Guana

Report on Science - 1994

The Conservation Agency

The Conservation Agency

Exploration, Education, and Research

President
James D. Lazell, Ph.D.
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6 Swinburne Street
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30 November 2003

Dr. Henry Jarecki
10 Timber Trail
Rye, New YOrk 10580

Dear Henry:

Note the date above. It seems none of us can find any one of the (minimum) three copies of my original report for 1994. I have reconstructed one from the files.

It starts off with the strange story of the mysterious boa. The pretty little constrictor that Major Chapman Grant said — back in 1932 — lived on Guana Island, which he collected on Tortola, and which was subsequently named for him, still has never been rcconfirmed. Miguel Garcia and Manuel Leal came and hunted in 1994, but it rained every night: they thought the habitat superb and their failure to find the boa inconclusive.

The boa is common on Tortola and specimens continue to accumulate at the Community College (from road, cat, human, and electric fence kills). There are about two dozen in jars there now. We still have never been able to get a CITES or USFW permit to move these to a major public museum — where they belong. The relationship of the BVI boa, granti, to other insular populations remains unstudied, and — until we can get them out — unstudiabe.

The white-crowned pigeon plans slope off into a bit about lizards, but that is the end of them for 1994: Surprise!

Turns out to have been a great termite year, though.

All the best,

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DEPARTAMENTO DE RECURSOS NATURALES Y AMBIENTALES

May 10 1994

VIRGIN ISLAND BOA : MORE SEARCH IS NEEDED IN THE BVI

by Miguel Garcia

The Virgin Islands Boa, *Epicrates monensis granti* exhibits a patchy distribution throughout the Puerto Rico Bank (Nellis et al. 1984; Mayer and Lazell 1988). Nevertheless, new populations have recently been discovered on Culebra Island and in Puerto Rico mainland (Hedges and Thomas 1990;Tolson 1990). These recent sightings indicate that the species may be more widely distributed than previously thought.

The U.S. Fish and Wildlife (1986) proposed a recovery plan for *E. m. granti*, which prioritized among other activities the searching for new populations and their status. Since July 1991, the Puerto Rico Department of Natural Resources (DNR) in cooperation with the Toledo Zoological Society and the U.S. Fish and Wildlife Service, has been conducting under the Endangered Species Program a project to determine the current status and distribution of all *E. monensis* populations in the Commonwealth of Puerto Rico, with the exception of the Cayo Diablo (off eastern Puerto Rico) population. However, little has been done in relation to the frequent *E. monensis* sightings from the British Virgin Islands, where more than a dozen of sightings and roadkills have been reported during the past two years (Lazell per. com.). Although most of the boa observations in the BVI are from Tortola, two instances of boa encounters have occurred on Guana Island

AVE. MUÑOZ RIVERA, PDA. 31/2, SAN JUAN, P.R. / APTDO. 5887, PUERTA DE TIERRA, P.R. 00906 / TEL. (809) 724-8774

(Lazell per. com.). Thus, the status of this species in Guana Island should be assessed immediately. This activity should be also performed in Tortola, but logistical problems such as time, funds and personnel preclude the initiation of this task.

PLAN

It is our experience that finding *E. monensis* on big islands (relatively speaking) is extremely difficult, especially when suitable boa habitat conditions, like vegetation continuity and high anole lizard abundances (Tolson 1988) are widespread, as they are in Guana Island. Boa searches should be conducted at night, when boas become active, foraging on sleeping anole lizards (Tolson 1988). This methodology has proved to be successful, even on places where boas are hard to detect, like Mona Island (García and Tolson unpub. data). Daytime is used to identify suitable boa habitats.

During my previous survey on Guana Island, I concentrated my effort on the North Beach area. This site needs to be surveyed again, together with any other beach front with good sea grape groves. Also the trail ending in the Long Point must be checked.

I understand that at least one week (two persons) must be devoted to reasonably cover Guana Island. Checking on my tentative agenda for October 1994, I would like to arrive at Guana Island on Friday 7 and stay there until the 14. I do not need any money for equipment or materials, however, I do need from you to cover my air fare from Michigan to Beef Island (round trip) and the air fare PR-Beef Island (round trip) of my colleague, Mr. Manuel Leal (an outstanding young herper from Puerto Rico, C.V. included).

