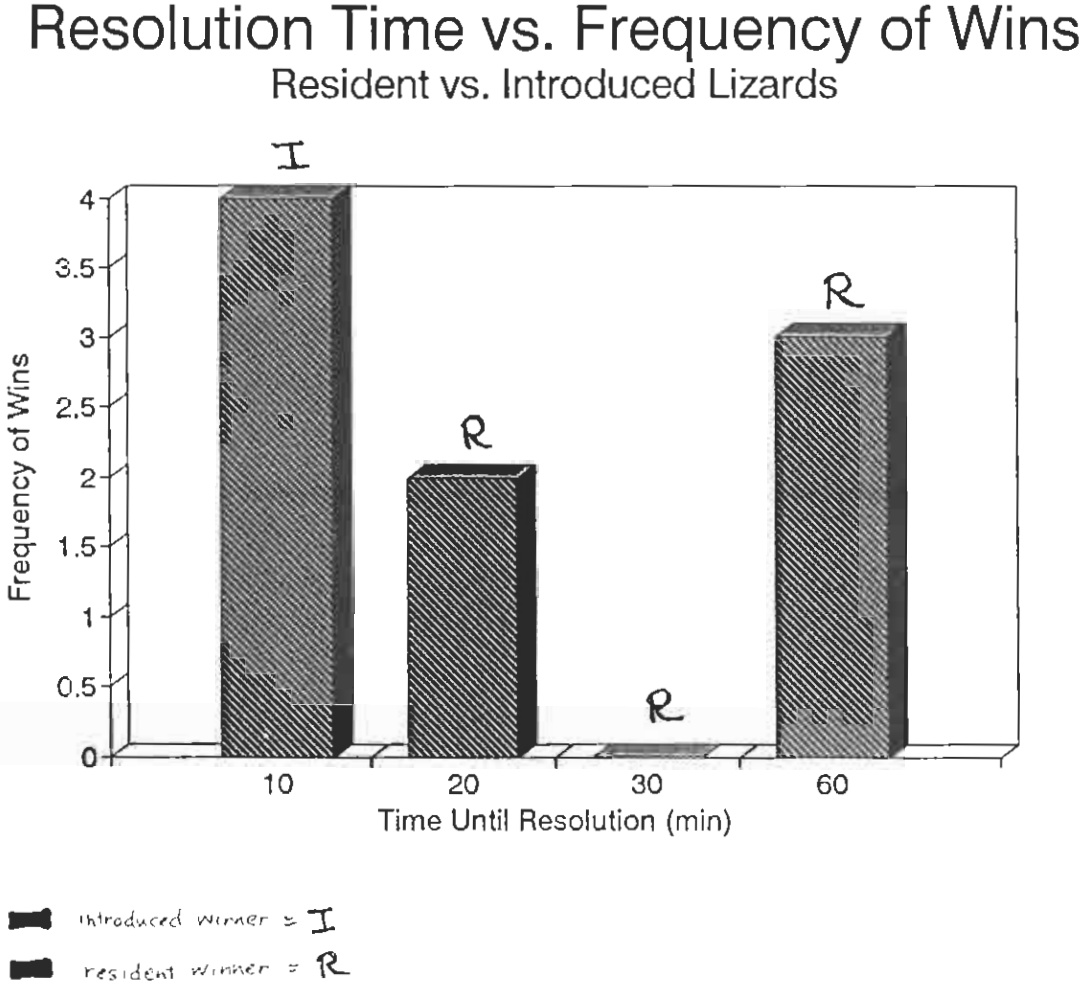

Resident vs. Introduced Crest Length
with respect to winner
Introduced Crest Length (cm)
Resident Crest Length (cm)
0
1
2
3
4
5
6
7
introduced winner
resident winner
ambiguous

Table 2


Resolution Time vs. Frequency of Wins
Resident vs. Introduced Lizards
I
R
R
R
Frequency of Wins
0
0.5
1
1.5
2
2.5
3
3.5
4
10
20
30
60
Time Until Resolution (min)
Introduced Winner = I
Resident Winner = R

Journal of Herpetology, Vol. 31, No. 1, pp. 149–155, 1997

Courtship Behavior and Vocalizations of the Frogs *Eleutherodactylus antillensis* and *E. cochranae* on the British Virgin Islands

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In addition to an advertisement call that may function both in mate attraction and intermale spacing, male frogs of many species produce a variety of other calls that are used in short-range encounters with females and other males (Wells, 1977a, b, 1988; Gerhardt, 1994). Vocalizations used during courtship are poorly documented for most species, although they may play an important role in the courtship of particularly those species in which the male leads the female to a nest site.

Frogs of the genus *Eleutherodactylus* form a dominant component of terrestrial anuran communities in many neotropical areas (Miyamoto, 1982; Drewry and Rand, 1983; Lynch and Ruiz-Carranza, 1985). The few species examined in detail show a diversity of vocalizations, including calls associated with the defense of retreat sites (Stewart and Rand, 1991, 1992) and with courtship and mating (Townsend and Stewart, 1986; Michael, 1996). Based on observations in captivity, Michael (1996) described courtship calls that differed in structure from the advertisement calls for three species of *Eleutherodactylus* (*coqui*, *cochranae*, and *antillensis*) and noted that their courtship calls were more similar than their advertisement calls. The observations were based on courtships by only two male *E. cochranae* and one male *E. antillensis* (in interspecific courtship with female *E. cochranae*), precluding detailed analysis of the calls.

We describe the behavior and vocalizations by *E. antillensis* and *E. cochranae* during courtships under natural conditions on the British Virgin Islands. The two species are closely related based on electrophoretic analysis (subgenus *Eleutherodactylus*, *auriculatus* section; Hedges, 1989). Our observations complement those recorded in captivity (Michael, 1996) and provide a context for the vocalizations.

We observed courtships of *E. antillensis* on 10 occasions in October 1993 and 1994 on Guana Island, British Virgin Islands, and recorded calls of males on audio-tape in six of these cases. The microphone was approximately 20–80 cm from the male, and light from a headlamp directed away from the frogs provided illumination for observations. We compared calls emitted during courtship to advertisement calls of 10 male *E. antillensis* recorded on Guana Island in 1994 as a part of a study on call variation among islands. In 1993, we used a Panasonic® tape-recorder with a built-in microphone (RQ-320), whereas in 1994 we used a portable JVC® cassette recorder (CD-1636) and a Sony Professional Walkman® (WM-D6C) fitted with unidirectional Audiotechnica® shotgun microphones.

We analyzed all calls on a DSP Sona-Graph® Model 5500 (Kay Electronics). We randomly selected five long (≥5 notes) and five short (≤5 notes) courtship calls emitted by each *E. antillensis* male during three stages of the courtship for detailed analysis. The stages, which corresponded to the sequential progress of the courtship, were (1) male and female in vegetation or on the ground, (2) male under leaf litter, and (3) both male and female under leaf litter. For comparisons, we also selected 10 advertisement calls from the second minute of each 5 min recording of vocalizations of males that had not been approached by females. We measured the dominant frequency of each note, time from the start of one note to start of subsequent note (note period), and total duration for each call, and calculated the average for each individual male. We applied Bonferroni's correction when using the same set of data in two analyses (Snedecor and Cochran, 1980). As a result, α was set at 0.025 when comparing characteristics of the advertisement and courtship call.

In October 1995, we observed a courtship of *E. cochranae* on Tortola, British Virgin Islands, and recorded

vocalizations using a portable cassette recorder (Sony Professional Walkman®, WM-D6C) with Electret® (SME V-6502) unidirectional condenser microphone. For analysis, we randomly selected five courtship calls before and after the pair disappeared into the presumed nest site. We compared these calls to advertisement calls emitted by five other males from the same site in the absence of females based on five randomly selected calls/male.

We observed courtships of *E. antillensis* on 11, 21, 22, and 23 October in 1993 and on 8 and 17 October in 1994. The courtships began between 2100 h and 1010 h, usually after a heavy rain. On the night of 17–18 October 1994, following a heavy downpour after 10 d with no rainfall, we noted six courtships (but obtained detailed observations for only two of these simultaneous events). In contrast, searches on eight nights between 7 and 20 October 1994 in the same area resulted in the location of only one courtship. The observations lasted from 20–130 min and were terminated when the pair either entered a presumed nest site under leaf litter or was otherwise lost from sight.

In the absence of females, male *E. antillensis* emitted two-note advertisement calls (Drewry and Rand, 1983) and occasional "trill" calls (series of repeated notes) that were interspersed among the advertisement calls (Drewry, 1970). In nine of 10 courtships of *E. antillensis*, a female approached a male that was emitting these calls at an elevated site in the vegetation. The female hopped close to the male, sometimes landing on top of him. The male immediately moved downward, usually approximately 50 cm, and changed his call from the advertisement call to a multi-note courtship call (Fig. 1). The process was repeated until the pair reached the ground (N = 7; in the remaining cases, the frogs were disturbed by the observers or were already on the ground). On the ground, the male moved slowly, emitting series of courtship calls, and the female crawled closely behind him as if in slow motion. The male then moved under the leaf litter, followed by the female. The male continued to emit multi-note calls.

In addition to courtship calls, male *E. antillensis* continued to produce advertisement calls and trill calls throughout the courtship. The male typically emitted a courtship call immediately after the female moved towards him, but reverted to the advertisement call if the female did not move or was lagging far behind. The courtship call seemed much softer than the advertisement call, but we did not measure sound pressure levels.

Twice, we observed two gravid females orienting towards the same male *E. antillensis* in early courtship. In both cases, the second female left after the other began to follow the male. Once, a second, silent male joined in a courtship, moving behind a female that was following a courting male on the ground.

We located a globular egg-mass at the site where a pair of courting *E. antillensis* had disappeared under the leaf litter on two occasions (23 October 1993 and 17 October 1994). The distance from the original calling site of the male to the nest was 450 cm and 580 cm for the two observations. By raking through the leaf litter, we found a third egg mass on 20 October 1994. All egg-masses were about 2–3 cm under the leaf litter in an open (i.e., not directly under plants) moist location in the forest. We examined the nest site on 17 October 1994 about 4 h after the courting pair had disappeared under the leaf litter. Both the male and female were by a newly-laid egg mass. No frog attended the other two egg masses or this nest on checks during subsequent days.

The number of notes in the courtship calls emitted by male *E. antillensis* during courtship varied from 1 to 22 (Fig. 2). Two-note advertisement calls and trill calls, also emitted by males during courtship, could be distinguished from the courtship calls by their dominant frequency and are not included in Fig. 2. The mean number of notes/call tended to increase as the courtship progressed from when the pair was in vegetation ($\bar{x}$ = 3.8, SD = 1.5, N = 94 calls by five frogs) to when the male or both frogs were on the ground ($\bar{x}$ = 5.7, SD = 2.2, N = 296 calls by six frogs) and to when the male ($\bar{x}$ = 9.0, SD = 2.6, N = 70 calls by two frogs) or both the male and female ($\bar{x}$ = 8.7, SD = 1.8, N = 30 calls by two frogs) were under the leaf litter. However, both short and long calls occurred at all stages of courtship (Fig. 2).

About 80% of the courtship calls by *E. antillensis* (397 of 489 calls by six males) began with two introductory notes. These were very similar to the notes in the two-note advertisement call both in the dominant frequency (Students t-test: $t = -0.338$, $P = 0.741$ for comparisons of 1st notes, $t = -0.381$, $P = 0.709$ for comparisons of 2nd notes) and the note period ($t = 1.158$, $P = 0.266$; Table 1). In early courtship (before the pair disappeared under leaf litter), the third and subsequent notes of the courtship call resembled the first note of the advertisement call but were somewhat higher in frequency ($t = 3.09$, $P = 0.008$; Table 1) and often showed a downward frequency sweep (Fig. 1). The mean note period between the remaining notes in the courtship call was longer than that between the notes in the advertisement call ($t = 4.778$, $P \leq 0.001$).