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23.i.94

Dr. Peter Tolson
Toledo Zoological Society
2700 Broadway
Toledo, OH 43609

Dear Peter:

Glad to hear all that. Yes, my figure was based on the proposal I saw. I had heard it had been funded -- I assumed at or near the level requested. That was years ago, of course. At that time, I requested a permit for E. monensis and my application was statedly rejected because you already had the permit and USFW would not grant two. Clear implication was that until you no longer held a permit I could not get one.

Of course we do keep the salvaged snakes. I sent one to BMNH, requiring no customs clearance. I, too, worked with Fred Kraus to get that other one to UMMZ. In the end it was confiscated by the then BVI conservation officer, claiming the BVI was going to set up a museum (also took a roadkill Anegada pinguis). The new BVI conservation officer is totally opposed to preserving or keeping specimens; rumor central has it he threw these away. We have a tiny, exhibit "museum" on Guana, and the new community college might take an interest, but this hardly solves the real problem. Specimens need to be in a major public museum, accessible to all herpetologists. I will reapply for a salvage permit restricted to the BVI, but I won't get it unless you specifically state that you need access to these specimens and have no other way to get them.

Alternatively, do you have a salvage permit? If so, why not just salvage these? I will give you their tag numbers and data; you report them on your permit; and they will magically appear in the U.S.A.

Have you -- has anyone -- looked further into geographic variation in the PR Bank nominal granti? If meristic characters sort populations you should have plenty of data by now.

All the best,

James Lazell, Ph.D.

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26.i.94

Dr. Bertrand Lettsome
Conservation Officer
Ministry of Natural Resources
Road Town, Tortola
British Virgin Islands

Re: Boa, Epicrates monensis granti

Dear Dr. Lettsome:

I would like to apply for an export permit for salvaged carcasses of the small, native boa in the BVI, noticed above.

From time to time, bodies of these snakes are found dead on roads, or killed by people, or electrocuted on fences. Some years ago, I sent a roadkill to the British Museum (Natural History). Most recently, Fred Kraus -- then doing botanical work on Guana Island -- got another dead one (cause of death unknown to me), which was taken by Julie Overing to your offices to serve as an official voucher specimen within the BVI. I believe other specimens are around, in bottles of preserving fluid, or frozen.

While I believe it is useful to have a specimen on hand in the BVI (such as the one Ms. Overing got from Dr. Kraus), I believe it is equally useful to put additional salvaged specimens in a major museum for worldwide public access. There are great taxonomic questions about the identities of monensis group boas from the various different islands (Tortola, St. Thomas, Cayo Diablo, Puerto Rico, and Mona being the largest). These questions can only be answered by examination and comparison of actual specimens.

If you look favorably upon my request to gather up and properly preserve and document BVI boas found dead in the field, I would specifically propose deposition in the Museum of Comparative Zoology at Cambridge, Massachusetts, U.S.A. This is the world's largest herpetological collection, superbly curated, and wide-open to qualified researchers and students from all over the world. The original type specimen of your boa, Epicrates

monensis granti, from Tortola, is in this museum, as are other specimens from St. Thomas and Mona (at least).

I hope you will consider exhibiting the specimen at your offices, and consider some educational publicity about these harmless, beneficial, rodent-eating snakes. They seem to be fairly common on Tortola, but they remain very little-known to most of the public -- either in the BVI or the world.

Sincerely yours

James Lazell, Ph.D.

cc: Dr. John Cadle, Museum of Comparative Zoology.
Dr. Peter Tolson, Toledo Zoological Society.

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24 March 1994

WHITE-CROWNED PIGEON RESTORATION: BRITISH VIRGIN ISLANDS

The white-crowned pigeon, la paloma cabeza blanca (Columba leucocephala) was formerly abundant in the British Virgin Islands but extirpated there as a breeding bird before 1970 (Mirecki, D.N. 1976. Report of the Cambridge Ornithological Expedition to the British Virgin Islands. Churchill College, Cambridge, UK: 44 pp.) It is the hope of The Conservation Agency to restore a breeding population to these islands.

Reasons for Extirpation

Mirecki, (op. cit.) gives no reason for his statement that this species "declined drastically" to the point where only it is now "only a casual visitor." Local authorities vary in opinions. Wiley (1985. Bird Conservation 2:107-159) listed habitat loss as the principal cause of general avian decline in these islands, but it seems as though suitable habitat remains in the BVI, especially on Guana Island. I have made direct comparisons of Puerto Rican breeding habitat with that available on Guana, and the Guana site seems less disturbed and more diverse. Wiley (op. cit.) listed shooting as the second greatest cause of loss, and this seems likely to me. White crowned pigeons are delicious, in strong contrast to the scaly-naped pigeon (Columba squamosa), which local people disdain as food. After World War II, it is said that guns were plentiful and hunting was a regular thing. Prior to making all hunting illegal in 1972, the white-crowned pigeon, West Indian whistling duck, masked duck, and roseate flamingo all disappeared. Populations of bridled quail dove and Bahama duck were reduced to rarity, but these species have come back and are now common on some islands (Lazell, J. 1989. Guana: a natural history guide. The Conservation Agency, Jamestown, Rhode Island, USA:

20 pp). Because gun ownership and hunting are now both illegal throughout the BVI, we believe restoration can succeed.

Restoration Site

We believe the Guana Island Wildlife Sanctuary is an ideal site to attempt restoration. The island is 340 ha and has extensive mangrove swamp bordering a salt pond. The staff will feed and care for the birds throughout their acclimatization period, and can continue to supply food indefinitely. According to Dr. George Proctor, P.R.D.N.R., who has conducted detailed studies of Guana's flora over a span of several years, the vegetation there today is in unusually fine condition and species diversity is remarkably high. We believe the Island's resources can easily support a population, especially if subsidized in the early stages.

Plan

We would like to bring six (6) fledgling white-crowned pigeons from Puerto Rico, or Culebra, or any other island within the purview of P.R.D.N.R., to Guana Island in the spring of 1994. The birds would be reared in captivity until able to fend for themselves, then released in the immediate area of the Island manager's home. The birds would continue to be fed around this building so as to monitor their health. We hope this nucleus would breed as soon as 1995, and possibly attract some of the reported strays from other populations.

To complete this project we need to obtain the young pigeons and a veterinary certificate, presumably from Dr. Juan Torres or Dr. Guzman, of U.S. Dept. Agriculture, or both, and the cooperation of the BVI Ministry of Natural Resources. This Agency will cover any costs involved provided they are not too great!). We will make plans and ascertain costs well in advance.

James D. Lazell, Ph.D.

phone:(414) 595-2074 email: mayerg@cs.uwp.edu fax:(414) 595-2056

28 April 1994

Dr. James D. Lazell, Jr.
Guana Island Club
Box 32
Road Town, Tortola
BRITISH VIRGIN ISLANDS

Dear Skip:

1. Pigeons: I didn't realize the white-crowned pigeon plan was so near fruition. This is really very good news. Sounds as if Jorge and company have been cooperative. I hope that everything has gone well with transporting them.

2. Thorpe: I didn't know that he had explicitly said that subspecies should only be recognized in cases of secondary contact, but that has been the practical result of much of his work. For example, his revision of European Natrix sunk all (or almost all) the subs except for the major western vs. eastern split which likely corresponds to a glacial Iberian vs. Greek refuge split, with current secondary contact in Germany and elsewhwere. Forms within the western group were sunk, and likewise for the east. I think that one of the major issues for him is the width of the cline connecting two intergrading forms relative to the size of the range of the "pure" forms. He seems to only want to recognize subs when the intergrade zone is quite narrow; otherwise, he prefers to think of it as a cline. As a matter of history and population genetics, it may be true that narrow intergrade zones between differentiated forms are most likely to have occurred in zones of secondary contact. I think it would be wrong to confound, as he apparently has done, the pattern of geographic variation with the the cause of that pattern, however. I think that subs should be recognized when the criteria of diagnosability and intergradation are met, regardless of the origin of the pattern. I am not entirely unsympathetic to his concern that intergrade zones be narrow in some sense, but I think there are cases where relatively broad and sometimes complex imtergradation can be best summarized as subspecies, e.g. chrysolepis in South America (based on PV & EEW), or conspersus on Grand Cayman (where two of the most strikingly divergent anoles I've seen intergrade over more than 50% of the island, the "pure" zones being modest for one sub, and downright small for the other). I think Thorpe's work on geographic variation has in general been quite good, although I have not agreed entirely with his taxonomic positions. It seems that his taxonomic views now diverge even more strongly from my own, and doubtless from yours as well. I have Thorpe's Nature

paper from 2-3 years ago; if you have more recent refs on this, let me know so I can see what he's saying now.

3. Other birds: As always, keep an eye out for mockers. Also, see if you can confirm the presence of Dendroica petechia, especially in the mangroves. As you know, I am very suspicious of last fall's sightings; I believe them most likely to have been migrants, perhaps petechia, more likely another species, from North America.