The dominant frequency of the courtship calls of each of the two *E. antillensis* males that we recorded both in early and late courtship changed when the male or both the male and female were under the leaf litter in the presumed nesting site (Fig. 1). The dominant frequency of the first note of the call was similar to that earlier in courtship (Frog 1: $t = -1.06$, $P = 0.30$; Frog 2: $t = 0.85$, $P = 0.42$), whereas the second note was lower (Frog 1: $t = 5.02$, $P < 0.001$; Frog 2: $t = 4.34$, $P = 0.002$) and the repeated set of notes was higher (Frog 1: $t = -9.17$, $P < 0.001$; Frog 2: $t = -2.58$, $P = 0.021$) than the respective notes in the call when the pair was above ground (Student's t-test for unpaired observations; each call treated as an independent observation for the purpose of analysis). A comparison of the mean dominant frequencies of the calls of these two males in late courtship to that of all six males in early courtship suggests a similar pattern (Table 1). The frogs were not visible at this stage, and the behavior associated with the vocalizations is unknown.

On 7 October 1995 at 2345 h, we observed a courtship of *E. cochranae* in Sage Mountain National Park on Tortola, British Virgin Islands. When first sighted, the male was emitting a typical one-note advertisement call (Drewry and Rand, 1983) from a crack in a small, dead tree 170 cm above the ground. The male emerged from the crack, facing towards a plump fe-

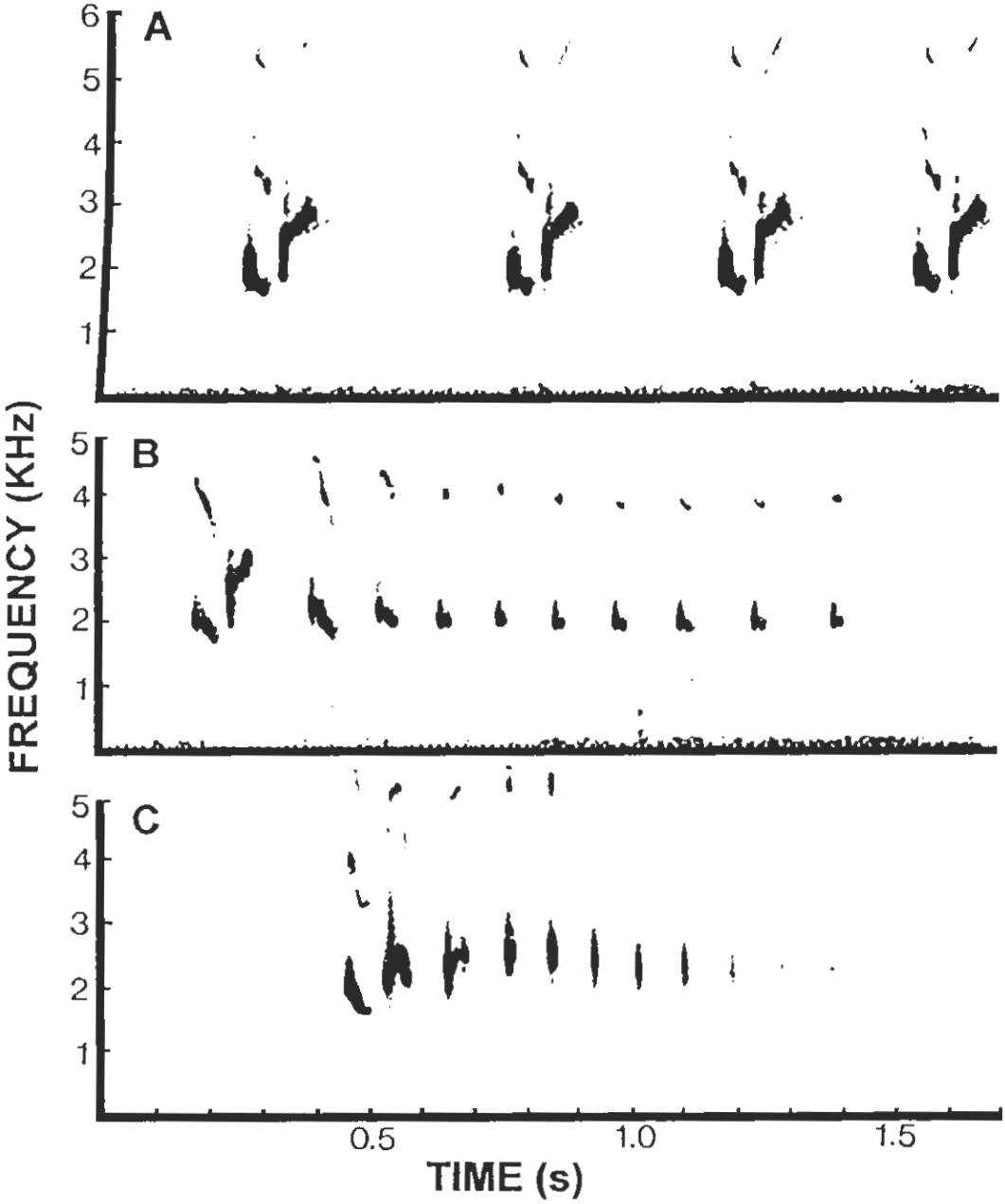

FIG. 1. Sonagrams of advertisement and courtship calls of male *Eleutherodactylus antillensis* recorded under natural conditions on Guana Island, British Virgin Islands. A—advertisement call, B—courtship call in early courtship when the frogs were above ground, C—courtship call in late courtship when the male or both frogs were under leaf litter.

male who was climbing up the trunk. The female approached the male, and the pair then disappeared into the crack with the male leading the female. The vocalizations of the male consisted of multi-note calls that showed a downward frequency sweep (Fig. 3). The mean number of notes in these calls was 3.3 ± 3.4 (range: 1–24; N = 126 calls; Fig. 4). All calls emitted by the male in the presence of the female were included in Fig. 4, as we could not distinguish possible one-note courtship calls from the one-note advertisement call by their dominant frequency. Most calls began with a long introductory note that resembled the advertisement call. The dominant frequency of this introductory note varied from 3270 Hz to 4200 Hz ($\bar{x}$ -

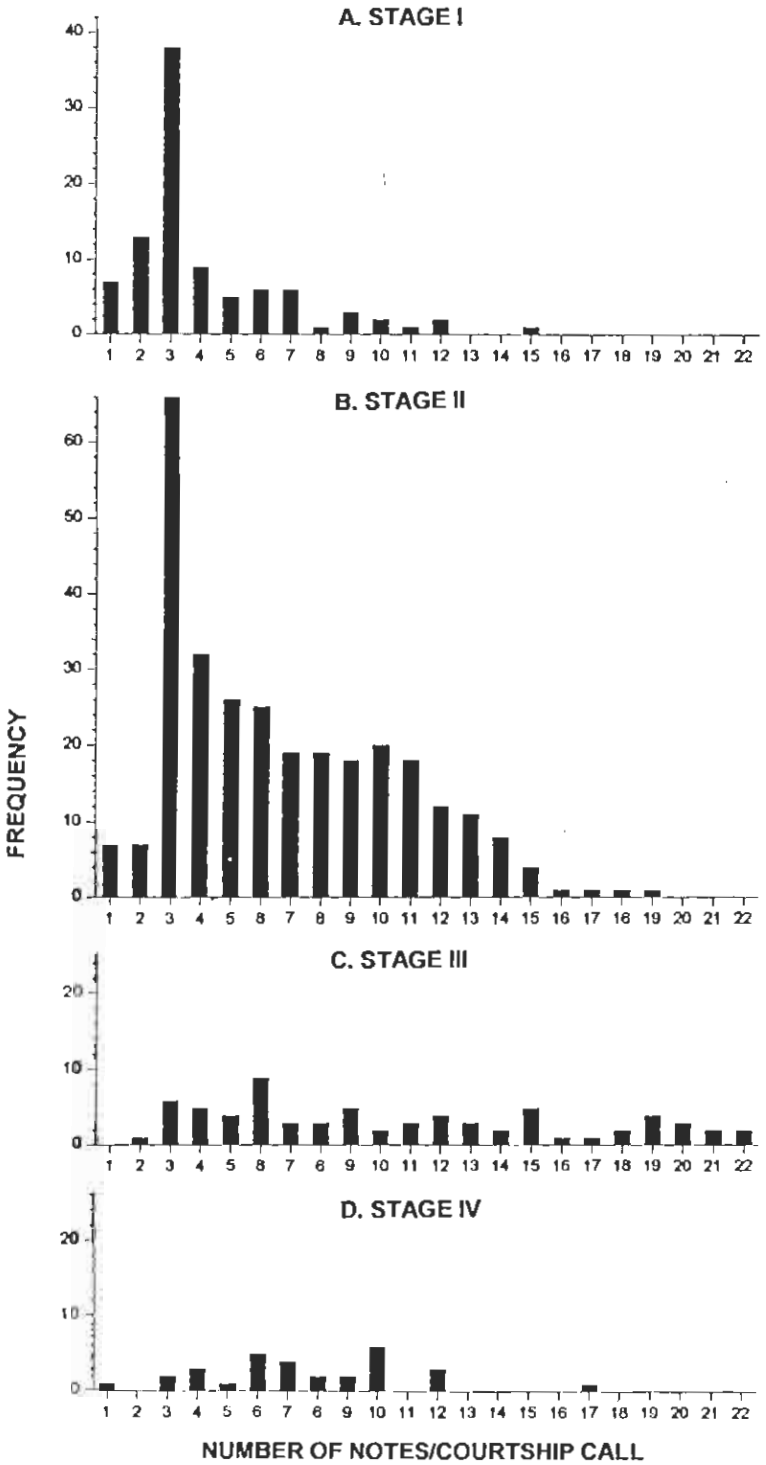

FIG. 2. Number of notes per courtship call of males during different stages of the courtship of *Eleutherodactylus antillensis*. Stages I–IV represent the temporal progress of the courtship. Stage I—male and female in vegetation, N = 94 calls by five males; Stage II—male, or male and female on ground, N = 296 calls by six males; Stage III—male under leaf letter, N = 70 calls by two males; Stage IV—male and female under leaf litter, N = 30 calls by two males.

TABLE 1. Dominant frequency and timing of notes in calls of *Eleutherodactylus antillensis* in the absence and presence of a receptive female. Note1—1st note of advertisement call or introductory to courtship call, Note2—2nd note of advertisement call or introductory to courtship call, Set–remaining, repeated notes in courtship call. The note period is the time from the start of one note to the start of the subsequent note.

Context	Dominant frequency x̄(SD), Hz			Note period x̄(SD), s			No. frogs (N)	No. calls
	Note1	Note2	Set	Note1	Note2	Set		
Advertisement call in the absence of female	2026 (99)	2827 (101)	—	0.066 (0.004)	—	—	10	50
Early courtship (male and female above surface)	2010 (92)	2805 (131)	2174 (80)	0.069 (0.004)	0.190 (0.025)	0.160 (0.064)	6	53
Late courtship (male or pair under leaf litter)	1960	2398	2395	0.095	0.122	0.171	2	33

3690 Hz, SD = 392 Hz, N = 10 calls; mean dominant frequency of advertisement calls of five other males = 3864 Hz, SD = 139 Hz). The remaining notes of the courtship call were shorter and had a lower dominant frequency (x̄ = 3249 Hz, SD = 177 Hz) than the first note (Student's t-test for paired samples: t = 3.64, P = 0.005, N = 10). Seven of 126 calls, all at the end of the courtship when the pair was in tree cavity, lacked an introductory note. The male continued to emit these vocalizations sporadically throughout the evening while the pair was in the cavity.

The following evening, the heads of two frogs, a small one on top of a larger one, protruded from the cavity, suggesting that the frogs were still in amplexus. On 14 October, the head of one frog was visible in the cavity, but no frog was in attendance the following night.

In the presence of receptive females, males of *E. antillensis* and *E. cochranae* emitted courtship calls that differed both in the timing and frequency from the advertisement calls of the species. These calls were similar in basic structure to the courtship calls of the two species recorded in captivity (Michael, 1996), although differences in details existed. The calls of both species were longer than those in captivity (maximum number of notes/call = 22–24 versus 10 as reported by Michael), and most calls began with introductory notes that resembled the advertisement calls of the species. The presence of these introductory notes may be important in multi-species assemblages under

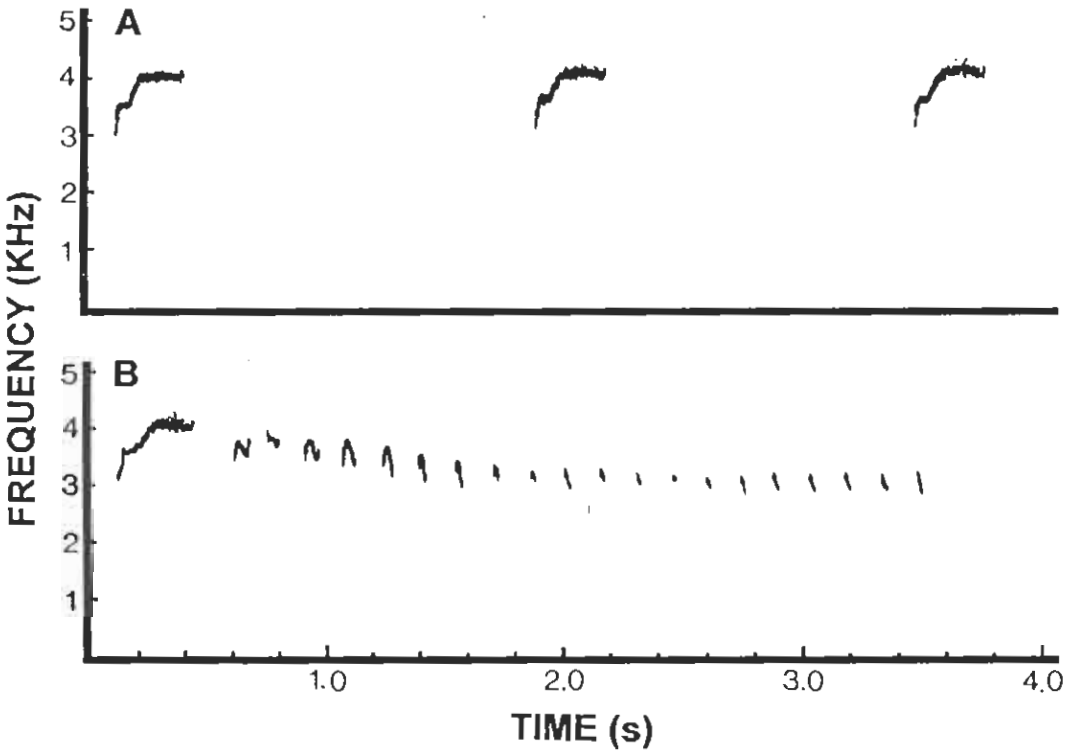

FIG. 3. Sonagrams of advertisement and courtship calls of male *Eleutherodactylus cochranae* recorded under natural conditions on Tortola, British Virgin Islands. A—advertisement call, B—courtship call.

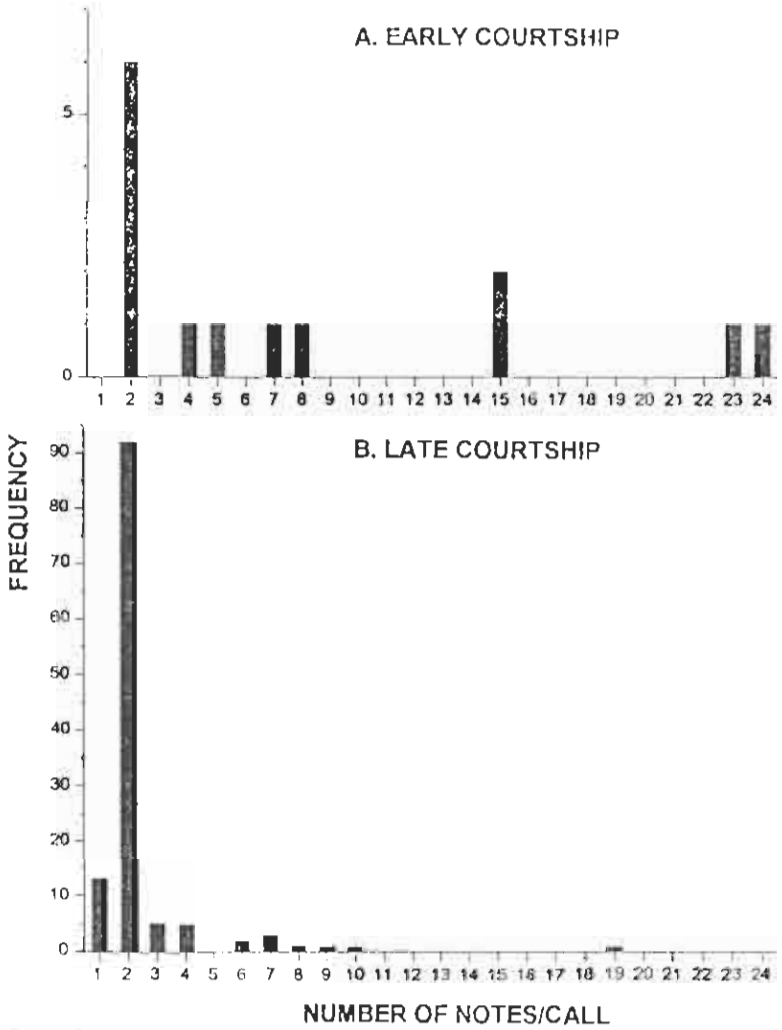

FIG. 4. Number of notes per call emitted by one male *Eleutherodactylus cochranae* before (A) and after (B) the courting pair disappeared into the presumed nesting cavity. A—N = 14 calls, B—N = 111 calls.

natural conditions if they encode information on species identity, as advertisement calls of many species are known to do (Littlejohn, 1981).

Our observations also suggest that the dominant frequency of the courtship call of male *E. antillensis* changed when the male or the pair was under the leaf litter in the presumed nesting cavity. The rise in the dominant frequency in the latter stages of the courtship may be an artefact of recording them through the leaf litter or from the vocal sac pressing against the surrounding leaves. However, these factors are unlikely to fully explain the observed shift because (a) the leaves would be expected to filter out higher, not lower dominant frequencies, and (b) the first introductory note of the courtship call, which had a similar dominant frequency as the repeated notes when the frogs were above ground, remained unchanged after the male burrowed into the leaf litter. The call males emitted under leaves appears very similar in structure to the retreat/nest defence call of *E. coqui* (Stewart and Rand, 1991). We suggest that the male shifts from a call that elicits a female to follow him to a call that may contain information on his subsequent ability to defend the nest.

Although males of many other species increase their calling rate or modify their advertisement call to some degree when approached by a female (reviewed in Wells, 1977a, 1988), sonagrams of structurally distinct courtship calls have been published for only a few species. These include several species of burrowing frogs of the genus *Pseudophryne* that deposit their eggs in terrestrial nest sites (Pengilley, 1971; Woodruff 1976), and *Centrolenella fleischmanni*, also a species with non-aquatic nesting habits (Greer and Wells, 1980). Distinct courtship calls also occur among dendrobatid frogs, including *Colostethus trinitatis* (Wells, 1980, 1988). Townsend and Stewart (1986) described the call of male *E. coqui* during courtship as being noticeably softer than the advertisement call but did not report that these calls were structurally different from the ad-

vertisement call. In captivity, *E. coqui* emitted multi-note calls of relatively low dominant frequency when compared to the two-note advertisement call of the species (Michael, 1996). We suspect that courtship calls are common, if not ubiquitous, among *Eleutherodactylus* and perhaps also among other terrestrially-breeding species in which the male leads the female to a nesting site. If multi-note calls are attractive to females, then the low number of notes in the advertisement calls of *E. antillensis*, *E. cochranae*, and *E. coqui* is puzzling, as those males that added notes to their calls could potentially enhance their ability to attract mates. Experimental studies of the relative responses of females and males to the coortship and advertisement calls may prove a fruitful field for investigating the constraints and driving forces that have shaped the evolution of vocal communication in these frogs.

Acknowledgments.—We thank the Conservation Agency (Jamestown, R. I.) and the Falconwood Foundation for their support of the study. Smithsonian Tropical Research Institute provided additional support in the form of a Short Term Visitor's Award to K. Ovaska. Renewable Resources Consulting Services Ltd. generously allowed us to use their office facilities. We are grateful to Skip Lizell who invited us to study frogs on Guana Island and whose continuing support made this study possible. We also thank Stan Rand, who advised us on sound analysis, let us use his sound equipment, and shared his observations and ideas on *Eleutherodactylus* vocalizations with us. He also provided useful comments on an earlier version of this paper. We are grateful to Scott Michael, who generously provided us with copies of his unpublished manuscripts.

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Accepted: 29 October 1996.

Journal of Herpetology, Vol. 31, No. 1, pp. 155–161, 1997

In Press in Animal Behavior, as of Sept '96

Vocal behaviour of the frog Eleutherodactylus antillensis on the British Virgin Islands

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Abstract. The vocal repertoire of male Eleutherodactylus and the communicative significance of two calls (the trill and courtship call) were investigated under natural conditions on the British Virgin Islands. Interspersed with two-note advertisement calls, males emitted trill calls, which typically consisted of an introductory note followed by 5-13 rapidly repeated notes. The introductory note and the repeated notes were within the range of the dominant frequencies of the first and second note of the advertisement call, respectively, but their mean frequencies were 5-8% higher. The temporal and spectral characteristics of the trill and advertisement calls were similar on three islands (Tortola, Virgin Gorda, Guana), except that the number of repeated notes in the trill was higher on Guana. Body size and air temperature had no detectable effects on the call characteristics. In response to experimental playback of trill calls (either synthesized or constructed from actual calls), males emitted more trills in the period following the stimulus than during the baseline. During the playback itself 5 of 22 males produced an unusually high number of trills that alternated with the stimulus trills. These responses suggest that the trill is an aggressive call used in the defence of a calling territory. In response to the playback of courtship calls (multi-note calls that males emitted only in the presence of a female and that differed from the trill call mainly in the dominant frequency of the repeated notes), 11 of 28 males left their perch sites and 9 approached the speaker. In contrast, males never left their perches during the baseline or the trill stimulus. These responses suggest that males may exploit the courtship calls of rivals to obtain mates through interference.

The presence of an advertisement call that may function both in mate attraction and inter-male spacing is an almost ubiquitous feature of anuran communication systems (Wells 1977a). Males of most species also roduce a variety of more specific calls that are used in encounters with females or other males (Wells 1977a, b, 1988; Gerhardt 1994). The examination of the structure and function of these calls provides a potentially fruitful field for investigating the constraints and driving forces that have shaped vocal communication within groups of frogs.

Frogs of the genus Eleutherodactylus are widespread in the neotropics and form a dominant component of many terrestrial anuran communities (Drewry 1970; Miyamoto 1982; Drewry & Rand 1983; Lynch & Ruiz-Carranza 1985). The relatively few species so far examined have complex and diverse vocal communication systems, including graded aggressive signalling (Stewart & Rand 1991, 1992), intricate avoidance of acoustic interference (Brush & Narins 1989; Zelick & Narins 1983) and courtship vocalizations (Michael, 1996; Ovaska & Caldbeck, in press). In E. coqui, the species that has been studied in most detail, variable use of the two notes of the advertisement call results in a complex system of vocal communication (Narins & Capranica 1978; Stewart & Rand 1991).

The calls of E. antillensis form a conspicuous feature of the night sounds on most islands of the Puerto Rico Bank in the Caribbean Sea, but the communicative significance of their various vocalizations is poorly known. We investigated the vocal

repertoire of male E. antillensis on the British Virgin Islands, located in the northeastern extremity of the Puerto Rico Bank. The species is present on a number of islands, including Guana, Tortola and Virgin Gorda, where we conducted our study (Lazell et al. 1983; Mayer & Lazell 1988). Populations on the different islands have been isolated from each other by physical barriers for approximately 8,000 - 10,000 years. Rising sea levels after the last glacial maximum, when the entire Puerto Rico Bank was united into a single land mass, resulted in the fragmentation of the bank into numerous islands (Heatwole et al. 1981). The extent of subsequent over-sea dispersal by frogs among islands is unknown.