4. Mabuya: I have been working on the manuscript. Two questions for you: First, there is some nomenclatural underbrush that needs to be cleared out regarding "mabouya" Lacepede. It is clearly non-binomial, and I've included a sinking of it in the MS, making sloanii the earliest name. I'm pretty sure you approve of this action, and it seems better to include it in a paper on real skinks, rather than as a separate purely nomenclatural paper. Second, I don't know what sex the specimens are. Should we a) sex them (do you know how) or b) just go ahead without sexing them?

5. Anoles: Yes, I would like some live anoles; they would be quite useful for biochemical work this summer. Ideal would be 10-12 crist, 3-4 of strat and pulch, plus one of any other lizard you could get (Hemidactylus, Ameiva, or a few Sphaeros). Anything you can get short of this would, of course, be useful and much appreciated; I know the trip is primarily for pigeons and you'll probably have little time for lizard catching. By the way, Joe and I have talked Alsophis; tissue from them would be good too (oops, I mean Liophis).

My home phone is 414-634-6799; I thought you had the number, and it has been working. Give my regards to everyone on Guana.

Best wishes,

Gregory C. Mayer
Assistant Professor
Biological Sciences

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14.v.94

Ms. Rosmond DeRavariere, Director
National Parks Trust
P.O. Box 860
Road Town, Tortola, B.V.I.

Dear Rosmond:

I thought it best to follow up our conversation of 29 April with some thoughts in writing on the Natural Resources Management and Training program being worked out jointly with H.L. Stoutt Community College and Hocking College.

The most important bird to try to study at Sage Mountain is the bridled quail dove or "marmy dove," Geotrygon mystacea. This bird occurs or occurred on several islands from St. Kitts west to eastern Puerto Rico, but it has declined to rarity or disappeared from much of its range. There is still a good population on Tortola, centered on Sage Mountain with birds roaming down the ghuts almost to sea level.

Bridled quail doves walk along the forest floor and are reluctant to fly. They can be herded into mist nets set on the groud. They are easily marked with colored plastic leg bands -- up to four, two on each leg, per bird. Color combinations allow individual recognition in the field, so that home range and density estimates can be made.

Bats are also best caught in mist nets, but set much higher up. Lianna is experienced with local bats. As our paper on them suggests, Tortola will be a very important place to collect bats, and major new discoveries are predicted.

Frogs will prove enormously difficult to work with. There are four species on Sage Mountain, but two are rare there: Cochran's piping frog (Eleutherodactylus cochranae) and the white lipped frog (Leptodactylus albilabris). The two that are common, Eleutherodactylus antillensis and E. schwartzi (bo-peep) are easy to hear but hard to find except on rainy nights. Even so, most individuals spend most of their time high up -- 20 to 50 feet -- in trees. Of course, identified specimens and good data would be wonderful, but getting either will be frustratingly hard.

Lizards -- especially the three species of Anolis -- are a much better bet. Sage Mountain is by far the highest and wettest site where Anolis cristatellus wileyae, A. stratulus, and A. pulchellus occur together. All are common there. They have been studied extensively elsewhere, as on Guana Island, but never at Sage Mountain or any ecologically similar site. We have typically looked at components of niche segregation and resource partitioning including activity periods, perch heights, perch diameters, home ranges and territories, and population densities. Data generated on these anole lizards at Sage Mountain would be directly comparable to a large body of existing (but largely unpublished) data, and extremely valuable. These lizards are diurnal, active, and actually fun to observe and study.

Please make and distribute copies of these comments, and copies of the enclosed papers too, to any-and everyone interested or involved in this project. I would enjoy being directly involved myself, if there was an appropriate role for me. I shall, in any case, be extremely interested in hearing how things develop.

All best wishes,

James Lazell, Ph.D.

P.S.: I cast my vote for bridled quail dove as the B.V.I. national bird, but surely Rowan Roy gets at least three votes to my one!

Dear Skip,

Hi! I'm still hoping to get some nice slides of BVI fauna from you. I developed the boa pictures with bad chemicals and everything is psychadellic yellow and green. Meanwhile, Everton made me a nice, heavy, well-fitting top to the boa aquarium so that I didn't have to use books anymore. The snake promptly escaped (on Tortola) and was probably eaten by my neighbor's cat.

I hear that Numi may be here in March. I've been trying to e-mail her but all my messages are returned. If you see her, please ask her to e-mail me at my regular address so I can respond.

I'm organising a nature and environment seminar series this semester at the college, and I've lined up all kinds of people from the community to give talks. I'm planning to talk about the native fauna of the BVI and was hoping I could get copies of some of your nice slides. I would need them by the middle of March. I especially need the following critter photos (if you have them):

boa
Liophic exiguus
tortoise
bridled quail dove
amphisbaena
typhlops
frigate
booby
humming bird
kingbird
any wading birds or sea birds other than pelicans
pintail ducks

If you have any of these and could provide copies for me I would greatly appreciate it.

I'm hoping to run a marine conservation short course (one week, intensive) this summer. Should be interesting, and I'll let you know how things work out.

Love, Lianna

BVI Salt Pond ecology:

Since 1987 I have been sporadically collecting ecological data from salt ponds in the BVI. Although this data has unfolded some interesting and previously unknown ecological cycles, some of which I presented at the BVI symposium in 1991, I do not yet have repeated data from all seasons and from a number of different ponds. The only pond I have continuous year-round data from is Guana's, while my data from other ponds is occasional, with none or few repeated samples per pond. Documenting the ecological cycles of hypersaline ponds in the VI is extremely interesting research not only because there is very little known about these harsh and unique habitats, but also because they are the sole local breeding habitat for a number of native birds, including the endangered Caribbean Flamingo and the Bahama Pintail Duck, and because the ponds play an important role in nutrient recycling and sediment deposition, upon which nearby coral reefs depend. I would like to spend the next two years collecting extensive ecological data from ten to fifteen ponds on a number of different Virgin Islands, each sampled on a monthly basis. Included in this sampling will be bird censuses, plankton sampling and identification, water chemistry analysis, and rainfall measurements. Margaret Collins is trying to locate experts in crustacean identification to assist me in identifying some of the more difficult invertebrate fauna.

Over the course of the two years, I project that this research will cost about $50,000. This will include equipment (water analysis chemistry kits, dissolved oxygen meter, atmospheric weather monitoring stations, collecting equipment, laboratory chemicals and other supplies, etc.), weekly travel to islands with salt ponds targeted for sampling in this study, photography and accurate mapping of salt ponds, reference materials, two trips to U.S. for library and museum collections research, applied correspondence costs (phone, shipping, consulting costs for having species identified), etc.

I hope to cover most of this cost by obtaining two grants from U.S. granting agencies. I plan to apply for funding from the MacArthur Foundation, the Audubon Society, The Nature Conservancy, and possibly from the US Department of Natural Resources or the Fisheries Department in the USVI. However, I will not be able to secure this funding for more than six months, and I would like a $5,000 start-up donation from The Falconwood Corporation. In addition, I would like the support of the Guana Island Wildlife Sanctuary in the use of their scientific equipment, library and limited access to phone (especially St. Thomas line), fax, and Rushit mailbox. I would greatly appreciate the opportunity to stay on Guana through the October science month, after which I will reside on Tortola, and use the scientific facilities on Guana occasionally (perhaps on weekends). Staying on Guana during the terrestrial science month will allow me to interact with

other scientists, get insight on my research and methods for grant-seeking, and have access to research facilities.

Lianna Jarecki, 1994

23.i.94

James L. Ortiz
31162 Boca Raton Pl.
Laguna Niguel, CA 92677

Dear James:

We certainly do need a spider study! For years I have tried to interest my one-time protegé and good friend Jonathan Coddington in Guana, but he says he is too busy. Then, mantispid expert Kevin Hoffman (Clemson U.) said he would come make a start, but the years go by and it's always "next year."

Even if Hoffman does come, we could certainly use two of you. Although no one has ever come explicitly to work on spiders, lots have been collected by the long list of entomologists who have worked the island. I have put a few (like the "tarantula" Cyrtopholis bartholomei and a gorgeous yellow, orange, and green little orthognath) in MCZ. Scott Miller, now chairman of entomology at Bishop Museum, Honolulu, no doubt put spiders in USNM, and probably has more now at BPM.

Mike Ivie, Montana State, got lots of spiders as catch incidental to his beetle trapping. Jason Cryan and Charles Bartlett, at NCSU, Raleigh, got more.... So, there are lots of extant specimens.

What I would suggest is that you contact those folks and borrow their specimens for identification. Are you on staff at a museum now? If so, borrowing specimens should be easy. All those people need their spiders identified. Then, get back to me with an initial list, noting any items of special interest. If you have a good proposal, there should be no problem coming next October.