We compared the characteristics of two calls (the trill and the advertisement call) of males from Guana, Tortola and Virgin Gorda. To obtain information on the communicative significance of the calls, we examined the responses of males to two categories of natural and synthesized calls: the trill call (occasional call of Drewry 1970) and the courtship call (Michael 1996; Ovaska & Caldbeck, in press; Fig.1). Both of these calls begin with 1-2 introductory notes, which are followed by a set of repeated notes. The calls differ mainly in the dominant frequency of the repeated notes. Under natural conditions, males intersperse trill calls among the advertisement calls at irregular intervals, and we predicted that the trill represents an aggressive call that functions in inter-male spacing. Males emit the courtship call only when leading a receptive female to a nest site (Ovaska & Caldbeck, in press).

If the trill call is an aggressive call, we predicted that the playback of recorded trills would elicit responding trills by male E. antillensis. Such behaviour has been observed in playback experiments of aggressive calls in other species (e.g. Wells & Schwartz 1984; Schwartz 1986; Stewart & Rand 1991). In contrast, we predicted that males would either ignore the playback of the courtship call or silently approach the speaker, based on the suggestion that males exploit the courtship calls of rivals to locate courtships that they can interrupt to obtain matings (Wells 1988).

METHODS

Trill Call

To obtain information on the spectral and temporal properties of the trill call, we recorded calls of individual male E. antillensis on Tortola, Guana and Virgin Gorda on the British Virgin Islands. We determined the repetition rate of trills in relation to advertisement calls in 25-min tape recordings of 12 males recorded between 1900 and 0100 hours on 11-21 October 1993 on Guana (n=6) and Tortola (n=6). To determine the timing and spectral characteristics of the trill, we recorded vocalizations of 28 males (n=10 on Guana and Tortola, 8 on Virgin Gorda) for 5-10 min for a minimum of three trill calls per male in October 1994 using either a portable JVC (CD-1636) cassette recorder or a Sony Professional Walkman (WM-D6C) fitted with unidirectional Audiotechnica condenser microphones. The microphone was approximately 50 cm from each male. At the end of the recording, we measured the snout-vent length (SVL) to the

nearest 0.1 mm and weighed each male to the nearest 0.1 g. We also recorded the air temperature.

We determined the timing and dominant frequency of 3 trill calls/male (randomly selected from the beginning, middle and end of the recording in cases when the number of trills was greater than 3) using a DSP Sona-Graph Model 5500 (Kay Electronics). We measured (a) number of notes in the introductory sequence, (b) number of repeated notes, (c) call duration, (d) time from the beginning of the last introductory note to the beginning of first repeated note, (e) mean time from the beginning of each repeated note to the beginning of the subsequent repeated note, (f) dominant frequency of the introductory note(s) and (g) dominant frequency of the repeated notes. For comparative purposes, we randomly selected 5, two-note advertisement calls from the second minute of the recording of each male for analysis. The parameters measured were (a) call duration, (b) time from the beginning of the first note to the beginning of the second note, (c) dominant frequency of the first note and (d) dominant frequency of the second note.

Playback Experiments

To examine the communicative significance of the trill and courtship calls, we presented individual calling males with a series of both call types sequentially under natural conditions and recorded their responses. The stimulus calls were prepared by (a) using vocalizations of two individual male E. antillensis recorded on Guana Island in

October 1994 (referred to as real trill and courtship stimuli) and (b) synthesizing series of sounds that imitated these calls in the timing and dominant frequency (referred to as synthetic trill and courtship stimuli). Each sequence began with three advertisement calls and was followed by either two courtship or two trill calls, each of which consisted of 8 repeated notes (Fig. 2). In both the real and synthetic stimuli, the interval between the advertisement calls and the repeated notes was 1 s, and that between the two sets of repeated notes was 2 s. The interval between the end of the second repeated set and the first advertisement call was also 2 s. Each 8.5-s sequence was iterated for 2.5 min to produce a stimulus tape.

The dominant frequencies of the first and second notes of the synthetic advertisement calls were set at 2200 Hz and 2800 Hz, respectively. The synthetic trill and courtship calls differed only in the dominant frequency of the repeated notes (2800 Hz in trill, 2200 Hz in courtship call). The dominant frequency of the lower note was based on the mean of repeated notes of courtship calls by 6 males (Ovaska and Caldbeck, in press) and is within the frequency range of the first note of the advertisement call of the populations examined. The dominant frequency of the higher note in the stimulus was based on the mean of the second note of the advertisement call by 28 males. The calls were synthesized on a Commodore Amiga computer (J. Schwartz, unpublished software).

We tested the responses of 28 males to the stimulus calls between 1930 adn 0100 hours on 5-15 October 1995 on Sage Mountain, Tortola (n=21), Beef Island (connected by bridge to Tortola, n=1), and Guana (n=6). We recorded the vocalizations of each male during five consecutive 2.5-min periods: (a) undisturbed baseline, (b) playback of either the trill or courtship call stimulus, (c) interval with no playback, (d) play-back of the remaining call stimulus (trill or courtship) and (e) no playback. One-half of the males were presented with the synthetic call stimuli and the other with the real call stimuli. The real trill and courtship stimuli used in any one trial were derived from calls of the same individual source male. The order of presentation of the trill and courtship stimuli to individual males was randomly determined within the limits that each call type was played first to an equal number of males.

We broadcast the stimulus calls using a portable Sony cassette recorder (TCM-9) connected to a Genexxa (SM200) speaker. The speaker was positioned about 2 m from the frog to be tested ($\bar{x} \pm$ SD =185 $\pm$ 22 cm, n = 28). The maximum amplitude of the stimulus calls at the location of the target frogs was about 70 dB SPL (c-weighting), which corresponded to the peak amplitude of the advertisement call of E. antillensis at a distance of 1 m, as measured using a Realistic Sound Level Meter. C-weighting was used because this setting on the instrument used corresponded most closely to a flat or no-weighting option. We recorded vocalizations of the frogs from a distance of about 2 m using a Sony Professional Walkman (WM-D6C) fitted with an Electret (SME V-6502) unidirectional condenser microphone. Initially, we carried out the experiment in

darkness. Because we could not always locate frogs that had left their calling perches during the experiment, however, we subsequently observed their behaviour with illumination from a headlight directed away from the frogs. The frogs appeared undisturbed by the dim light. At the end of each trial, we measured and weighed the frog and recorded the air temperature. We also measured the distance from the location of each frog to its original calling site and to the speaker.

Data Analysis

We used multiple regression analysis to examine the influence of (a) island, (b) body size (SVL) and (c) air temperature on the 10 call characteristics measured (call duration of the trill was omitted because it is a function of another variable, the number of repeated notes). We used the mean per male for each of the independent variables in the analyses. We compared the dominant frequency of (a) the introductory note of the trill call to the first note of the advertisement call and (b) the repeated notes of the trill to the second note of the advertisement call using Student's t-test for paired samples (2-tailed). We used the mean values for each male, and the data conformed with the assumptions of parametric statistics.

To analyze the vocal responses of males to the trill stimulus in the playback experiments, we compared the number of trills males emitted during the baseline period to that during (a) the trill stimulus and (b) the period following the trill stimulus (no stimulus), using the Wilcoxon signed ranks test for paired samples. Because the same

variable (number of trills during the baseline period) was used in two analyses, we used Bonferroni's correction and reduced α to 0.025 (Snedecor & Cochran 1980). We compared the number of spontaneous advertisement calls produced during the baseline period and those elicited during playback of the courtship stimulus using Wilcoxon signed ranks test for paired samples. All descriptive statistics are expressed as $\bar{x} \pm$ SD.

RESULTS

Characteristics of the Trill

Males emitted trills relatively infrequently during bouts of calling, although rates of both trills and advertisement calls varied widely. An average of 0.4% of the calls of individual males were trills during 25-min recordings in October 1993 (4.6 $\pm$ 3.1 trills to 1159 $\pm$ 454.2 advertisement calls; n = 12 males).

The trill call typically consisted of an introductory note followed by a set of 5-13 repeated notes of a higher dominant frequency (Fig. 1a). The lower introductory note, which was not present in 16% of the 77 trills by 28 males recorded in 1994, was slightly higher than the first note of the two-note advertisement call (2192 $\pm$ 182 Hz versus 2025 $\pm$ 76 Hz; t = 4.4, df = 1, n = 28, $P < 0.001$, all islands combined). Two trills by one male had two introductory notes. The repeated note was higher than the second note of the advertisement call (2908 $\pm$ 84 Hz versus 2763 $\pm$ 105 Hz; t = 5.7, df = 1, n = 28, $P < 0.001$, all islands combined). Occasionally (2 of 12 males in 1993, 2 of 29

males in 1994), a male repeated the higher-frequency note during a period of intense calling without producing a complete trill call (Fig. 1c).

The call characteristics measured differed significantly among the three islands (adjusted $r^2 = 0.51$, $F_{10,14} = 3.49$, $P = 0.02$. This difference was largely due to variation in one variable, the number of notes in the repeated series of the trill (partial $r^2 = 0.40$, $P = 0.0006$). The other call variables explained little of the total variation among islands (partial $r^2 < 0.03$ for each individual variable). The mean number of repeated notes in the trill was greater on Guana (9.9 ± 1.4) than on Tortola (7.5 ± 0.6) and Virgin Gorda (6.7 ± 0.8).

The SVL of males, which ranged from 23.5 to 34.0 mm, had no detectable effect on the call characteristics measured (adjusted $r^2 = 0.0001$, $F_{10,12} = 0.75$, $P = 0.67$. Similarly, air temperature had no detectable effect on these characteristics (range: 22-26°C; adjusted $r^2 = 0.18$, $F_{10,13} = 1.52$, $P = 0.24$).

Responses to Playback of the Trill Stimulus

The air temperature during the playback trials varied from 23 to 28°C (24.5 ± 1.2°C), and the frogs were perched on vegetation at heights of 30-225 cm (121 ± 63 cm) at the beginning of the trials. The body size of the males tested ranged from 26.0 to 30.0 mm (29.8 ± 1.4 mm) in SVL.

All males that were presented first with the trill, rather than the courtship stimulus, remained on their calling sites throughout the playback of the trill stimulus. However, six of the 14 males that were presented with the courtship stimulus first had already left their calling sites before the playback of the trill stimulus began and were omitted from the analysis. Thus, the sample size was reduced to 22 males. The number of trills was 0.9 $\pm$ 1.0 during the baseline period, 2.2 $\pm$ 3.1 during playback of the trill stimulus and 1.8 $\pm$ 1.7 during the period following the trill stimulus. Males produced significantly more trills in the 2.5-min space following the trill stimulus than during the baseline period (z = 2.42, P = 0.016, n = 14 males with non-zero values; Wilcoxon signed ranks test for paired samples). The synthetic and real trill stimuli were equally effective in producing this response (P = 0.64; binomial test). However, the number of trills during the playback of the trill stimulus itself was similar to that during the baseline period (z = 1.55, P = 0.12, n = 16 males with non-zero values; Wilcoxon signed ranks test for paired samples).

Five of 22 males behaved as predicted and emitted an unusually high number of trills (6-10) during the playback of the trill stimulus. In contrast, the highest number of trills any male emitted during the baseline period was 3. These males placed most of their trills (29 of 39 trills; n = 5 males) after the second stimulus trill, and only four of the total of 39 trills overlapped with the stimulus trills. In contrast, the responding trills frequently overlapped (in 18 of 39 cases) with the advertisement calls that began each

iteration of the stimulus sequence. One of these males was presented with the synthetic trill stimulus and four with the real trill stimulus.