Usually we provide room and board (pretty luxurious!), all beer, fruit juices, and soda pop, and wine with dinner. We also schedule trips to other islands -- all included. Most participants pay their own airfare, but we have six tickets in the budget. Competition for the tickets is stiff and they usually go based on need. For example, a good student doing a Ph.D. thesis that includes Guana has a better than a comfortably retired emeritus professor with a Guggenheim.

In addition to a proposal, I will need a CV and list of publications. In entomology, I defer all staffing decisions to Scott Miller (who has coordinated that program since our beginning in 1982). Of course, spiders are not insects, but I would still need outside advice.

I hope to hear from you soon.

Best wishes,

James Lazell, Ph.D.

Roy Snelling

COLLECTING ON GUANA ISLAND: I managed two visits to Guana Island in the British Virgin Islands, one from mid-April to mid-May, the other mid-June to mid-July. Bees were very scarce for most of the period of the first trip, which was at the conclusion of the dry season. We had some rain about a week before I left and things were just getting interesting. *Centris smithii* Cresson was present in some numbers in the more xeric area of the island (Long Man's Point); females were nesting in very hard-packed soil of a trail; males patrolled the area, but were scarce. Females were collected only at flowers of *Stigmaphyllon periplocifolium* (Malpighiaceae). I also found some specimens of *C. haemorrhoidalis* on the same plant. A new record for the Island is *C. decolorata* (Fabricius); this bee is found over much of the Caribbean, most often nesting in sandy areas. I got one female in April on *S. periplocifolium*, then several males in late June on flowers of *Melochia tomentosa*; males of *C. smithii, C. haemorrhoidalis*, and *C. lanipes* were also taken on the same flowers.

Collecting during both trips was challenging, to say the least. There simply weren't that many bees around. There were also few native plants in bloom.

I did come up with two additional species, however, for Guana Island. The first of these was *Mesoplia rufipes* (Perty), a very handsome ericrocidine parasitic on *Centris* species. It apparently ranges from Cuba and Hispaniola south to northern South America, as well as being present in Central America. Its presence on Guana can hardly be considered a surprise.

The same cannot be said of the second novelty collected there in late June. A single male of *Hylaeus (Paraprosopis) wootoni* (Cockerell) was collected from flowers of *Ipomoea pescaprae* at White Beach. This bee is native to the southwestern United States and adjacent Mexico, extending from Colorado and Coahuila to the Californias. It will be interesting to see whether or not this bee will become established on Guana.

RESEARCH PROJECTS: Foremost is the effort to finish up on the massive rewrite of the Gerry Stage revision of *Hesperapis*. There have been too many delays and I want to get rid of the thing. My biggest difficulty is that the entire section on biology and nesting behavior needs to be rewritten and I just keep putting it off. There are also a few more illustrations to do, as well as all the maps.

Also high on the list of things to complete is the revision of the North American (incl. "Central America") *Hypochrotaenia*. The keys are pretty well done, but I still have to do the species descriptions (several new species) as well as all the illustrations and maps. Big job.

Two shorter projects: the *Centris* of the Greater Antilles (Bahamas to Virgin Islands) and the *Hylaeus* of the Greater Antilles. Some new synonymy in *Centris*; several new species of *Hylaeus*, including one from Guana Island and several from Cuba.

North Carolina State University

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May 25, 1994

Dr. James (Skip) Lazell
The Conservation Agency
3930D Marcom St.
Raleigh 27606

Dear Dr. Lazell:

The purpose of this letter is to formally request to be considered for participation in the Guana Island project and to inform you of my goals should I be accepted.

My goals are:

1). Find the nymphs and host plant(s) of *Deiroderes inermis* Ramos, a rare treehopper (Homoptera: Membracidae) found on Guana last year. Nymphs of *D. inermis* are unknown and needed to place the species in a subfamily.

2). Find nymphs (and host plants) of Kinnarids, which are virtually unknown (Lois O'Brien, personal Communication). Immature kinnarids have not been described. They are reported to feed on roots (L. O'Brien in Stehr, F. 1991).

3). Search for fulgoroids that were not recorded on the previous trip, especially by collecting in habitats not previously sampled. I will also try sweeping during the early evening hours to test a suspicion that some taxa are active only during the cooler hours of the day, as are some Ants (C. Collingwood, pers. comm.).