Responses to Playback of the Courtship Call Stimulus

Thirteen of the 28 males tested fell silent when the courtship stimulus began, and 11 left their perch sites during or immediately after the playback of this stimulus. Nine frogs approached to within 10-170 cm of the speaker (covering 43 $\pm$ 23% of the initial frog-to-speaker distance). The remaining two could not be located at the end of the playback series but could have been hidden by vegetation. One additional male also silently left his perch site, but we could not determine at what point in the playback sequence this occurred, because this trial was carried out in darkness. In contrast, males never left their perches during the baseline period. The synthetic and real courtship stimuli were equally effective in eliciting a silent approach (real = 5, synthetic = 4 cases). Those males that remained on their perches and continued calling emitted advertisement calls at the same rate as during the baseline period (109 $\pm$ 54 versus 105 $\pm$ 48 calls/2.5 min, respectively; z = 3.14, P = 0.975, n = 14; Wilcoxon signed ranks test for paired samples).

DISCUSSION

Males of E. antillensis have a vocal repertoire that consists of three basic call types: advertisement call, courtship call and trill call (Fig. 1). In addition, during intense bouts of calling, males occasionally repeated the second, higher frequency note of the advertisement call. The trill and courtship calls were composed of variable repetitions of notes that resembled but were not identical to those in the two-note advertisement call. This system is similar to the vocal repertoire of E. coqui, where variable combinations of the two basic notes of the advertisement call form a complex system used in aggressive signalling (Stewart & Rand 1991, 1992) and in courtship (Michael 1996). Both the trill and the courtship calls of E. antillensis consisted of 0-2 introductory notes followed by a series of rapidly repeated notes. In the courtship call, the introduction was identical to the advertisement call, but the dominant frequency of the repeated notes was slightly higher (about 7%) than the mean of the first note of the advertisement call (Ovaska & Caldbeck, in press). The trill usually began with one introductory note that was higher (by 8%) than the first note of the advertisement call and was followed by repeated notes that resembled but were also somewhat higher (by 5%) than the second note of the advertisement call. The repeated notes in both the trill and the courtship calls were within the range of the dominant frequencies of the notes of the advertisement call.

The results of the playback experiment suggest that the trill is an aggressive call. Males produced more trills in the 2.5-min space following the presentation of the

trill stimulus than during the baseline period. However, although we have frequently heard neighbouring males exchanging alternating trills during bouts of calling in nature, only five of the 22 males tested responded to the playback of this stimulus as expected. Such an alternation of aggressive calls have been reported for E. coqui (Stewart & Rand 1991) and E. urichi (Wells 1981). The location of the speaker, which was usually on the ground, in relation to the perch height of the frogs tested ($\bar{x}$ = 120 cm) could have influenced the effectiveness of the stimulus. Males would typically hear trills of other males from heights of 1-3 m in the vegetation rather than from the ground. The intensity of the stimulus could also have affected the responses, as it does in E. coqui (Narins & Capranica 1978).

Males emitted trill calls interspersed with advertisement calls at irregular intervals throughout the observation periods from dusk to after midnight. In contrast, the aggressive calls of male E. coqui are used in nest/retreat defence and occur mainly at dawn and dusk, although males may also emit these calls from outside their retreats in the absence of intrusions (Stewart & Rand 1991, 1992). The trill of E. antillensis may function in the defence of a calling territory and/or advertisement of the occupancy of a territory to other males. Whether it also functions in retreat defence is presently unknown.

Spectral and temporal characteristics of the trill and advertisement calls of E. antillensis were similar on Tortola, Virgin Gorda and Guana, except that the average

number of repeated notes in the trill was greater on Guana. In E. coqui, the aggressive calls of males form a graded system, in which the number of notes increases with the intensity of threat (Stewart & Rand 1991). The observed inter-island variation in the trill may have reflected differences in the calling environment experienced by individual E. antillensis. On Guana, the habitat differed from that on the other two islands in two obvious respects: the acoustic environment was less complex, because E. antillensis was the only species of Eleutherodactylus present (versus two species on Virgin Gorda and three species on Tortola), and the distribution of the frogs was very patchy on this relatively dry island. The information content in longer calls could potentially be lost in the complex acoustic environment on Virgin Gorda and Tortola. Alternatively, the patchy habitat distribution on Guana could have increased competition for suitable calling and retreat sites, resulting in aggressive signalling of increased average intensity. A high density of calling males could similarly result in increased competition and more frequent and/or longer trill calls. Playback experiments with trill stimuli of different duration in combination with high and low background noise could be used to test these hypotheses.

Upon experimental playback of the courtship call, about 40% of the males tested, silently approached the speaker. This result suggests that males may use calls of their rivals to locate courtships in which they can interfere. Supporting this hypothesis, the operational sex ratios of E. antillensis on each island were heavily biased towards males, and males occasionally interfered in courtships of rival males

(K.E. Ovaska & J. Caldbeck, unpublished data). Opportunistic interference in courtships of rivals on neighbouring territories is a common male mating strategy in E. johnstonei (Ovaska & Hunte 1992), but whether males use acoustic cues to locate courtships is unknown. Males of E. antillensis (Michael 1996; Ovaska & Caldbeck, in press), as well as E. coqui (Townsend & Stewart 1986) and many other species (reviewed in Wells 1988), decrease the amplitude of their calls when courting a female. Wells (1988) suggested that the softer calls may be advantageous to males in reducing the probability of detection by rivals. The results of our experiment support this hypothesis.

Michael (1996) pointed out that the courtship calls of three species of Eleutherodactylus (coqui, cochranae, antillensis) are similar, although their advertisement calls are markedly different. The aggressive calls of the few species of Eleutherodactylus so far examined also show similarities in basic structure. The retreat and nest defence call of E. coqui, which includes a rapid repetition of the second (qui) note of the advertisement call (Stewart & Rand 1991, 1992), resembles the trill of E. antillensis. Call intensity may complicate the pattern in E. coqui (Stewart & Rand 1991), however, and the first (co) note, if it exceeds a threshold of loudness, may also function as an aggressive call directed toward other males (Narins & Capranica 1976, 1978). The aggressive encounter calls of male E. urichi (Wells 1981) and E. cochranae (S.F. Michael, personal communication) also consist of rapid repetitions of short, relatively high-frequency notes. The comparison of aggressive and courtship calls from a wider

range of species and from different species groups of Eleutherodactylus would provide a fruitful field for further study.

ACKNOWLEDGMENTS

The Conservation Agency (Jamestown, Rhode Island) and the Falconwood Foundation provided financial and logistic support that made this study possible. Smithsonian Tropical Research Institute provided additional support in the form of a Short Term Visitor's Award to K.O. Renewable Resources Consulting Services Ltd. generously allowed us to use their office facilities. We thank Skip Lazell who invited us to study frogs on Guana Island and who unwaveringly continued to support our activities throughout the study. We are also grateful to Stan Rand, who provided invaluable assistance in synthesizing frog calls and preparing stimulus tapes for the playback experiments. We also thank Gad Perry, who helped us in the field on numerous occasions, and Trisha McNeil, who provided field assistance in the initial stages of the study. Scott Michael generously shared his unpublished data and manuscripts on Eleutherodactylus behaviour with us.

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FIGURE LEGENDS

Figure 1. Sonagrams of different calls categories emitted by male Eleutherodactylus antillensis. (a) trill call; (b) two-note advertisement calls, (c) advertisement calls with second note repeated, emitted during intense bouts of calling; (d) courtship call. All calls were recorded on Guana Island in October 1994.

Figure 2. Sonagrams of stimulus calls used in playback experiments with male Eleutherodactylus antillensis. Each 8.5-s sequence began with three advertisements calls and was followed by either two trill or two courtship calls. (a) computer-synthesized (synthetic) trill; (b) computer-synthesized (synthetic) courtship stimulus; (c) trill stimulus composed of calls of a male E. antillensis (real trill); (d) courtship stimulus composed of calls of a male E. antillensis (real courtship stimulus).

FIG. 1

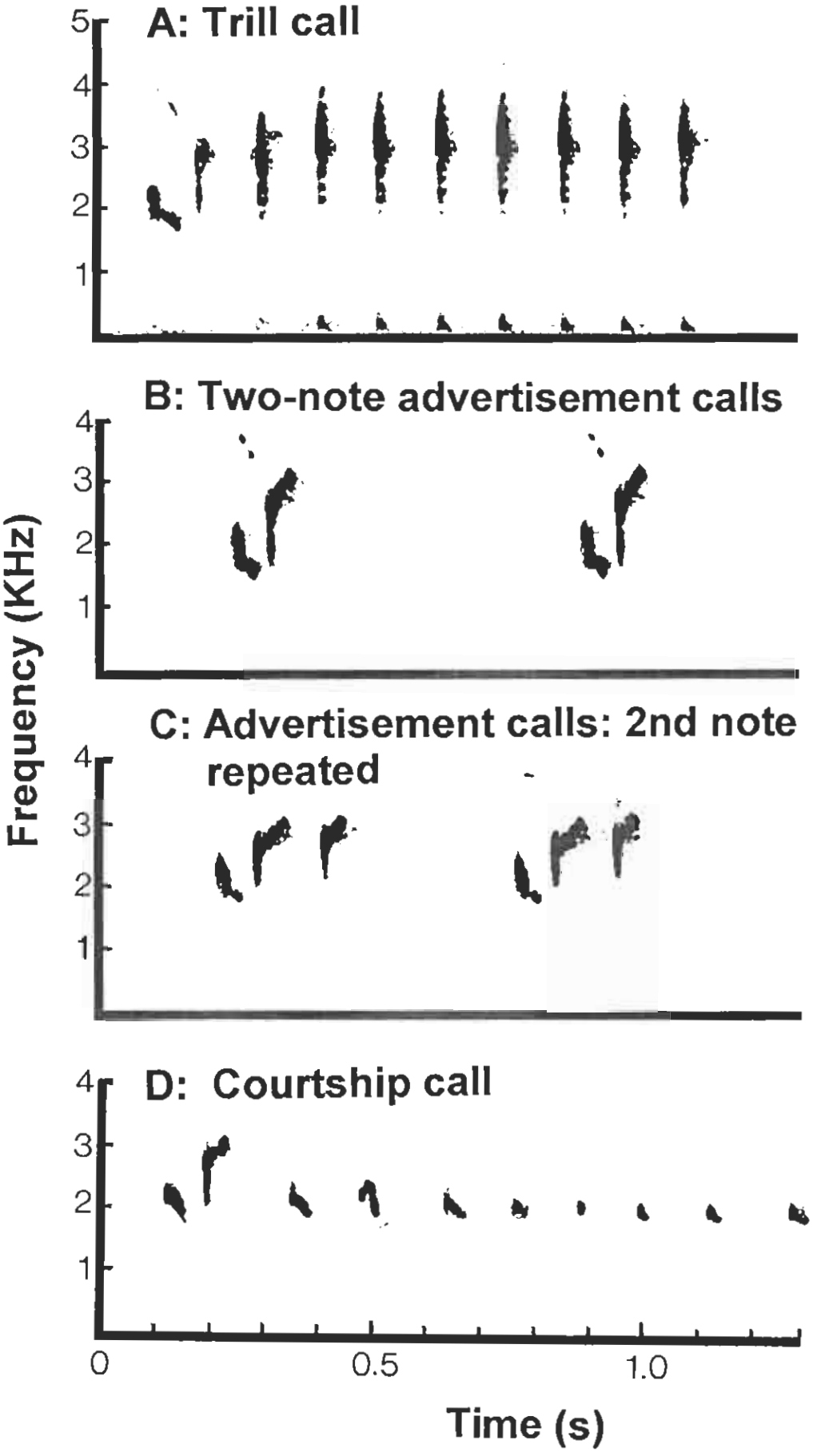
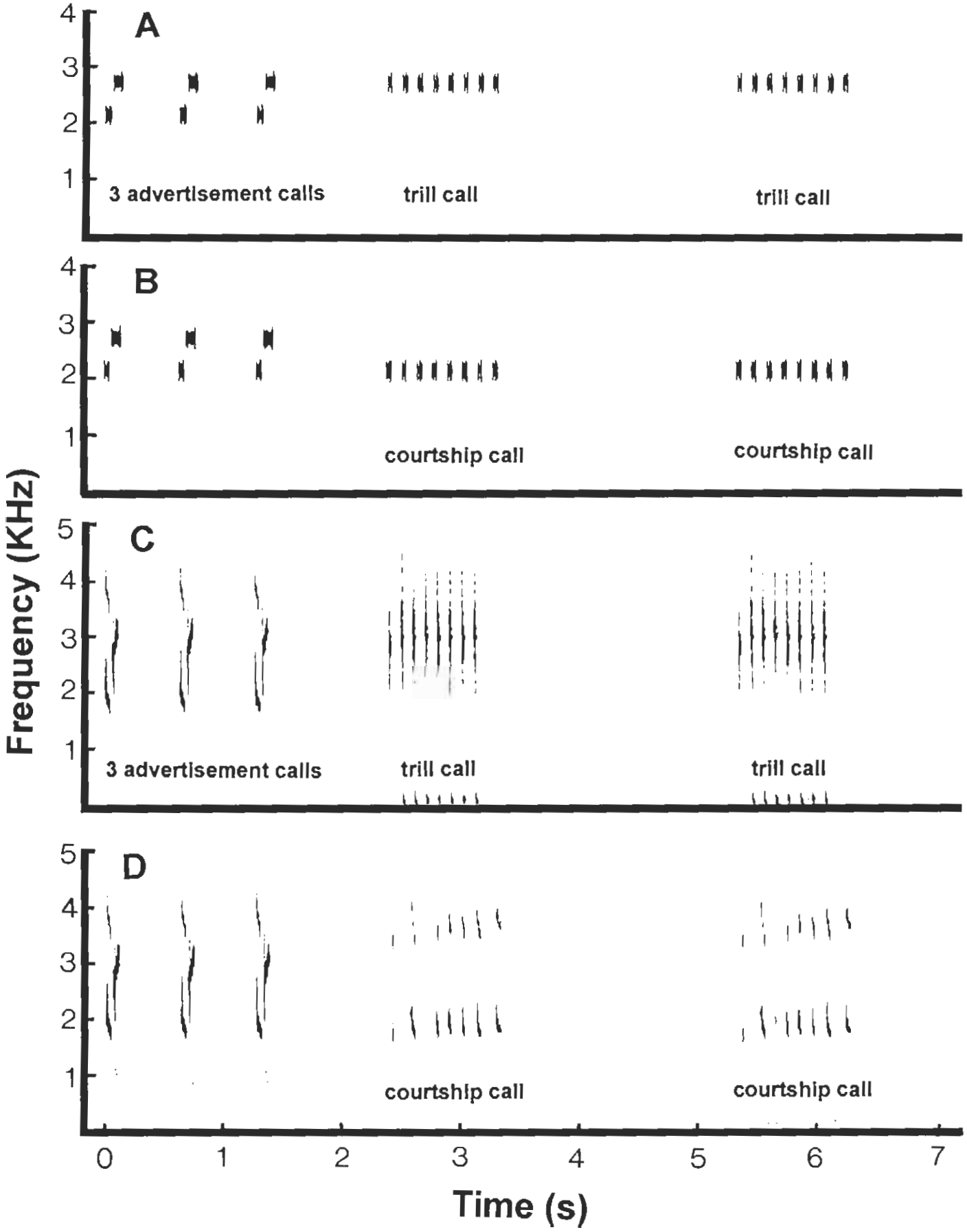

A: Trill call
B: Two-note advertisement calls
C: Advertisement calls: 2nd note repeated
D: Courtship call
Frequency (KHz)
Time (s)
0
0.5
1.0
1
2
3
4
5

FIG. 2


A
3 advertisement calls
trill call
trill call
B
courtship call
courtship call
C
3 advertisement calls
trill call
trill call
D
courtship call
courtship call
Frequency (KHz)
Time (s)
0
1
2
3
4
5
6
7

OCTOBER 1996 - GUANA ISLAND BIRDS

BANDING

Nets run on all days from October 3 to 22 with the exception of the 9th.

Nets - Placement

SP1 - Same place as last year on north side of Salt Pond from road to pond and at right angles to both.

Vegetation higher than net on both sides of net but all brushy and vegetation not much higher - 10 foot poles and 2 nets.

SP2 - On flat near Donkey Pasture and at east end of brush starting from road and running at right angles to road toward other road on flat.

Grass on one side with one bush and brush on other - brush higher than net but not by much. 10 foot poles and 2 nets.

SP3 - Same as last year - from road over fence into garden area and at right angles to road.

Vegetation higher than net on both sides although both ends flanked by grass. Hedgerow very wide and only a little higher than net. 10 foot poles - 2 nets.

SAND - A variety of nets set in the salt pond. All sets were one net running from shore out at right angles and another net running parallel to shore and end of first net. Forming either an L or a T.

Shore net always far enough on shore that shore pole was hidden in grass. Bottom trammel usually pushed down to ground. Birds sometimes flew in on their own but most of birds were pushed along until they were trapped in the L and then frightened so they flew into net.

LR - Laundry Room - Only run one day but set from laundry room along north side of path back toward hotel.

Essentially under tall tree with limited vegetation to north and then 15-20 foot high vegetation on downhill slope on opposite side of trail.

One net on 6 ft. poles.

SD - Satellite Dish - Also run one day. On trail between SD and LR with north end very close to dish. Running essentially N-S to catch birds going upslope toward peak.

Vegetation on both side a little higher than net.

One net on 6 ft. poles.

AH - Anegada House - Net set to protect lizard bags. Sort of weaved through the bags and vegetation and only portions of it useable. Just off SE corner of Anegada House.

Set under large tree in thick vegetation but with one open path leading to it at right angles to net.

One net tied to vegetation and covering area 3-7 feet off ground.

Pyramid Nets - Series of nets following trail around northern side of Pyramid. Numbered in order they were set out - 1 to 7 were progressively further away. 8 & 9 were closer to beginning of trail than 1 and 10 & 11 were at right angles to the trail and on trail to top of the Pyramid.

P1 - Starting from jct. with trail to water? disposal line.
1 net on 6 ft. poles - vegetation on both sides - side hill setting but fairly open.

P-2 - Another 40-50 feet out - as for P-1 but less open

P-3 - Another 40-50 feet out - as for P-1 but less open

P-4 - Another 40-50 feet out - as for P-1 but less open

P-5 - Another 150 feet out - as for P-1 but less open

P-6 - Another 150 feet out - as for P-1 but less open

P-7 - Another 50 feet out - more open and drier than any of the other nets.

P-8 - Under trees but fairly level and lush vegetation on both sides of net.
One net on 6 ft. poles

P-9 - Connected net 8 and net 1 - under trees at one end and open at other - slope progressively steeper as it approaches net 1
One net on 6 ft. poles

P-10 - A short ways up the trail toward Peak from Anegada House - cut at right angles to trail and extending to both sides.
Tall vegetation on both side, fair slope but set on a more level bench and on the main ridge line.
10 foot poles and 2 nets.

P-11 - Near the top of the peak on another level bench and running at right angles to trail and extending to both sides.
Shorter and drier vegetation on both sides. In some places the net extended above the vegetation.
Initially set on 10 foot poles at one end and 15 at the other. Then reduced to the standard 10 foot poles and 2 nets.

SPECIES BANDED

Total of 170 unbanded birds and 9 previously banded birds [1994 or 1995] were caught representing 20 species.

	Hatching Year	After Hatching Year
American Kestrel - 1		
Lesser Yellowlegs - 3		
Semipalmated Sandpiper - 2		
Spotted Sandpiper - 6		
Wilsons Plover - 8		
Zenaida Dove - 3		
Common Ground Dove - 9		
Mangrove Cuckoo - 1		
Caribbean Elaenia - 5		
Pearly-eyed Thrasher - 22		
Red-eyed Vireo - 2	2	
Bananaquit - 42 - 8 recaptures	27	15
Black and White Warbler - 4	3	
Blackpoll Warbler - 27	18	9
Black-throated Blue Warbler - 2	2	
Magnolia Warbler - 1	1	
Northern Parula - 2	2	
Kentucky Warbler - 1	1	
Northern Waterthrush - 3	3	
Black-faced Grassquit - 26 and 1 recapture		

TOTALS CAUGHT BY DATE AND NET

Oct.	T	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22
AH	**16**	**3**	**3**	0	**3**	**1**	0	**2**	0	0	**2**	0	0	**1**	0	0	**1**	0	0	0
SP1	**28-4**			**2**	**8**	**4**	**10**	**2**	**2**											
SP2	**6**		**1**	**1**	**2**	**1**	**1**	0	0											
SP3	**12-2**		**2**	0	**2**	**3**	**5**	0	0											
SAND	**19**					**4**		**7**	**2**	**1**		**3**							**1**	**1**
LR	**2**				0	**2**														
SD	**1-1**				**1**	0														
P1	**9-3**										**1**	0	0	**2**	**5**	0	0	0	0	**1**
P2	**5**										0	**1**	**2**	0	**1**	**1**	0	0		
P3	**5**										0	**1**	**1**	0	0	**1**	0	**2**		
P4	**4-1**											0	0	0	0	**2**	**1**	**1**		
P5	**5-2**											**1**	**1**	0	**2**	0	**1**	0		
P6	**5-4**											**2**	0	0	**2**	0	0	**1**		
P7	**4-3**													0	**1**	**3**	0	0		
P8	**14-8**													**3**	**6**	0	0	**4**	0	**1**
P9	**4-3**													0	0	**1**	0	**1**	**1**	**1**
P10	**25-10**															**11**	**3**	**4**	**4**	**3**
P11	**6-1**																	0	**3**	**3**

TOTAL **170-42**

Net open and number of birds caught = 0, 1, 2, etc.
Total number caught - North American migrants

NORTH AMERICAN MIGRANTS BY DATE AND NET

Oct.	T	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22
AH	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SP1	**4**		0	**2**	**1**	**1**	0	0											
SP2	**0**	0	0	0	0	0	0	0											
SP3	**2**	**1**	0	0	**1**	0	0	0											
SAND	**0**																		
LR	**0**			0	0														
SD	**1**			**1**	0														
P1	**3**									0	0	0	0	**2**	0	0	0	0	**1**
P2	**0**									0	0	0	0	0	0	0	0	0	0
P3	**0**									0	0	0	0	0	0	0	0	0	0
P4	**1**										0	0	0	0	**1**	0	0		
P5	**2**										0	0	0	**2**	0	0	0		
P6	**4**										**1**	0	0	**2**	0	0	**1**		
P7	**3**												0	0	**3**	0	0		
P8	**8**												**1**	**5**	0	0	**1**	0	**1**
P9	**3**												0	0	**1**	0	0	**1**	**1**
P10	**10**														**5**	**1**	**2**	**2**	0
P11	**1**																0	**1**	0
TOTAL		**1**		**3**	**2**	**1**					**1**		**1**	**11**	**10**	**1**	**4**	**4**	**3**
Nets	1	3	4	6	6	4	4	4	1	4	7	7	10	10	11	11	12	8	8

The Ornithological Council

SCIENTIFIC INFORMATION ABOUT BIRDS

28 May 1996

Dr. James D. Lazell
The Conservation Agency
6 Swinburne Street
Conanicut Island, RI 02835

Dear Skip,

Attached is the report on our visit to Guana Island, and to Great Tobago. It was another very successful trip, and nice that our visits overlapped. I hope all is going well with the pigeons!

Just before we left on Sunday, Henry asked me to come talk to the Chief Minister about the goats on Great Tobago. Mr. O'Neill thought the goats had all been shot and were no longer a problem. So I explained the situation to him, and the devastation. He asked for a copy of my report, so I am forwarding just the Tobago information to him. In fact, I wrote the report in a style which I could use with him - thus the recommendations for management which I included.

American Ornithologists' Union
Association of Field Ornithologists
Colonial Waterbird Society
Cooper Ornithological Society
Pacific Seabird Group
Raptor Research Foundation
Wilson Ornithological Society

I was thinking about the attempts to re-establish mangrove on Guana. A colleague has been doing this throughout the southern US and Caribbean for 20+ years and might well be able to give you some hints on solving the problem out there. He really knows how to do it: Robin Lewis, Lewis Environmental Services, Tampa FL, 813 889-9684.