4). Associate host plants (or habitats) with as many fulgoroid species found on the island as possible, especially those found on woody plants. I will review available sources (esp. Caldwell and Martorell, 1950, Auchenorrhyncha of Puerto Rico) prior to the trip.

5). In addition to the above, I will also be looking for some edaphic taxa (Diplura, Symphyla, etc.) for Dr. T. Allen (University of Delaware).

I expect that I will need some help identifying the plants. I will be looking into keys/guides to the flora of the BVI between now and October, and have some leads in that regard from a botanist here. I expect to either buy or make a half-sized plant press to use on Guana, but I am, as yet, undecided as to whether I should get a permit to carry plant material back to the U.S., or I should plan on getting everything identified on Guana. I could plan on leaving pressed plant specimens with somebody if need be. I would appreciate your thoughts on this matter.

In terms of exactly when I could go, I can suggest two options, dependant mostly on which is most convenient with you, Oct. 1 - 12 or Oct. 8 - 19. I think I would prefer the latter, although it probably would make little difference. These dates both include the Columbus day holiday (on purpose). I would like to overlap with Mike Ivie, if possible. I understand he expects to go to Guana early in the month. Thank you for your consideration and look forward to hearing from you again.

Sincerely,

Charles R. Bartlett

BISHOP MUSEUM

CELEBRATING A
CENTURY OF DISCOVERY

June 6, 1994

Mr Charles Bartlett
Dept. of Entomology
North Carolina State University
Box 7613
Raleigh, NC 27695-7613

Dear Mr Bartlett:

Thank you for your letter of May 25th. We will be happy to provide any *Pissonotus* for your studies. I have asked Keith Arakaki to handle your loan request.

With regard to specialists who might wish to borrow your Guana specimens, the two best contacts are:

Roy Snelling, Natural History Museum of Los Angeles County, 900 Exposition Blvd., Los Angeles, CA 90007. He is working on the ants, aculeate wasps, and bees of Guana. He has collected multiple times on the island.

Mike Ivie, Dept. of Entomology, Montana State University, Bozeman, Montana 59717. He is coordinating a group project on the Coleoptera of the Virgin Islands. He has extensive material now from Guana.

Several other entomologists have collected on Guana, but they are either working on taxa that require specialist collecting (e.g., termites) or they are not working on faunal papers.

I think I mentioned in an earlier letter that my Guana collections up to 1986 are deposited at the Smithsonian, and probably represent the largest single block of general collecting that has been done there.

If you collected Lepidoptera on Guana, I would like to see them with two limitations: I do not need butterflies that were already documented in the enclosed reprint and I do not want microlepidoptera in poor condition.

Thank you for your interest in Guana Island. Please keep me informed as your studies progress.

Sincerely,

Scott E. Miller
Chair, Natural Science

cc: J. Lazell
Internet: scottm@bishop.bishop.hawaii.org

The State Museum of Natural and Cultural History
1525 Bernice Street • P.O. Box 19000A • Honolulu, Hawai'i • 96817-0916
Telephone: (808) 847-3511 • Fax: (808) 841-8968

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DISPERSAL OF PLANT PESTS IN THE VIRGIN ISLANDS

SCOTT E. MILLER

Bishop Museum, Box 19,000-A, Honolulu, Hawaii 96817

On October 26, 1990, Greg Mayer, Tina Kuklenski, and Scott Miller sampled invertebrates from a large shipment of potted plants being unloaded at Guana Island, British Virgin Islands (BVI). Becker and Miller (1992) provide background on Guana Island. The plants, including many specimens of several species of palms, were being imported from nurseries in southern Florida for landscaping. The importers had apparently met all BVI regulations and had checked in with government authorities in Tortola before the barge proceeded to Guana Island. The shipment was infested with large numbers of insects and snails, some of which have been identified as follows:

Cockroach (Blattodea: Blaberidae)
Pycnoscelus surinamensis (Linnaeus), Surinam cockroach

Mealybug (Homoptera: Pseudococcidae)
Dysmicoccus brevipes (Cockerell), Pineapple mealybug

Ants (Hymenoptera: Formicidae)
Brachymyrmex obscurior Forel
Hypoponera opaciceps (Mayr)
Odontomachus ruginodis Wheeler
Paratrechina longicornis (Latreille), Crazy ant
Paratrechina pubens (Forel)
Pheidole morerens Wheeler

Snails (Mollusca)

Lamellaxis gracilis (Hutton)

Polygyra cf. P. cereolus (Muhlfeld)

Praticolella griseola (Pfeiffer)

Succinea cf. S. luteola floridana Pilsbry

Although some of these species are native to the Puerto Rican Bank, most are introduced species that are now widespread in the Caribbean region, including southern Florida (Godan, 1983). Most are known from the Puerto Rican Bank (Wolcott, 1950-1951). Several major agricultural pests are included, such as those with common names listed. The presence of this many invertebrates on this shipment indicates the ease of dispersal of agricultural pests.