Meanwhile its back to politics here! The Navy is killing off the last of the shrikes on San Clemente Island (17 left) and the Army wants to run tanks over 45,000 acres of red-cockaded woodpecker habitat in Louisiana.

Thanks so much for the opportunity to do this work. I really appreciate it. Just establishing adult and egg sizes has never been done for these species, so everything we have been able to do is extremely valuable. I am going to pursue a permit for, at least, taking blood for comparison to seabird species in other parts of their range. Are you interested in taking samples of land bird species which breed on Guana also? There are so few genetic data for island species. The Smithsonian molecular lab is glad to have the samples for future analyses. They like to have voucher specimens with them though, and that may be the rub - can I get a collecting permit!

Best regards,

Betty Anne Schreiber

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SCIENTIFIC
INFORMATION
ABOUT BIRDS

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Wilson Ornithological Society

ORNITHOLOGICAL VISIT TO THE BRITISH VIRGIN ISLANDS 11-19 MAY 1996

Betty Anne Schreiber, Ph.D.
Executive Director, Ornithological Council

Introduction

Guana Island and Great Tobago Island in the British Virgin Islands were visited during the above period by Dr. Betty Anne Schreiber and Mr. Gary Schenk to collect data on breeding seabirds and assess the status of the seabird populations (see attached report).

During this visit we also gave lectures to school groups on seabirds and their preservation, and assisted in setting up the Smithsonian Migratory Bird School Exchange Program between BVI schools and U.S. schools. We conducted a radio interview at ZBVI about the migratory bird program and our work on Guana Island. We met with several people to discuss bird conservation issues in the BVI and will provide these people with further contacts to conservation funding groups and further information on conservation education programs: the Director and staff of National Parks Trust; staff of the Department of Conservation and Fisheries; members of the Association of Reef Keepers.

The **Smithsonian Migratory Bird School Exchange Program**, called "Partners in Art", promotes an interest in birds and the environment through a cross-cultural exchange program between primary school classes. Migratory birds were chosen since children in the U.S. see these birds in the spring breeding season, and children in the Caribbean see the same birds during the irds' winter visit south. The goals of the program are to:

1) Stimulate cross-cultural exchange of impressions and information regarding migratory birds, a common thread linking the lives of the students.

2) Instill in students an appreciation for birds, bird migration and the natural world around them.

3) Increase awareness among students about the important roles birds play in the environment, such as eating insects which destroy crops, and dispersing pollen.

I met with elementary teachers at Athlea Scatliffe Primary School in Road Town and Bregado Flax Education Center on Virgin Gorda to discuss the program. They will begin participating this coming Fall. If the teachers like the program and it works well, it can be expanded to include more classes and schools.

I presented lectures at these schools on birds, their adaptations to life and their conservation needs.

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RESULTS OF ORNITHOLOGICAL VISIT BRITISH VIRGIN ISLANDS 11-19 MAY 1996

Betty Anne Schreiber
Ornithological Council

Guana Island

Following is a species list of birds observed during our visit. While work on seabirds was our main objective, we did make casual observations on all species present on Guana for future reference.

Brown Pelican	B	R	F[1]	
Magnificent Frigatebird		R	F	
Brown Booby		R	F	
White-tailed Tropicbird	B			
Red-billed Tropicbird	B			
Laughing Gull		R	F	
Sandwich Tern		R	F	
Roseate Tern		R	F	U
Great Blue Heron		R	F	
Little Blue Heron		R	F	
Green Heron		R	F	
Snowy Egret		R	F	
Greater Flamingo		R	F	
Semipalmated Plover		R	F	
Lesser Yellowlegs		R	F	
Black-necked Stilt	B	R	F	
American Oystercatcher		R	F	
American Kestrel	?	R	F	
Red-tailed Hawk		R	F	
Smooth-billed Ani	B	R	F	
Mangrove Cuckoo	?	R	F	
Yellow-billed Cuckoo		R	F	
Scaly-naped Pigeon	B	R	F	
White-crowned Pigeon	recently introduced			
Zenaida Dove	B	R	F	
Common Ground Dove	B	R	F	
Bridled Quail Dove	B	R	F	
Antillean Crested Hummingbird	B	R	F	
Green-throated Carib	B	R	F	
Antillean Mango		R	F	
Gray Kingbird	B	R	F	
Caribbean Elaenia	B	R	F	
Pearly-eyed Thrasher	B	R	F	
Bananaquit	B	R	F	
Black-faced Grassquit	B	R	F	

34 species observed on Guana Island

[1] B = breeds or recently reported to do so. U = used to breed, but no longer does so. R = roost on the island. F = feeds in the nearshore waters around the island or on the island

Brown Pelicans.

There were a total of 22-26 nests present on Guana at the time of our visit and more were under construction. Two nests had 10-12 week old chicks, 1 had just hatched chicks and other, unreachable nests, had either eggs or very small chicks. The predominance of nests with eggs or small chicks suggests that April-May is the beginning of the nesting season. Guana Island provides one of the last safe nesting sites of this species in the whole Virgin Islands and Puerto Rico area. Young of this species are commonly eaten by local people when they can get to the nests.

Data from measurements of 12 sets of eggs and from counts of diving success of feeding pelicans are being analyzed and will be provided.

Data were collected on age ratios of pelicans present: adults, subadults and immatures. The proportion of subadults and immatures in the population is a good indication of the productivity of the birds over the past several years.

Diving success rates of adults versus immatures were counted to determine if immatures are less successful at catching fish than adults. This has been found to be true in populations in Florida, indicating that there is a learning period for young birds, when they are less efficient at catcing fish.

Terns.

Several tern species nest or used to nest in the British Virgin Islands, but they are quickly being extirpated by human pressures. Terns nest on the ground on open beaches. They are very susceptible to mammalian predators, so that any island with goats, cats, sheep, etc. quickly loses its breeding population. Additionally, with increasing boat traffic in the Virgin Islands, tern nesting beaches are constantly disturbed by human visits. This causes desertion of existing active colonies and total lack of nesting attempts when terns return to find their colony site constantly disturbed.

There are few to no data on which terns still nest in the British Virgin Islands. Once a year the US Fish and Wildlife Service attempts to do a survey of nesting populations during one day in June, providing the only available data on the status of the terns. We counted 75 Roseate Terns roosting on Monkey Point and 100 Sandwich Terns roosting on the rocks at Long Man's Point. Both groups were courting and standing in pairs but moved on to attempt to nest elsewhere.

It might be possible to re-establish some tern nesting populations on Guana Island, which could provide the terns with potentially their only safe, undisturbed, nesting sites. The area on North Bay beach to the south of the vehicle access road would be an ideal tern nesting area. It would

need to be posted and reserved for bird use from May through August. This would leave most of North Beach, and all of White Bay, Muskmelon Bay and Bigelow Beaches open for human use. A re-introduction program could be designed for a specific tern species by using carved decoys and courtship calls broadcast over a speaker during the beginning of the nesting season.

Mangroves

Several bird species commonly seen on and around Guana Island nest in stands of mangrove. The number of nesting species on Guana could probably be increased if mangroves were grown successfully around the salt pond. As mangrove areas have been destroyed around the British Virgin Islands, there are few areas left for these species to nest.

The comparison between Guana Island and what we have experienced on Great Tobago is dramatic. Guana can serve as an example of what restoration could do for Tobago now that it has been made a National Park.

Great Tobago Island

We visited Great Tobago on 14 and 18 May, 1996, accompanied by personnel from the Department of Conservation and Fisheries who were kind enough to take us in their boat. The purpose of the visit was to assess the status and health of the seabird populations. Tobago has very significant colonies of Brown Boobies (*Sula leucogaster*) and Magnificent Frigatebirds (*Fregata magnificens*); in normal years there are an estimated 500 frigatebird nests and 150-250 brown booby nests (from previous counts). The hurricane last year destroyed many nests and disrupted the nesting cycle which should return to normal by Fall 1996.
These species have been little studied due to their remote nesting locations. As a result, there are few data on nesting numbers of birds, their nesting success, size of the birds, egg size, and chick growth rates. We are collecting data on all these parameters.

Unfortunately Tobago also has a population of an estimated 12-20 goats. We saw 5 just in the saddle area where hiked up over the island ridge from our landing site down into the frigatebird colony. Over the years these goats have not only eaten most of the vegetation of the island, they have prevented any new trees or bushes from beginning to grow by eating all new shoots. Goats hooves break up the surface soil and cause extensive erosion. Goats are an introduced animal which is not native to these islands. In general, it is a bad idea to introduce any new animal into a biological system which has existed in the absence of that animal for many, many years. Animal introductions usually cause the whole biological system of an area to fall apart: many natural species are killed off and fewer total species are supported by the ecosystem.

The presence of goats on Tobago has several ramifications:

1) Since the goats have eaten most of the vegetation and kept new vegetation from growing, there is extensive erosion of the soil into the surrounding reef area. This eroding soil smothers and kills the filter-feeding coral of the reef and kills larval fish and crustaceans.

2) With fewer bushes and trees on the island, there are fewer species of birds nesting. Many birds need trees or bushes in which to build nests. The few trees left are heavily used by frigatebirds, whose nests are crowded into very limited space. This crowding is killing the trees as frigatebirds pick them apart for nesting material and build nests in them.

3) Ground nesting birds, such as terns, gulls and brown boobies have their nests and eggs trampled by goats. Terns

and gulls have abandoned the island as a nesting site due to this disturbance. The remaining boobies who nest there are only able to raise young successfully if they build their nest under a tree where goats cannot walk.

Each of these problems diminishes the economic value of the area. When reefs are destroyed nursery grounds for many fish and crustaceans are lost. If fish and crustaceans have no nursery grounds they die, depleting the human food supply. Tourists and sailors come to an area like the British Virgin Islands because of their snorkling opportunities and natural beauty. When islands and surrounding reefs are destroyed, fewer people are likely to come and income to the local economy is lost.

Magnificent Frigatebirds

We estimated 480 to 500 frigatebird nests present on the island at the time of our visit. They were nesting in large old seagrape trees on the north east side of the island. Most nests had chicks and most chicks were within 4 weeks of fledging. About 10-20 chicks were of various ages younger than this. No nests with eggs were seen. During our visit last October there were about 500 pairs of adults courting, nest building and laying eggs. The birds appear to have had a very successful nesting season.

We measured 22 frigate bird chicks to determine the growth rates of young. These data are being entered in to the computer for statistical analysis. All chicks appeared to be healthy and only 5-6 dead young were found. Generally dead young are from newly mated pairs who don't quite get the system right their first time and this extent of mortality is normal.

Brown Boobies

There were about 40 brown booby nests present at the time of our visit. There were about 20 nests during our visit last October. Nests present during this visit were in all stages from adults incubating eggs to nests with large chicks. Since all nesting was destroyed by the hurricane last Summer, their nesting cycle and numbers may be off schedule this year. They nest on the slope of the north east side, on the lower half of the island, toward the water. Most nests were under trees or next to some obstruction that prevented goats from walking on them.

We measured growth in 12 chicks and were able to catch and measure 13 adults. Three clutches of eggs were measured. All chicks appeared healthy.

Ideally we need to measure at least 40 adults and 40 eggs of each species for comparison to these species nesting in other areas. If we are able to obtain that many measurements, we

will be able to do the statistical comparisons to other populations. Preliminarily, I can say that the brown boobies of Tobago are much smaller than those nesting in the Pacific. They also have different voices and bill colors. This means they are a unique genetic population.

Recommendations for Improvement of Tobago

1) Remove the goat population as soon as possible.

2) Do a general ecological study to determine what plant and animal species are present. To do this a botanist, herpetologist, mammologist and ornithologist should visit the island. Each scientist should be asked to produce a fairly complete list of species present and suggestions for restoration of the island flora and fauna. It may be possible to let restoration occur naturally, but it may also be necessary to remove some introduced species that compete with naturally occurring species.

3) Post the island with signs saying that it is a National Park and ask people to respect the wildlife. At a minimum, entry onto the south and east beaches should not be allowed from April through August when terns could be nesting.

4) Encourage interested researchers to do studies in the British Virgin Islands. This can provide an inexpensive way for government to obtain the information they need to help manage the islands since most researchers have funding from other sources. As part of obtaining a permit to conduct research on the islands, the governement could receive a report on the findings of the research. These reports would provide valuable information on how to manage the resources of these islands.

With the amount of boat traffic and number of visits to islands occurring in the British Virgin Islands, added to the damage done by goats, cats, dogs etc., I suspect that few seabirds still nest successfully and that the number of landbirds is diminishing. Ideally, several islands should be restored to their natural state and entry limited or prohibited if populations of nesting birds are to be maintained.

The Ornithological Council

PROVIDING SCIENTIFIC INFORMATION ABOUT BIRDS

27 March 1997

Dr. James Lazell
The Conservation Agency
6 Swinburne Street
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Dear Skip,

Hope is all is well with you guys. Spring has come to DC at last - all my daffodils are up, the red-shouldered hawk is on eggs and house sparrows are claiming all my nest boxes!

I have recently been able to get blood samples and adult measurements of boobies and frigate birds from the Cayman Islands and from Barbuda which is great. It will be wonderful comparative material for the Pacific Islands data.

With that in mind, I would like to return to Guana in October when the boobies and frigates should be incubating eggs and get more adult measurements and some blood samples. I have given up on getting a permit to band; have heard nothing from the government. Perhaps coming the week of 11-19 October if you are thinking of schedules yet!

Also, do you know if anyone has checked the cave for procellariiform bones? If this hasn't been done, I would like to set up a small dig and see what we can find. I would like to check on the status of the nesting pelicans and possibly get blood samples from them, too.

Had you ever thought about hosting a symposium on conservation needs in the BVI, or wider Virgin Islands? Might be interesting to think about. What brought this to mind is that at the upcoming meeting of the Society of Caribbean Ornithology we are having a symposium on the status and conservation of Caribbean seabirds (Aug. in Aruba). The status of each species will be reviewed and in a set of workshops we will address the problems of each country and needed action for conservation. We are trying to have representatives from as many Caribbean countries as possible present. I have told Nick Drayton about it in the hopes they will send someone, but if they do not, I would try to address

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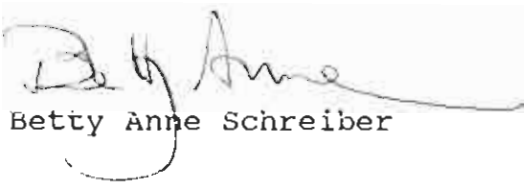
some of the problems so that there is a report from the BVI. Do you have any suggestions on this, or anything you would like to see included? We want to publish the proceedings. It seems to have a lot more effect on countries and help in getting aid when something about their needs is in print, especially from a scientific meeting.

Best regards,



Betty Anne Schreiber

VEGETATION RECOVERY MONITORING PROGRAM

Numi Mitchell and George Proctor

Degraded habitat is a problem on many Caribbean islands, Guana being one of them. Since the 1700's islands have been damaged by numerous human activities. Islands were first denuded by charcoaling, a selective cutting process which reduces hardwood tree species. After selective cutting vast areas were cleared for farming. Clearing led to loss of the humus layer because trees were no longer generating the leaves for the rich organic layer, and because rapid soil erosion occurred on the farmed slopes. The popular custom of burning to clear old cane trash caused further nutrient depletion. Free-ranging grazing animals (goats, sheep, cattle, burros, pigs) foraged and ate whatever they could find in regions that were not farmed. In such areas, opportunistic plant species, often exotics or species with distasteful or toxic secondary compounds, colonized denuded areas. By the 1800's the West Indies was left with mineralized nutrient-poor soils and low plant species diversity.

In the Virgin Islands, modern degradation of entire islands has been alleviated or reduced by private ownership (e.g., Guana, Mousquito, Necker). Public reserves on other cays (e.g., Saint John, Tortola, Prickly Pear) protect some percentage of their open space. Stewards of these properties concerned with restoring native habitats have a common problem, however: how to facilitate a process retarded by previous damage.

Here we propose to initiate a study on the Guana Island Wildlife Sanctuary, to begin to determine the requirements for restoring natural vegetative communities in denuded areas. In this first year we will set up a single experimental treatment with appropriate control. In this stage we hope to determine the effect of fencing out non-native grazers. Though this work will stand alone, we hope subsequently to propose additional treatments and empirically examine methods for speeding forest recovery. It would be ideal to combine this project with the biomass vegetative plot studies tentatively proposed by Gad Perry and Gordon Rodda.

METHODS

Five 10 x 10 m plots will be fenced in habitats that are wooded in native vegetation but which are currently heavily grazed by sheep or goats: North Bay woods, west side of Liao Wei Ping trail, east side of Liao Wei Ping trail, north shoulder of Quail Dove Gut, and the abandoned plantation on south shoulder of Quail Dove Gut. Five unfenced 10 x10 m control quadrats will be located close (5-10 m) to the fenced plots. Control quadrats will be placed in habitat similar to that in the adjacent experimental quadrat. Once established, both fenced and

unfenced plots will be botanically surveyed. All vegetation will be identified, and basal stem counts of all trees (> 2 m high and > 2 cm basal area), tree seedlings (< 1 m high), and saplings (1- 2 m high), as well as shrubs and herbs will be assessed. Humus depths (cm) will be taken at 1 m intervals in each quadrat.

Fencing will consist of 1.2 m high "field fence" (Southeastern International Sales, Miami, Florida). The fencing will be graduated with lowest tier smallest (8 cm high by 15 cm wide) and top tier largest (20 cm high by 15 cm wide). Graduated fencing will permit the passage of native animals (including iguanas of all sizes) but not goats, sheep, or their offspring. No treatment other than fencing will be applied to the experimental quadrats.

Follow up vegetative surveys will be conducted annually in October to determine whether there are differences between the fenced and control quadrats at each site.

BUDGET

60 fence posts (each post place 3 m apart in five fenced plots) @ $2.76 ea.	$165.60
1.2 m high field fence (12 gauge) 200 m (656 ft) @ $0.71/ft.	$465.76
Total	$631.36

These are US prices, presumably BVI prices will run 20-30% higher and there may be some shipping charge.

A new horizon for biological field stations and marine laboratories

For more than 100 years, biological field stations and marine laboratories (FSMLs) have provided opportunities for education and research to students and scientists. In recent years, however, funding for field biology has become constrained, pristine habitats are rapidly disappearing, instrumentation and other essential resources bear ever higher price tags, and the need for the services that FSMLs provide is increasing. Historically, these facilities have operated largely independently of each other. It is now time for FSMLs to establish links with one another, thus forming a network of field sites that can meet the needs of the future with their combined strengths and resources.

The fundamentals of biology and ecology are best taught at FSMLs. For decades, FSMLs have been contributing to the accumulating body of knowledge about basic biological processes. The close proximity to the natural environment means that the study of biology at an FSML has a degree of validity that is difficult to achieve in a purely indoor laboratory setting. This continuing emphasis on basic biological research must be maintained. The proposed formalized network of FSMLs would facilitate the fertile exchange of information that is so critical for furthering good basic science, which is fundamental to all successful resource management efforts.

The assessment of environmental effects is facilitated at FSMLs. Human activities add variation to natural trajectories of environmental change, often to the detriment of human well-being. Such variations may result in massive alteration of hydrological flow patterns and pathways; deposition of toxic pollutants in the atmosphere, watersheds and coastal environments; and chronic loss of genetic variability and other measures of biodiversity. The proposed network of FSMLs would provide an integrated and multi-scale sampling design for partitioning natural and human sources and consequences of environmental change. No other organized research or monitoring and evaluation effort of this magnitude has been attempted.

FSMLs encompass sites that retain natural biodiversity and, on the whole, are not dominated by humans. At these sites, species distribution and abundance data, and natural- and human-mediated influences effecting biodiversity (including the pervasive influences of non-native introductions) can be systematically evaluated in relation to various spatial and temporal scales, and comparisons can be made with adjacent sites where biodiversity is impaired. Moreover, by networking FSMLs, many of the uncertainties regarding effective biodiversity measures and sampling protocols can be evaluated and resolved.

FSMLs can assist in the evaluation of methods for sustaining healthy ecosystems. Such ecosystems can be assessed in terms of 'goods' and 'services', which are generated, for example, by the effects of human culture and values, and more specifically by institutions that contribute to the effective management of terrestrial, aquatic and coastal ecosystems. 'Ecosystem goods' include timber, fisheries, minerals, natural pharmaceuticals and other chemicals that are renewable and sustainable in naturally functioning ecosystems. Essential 'ecosystem services' include good air and water quality, biological productivity, aesthetics and recreation. FSMLs exist in a wide variety of environments, for example, alpine zones, forests, grasslands, marshes, riverine and lacustrine environments and coastal areas, and therefore comparative analyses of ecosystem function can be made at multiple scales. Technology can be provided proactively to management agencies and policy makers. Thus, directed information can provide the basis for adaptive approaches to sustaining vital ecological systems.

Many FSMLs, being well-integrated with local communities, are uniquely suited for technology transfer and interactive demonstrations of contemporary ecological science in principle and practice. The validity of inferences from ecosystem modeling can be determined from the long-term environmental data routinely collected by these stations. These data have no limit to their shelf life, increasing dramatically in value with age. Greater emphasis on partnerships in ecological research and problem solving is needed between FSMLs, management agencies, government, industry, and the public.

Research should be directed towards providing sound scientific information to guide the reversal of ecological impoverishment. There is an immediate need for information centers aimed at improving public understanding of the way natural systems operate – from molecules to watersheds – in order to guide restoration and rehabilitation efforts effectively. Networked FSML sites include access to relatively pristine areas and to adjacent habitats that vary in degree of impairment. Their interdisciplinary systems-science approach provides innovative hypotheses and mechanisms for comparative purposes. The broad distribution of these stations allows a continuum of data sets for intersite comparisons and intrasite experimental manipulation.

FSMLs have evolved from their original service mission supporting part-time visiting investigators and limited research capacity to full-time research centers. Many have well-developed physical and intellectual infrastructures, and long legacies in the analysis and synthesis of environmental data derived from the unique ecological attributes of the site and the concurrent application of state-of-the-art techniques and methodologies.

A network of FSMLs is poised to fill a vital role not only in generating new science in response to the research priorities listed above, but also in providing long-term data for quantitative evaluation of national or global environmental conditions. Long-term research programs already implemented at some FSMLs have clearly demonstrated the importance of field sites in documenting environmental baselines, and processes and responses produced by natural- and human-mediated environmental change. Moreover, most FSMLs already have a clear track record in the integration of ecological science and natural resource management at local to regional scales.

At a workshop held in March of 1995 the directors of 33 FSMLs discussed the future of their facilities[1]. As a result, progress has begun towards the implementation of a network of FSMLs as research and monitoring nodes spread across the USA and its coastal and territorial oceans, in a manner that allows a systematic resolution of research priorities at local, regional, continental and global scales. Efforts to broaden this FSML network internationally have begun with attempts to identify FSMLs globally. Funding must be secured to accomplish networking goals.

Susan Lohr

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Jack Stanford

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References

1 Lohr, S.A. *et al.* (1995) *A New Horizon for Biological Field Stations and Marine Laboratories* (Miscellaneous Publication No. 1) Rocky Mountain Biological Laboratory

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15 April 1997

Dr. Susan Lohr
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Re: A Caribbean RSMI.

Dear Dr. Lohr:

I have been meaning to write for nearly a year, but have been too much in the field. This Agency operates a field station at Guana Island, British Virgin Islands. Generally, we have a marine science program each July, and a terrestrial program each October.

I enclose a brochure about the Island and a list of our publications (perpetually incomplete). We welcome proposals for research.

Regular mail (address above) is the best way to contact us, but short messages can be faxed to (401) 423-2396 or e-mailed to wenhua@uriacc.uri.edu, or jcinjtown@aol.com.

Please add us to your network.

With best wishes,

Skip Lazell

James Lazell, Ph.D.