Introduced insect pests are a major problem for North American agriculture (Dowell & Krass, 1992; Sailer, 1978, 1983; U.S Congress, 1993). In addition to being agricultural pests, introduced insects and snails appear to be the primary cause of extinction for native invertebrates on islands (e.g., Howarth, 1990; Howarth & Ramsay, 1991). Vectors of human disease, such the Asian tiger mosquito, can also be spread by commerce (e.g., Francy et al., 1990; Mitchell et al., 1992). The recent spread of two giant African snails to Martinique is a stark example of the problem of continued pest dispersal (Mead & Palcy, 1992).

Identifications were made by K. Emberton (Academy of Natural Sciences of Philadelphia, snails), D.R. Miller (Systematic

Entomology Laboratory, U.S. Dept. of Agriculture, mealybug), R.R. Snelling (Natural History Museum of Los Angeles County, ants), and J. Strazanac (Bishop Museum, cockroach). Voucher specimens retained by specialists, except snails.

SUMMARY

Given the threat that introduced insects and snails present to agriculture, human health, and conservation management, and potential economic consequences of such introductions, island governments should create and implement policies for the inspection of agricultural materials, including provisions for fumigation and quarantine as necessary.

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The Biogeography of Ground Beetles of Mountains and Islands

GENERAL EDITORS

Dr. G.R. Noonan
Dr. G.E. Ball
Dr. N.E. Stork

Guana I., p. 114

5.

Geographical Distribution and Evolution of the Selenophori (Harpalini) and *Apenes* LeConte (Lebiini) in the Antilles (Coleoptera: Carabidae)

GEORGE E. BALL.

Department of Entomology, University of Alberta, Edmonton, Alberta Canada T6G 2E3

ABSTRACT

Based on evidence derived from: (1) distribution patterns of the Antillean taxa of selenophorine Harpalini (39 taxa in seven genera) and the lebiine genus *Apenes* LeConte (32 taxa); (2) degree of differentiation between islandic stocks and mainland relatives; and (3) the assumption that macropterous adults fly and can cross water barriers, two questions are discussed: when did the extant species reach the islands, and how did they do so?

Exogenous taxa (those with conspecific populations on both mainland and in the islands) of both groups apparently reached the islands relatively recently, because there are no detectable differences between islandic and mainland populations. Furthermore, the adults of all exogenous taxa are capable of flight, and probably flew to reach the islands, though transport by man cannot be ruled out. The principal route into the Antilles has been *via* the Lesser Antilles, with northern South America as the source area. A secondary route has been *via* the Bahamas, with southeastern United States as source area.

The autochthonous taxa – a monobasic genus confined to Hispaniola, species without mainland counterparts, and endemic subspecies with mainland relatives – probably reached the Antilles earlier. Such invasions were by way of Middle America, based on the relative proximity of this area to the Greater Antilles, where most autochthonous taxa are concentrated. Because of a range in distinctiveness among the autochthonous taxa, there seems to have been a range of arrival times, probably extending throughout the Tertiary Epoch.

Reduced size of water gaps between Middle America and the Greater Antilles would make the islands more accessible to mainland stocks, and especially those with volant adults, such as the selenophorines and *Apenes*. Geological evidence indicates that, at various times during the Tertiary Epoch, water gaps were smaller than they are now. Thus, members of the carabid taxa in question could have reached the Greater Antilles by oversea dispersal more easily than at present. The dispersalist hypothesis postulates that, in fact, such events and others that enhance flight potential, account for the populating of the Antilles.

A competing hypothesis, based on less convincing geological evidence, states that, in early Tertiary time, the antecedents of the Greater Antilles (the proto-Antilles) were part of an isthmus or chain of islands that more or less joined Middle and South America. If so, the phylogenetically more isolated selenophorine and *Apenes* stocks may have been part of the proto-Antillean biota, and thus reached their present islands either overland or by short flights from South America. This is the vicariance hypothesis.

Either the dispersalist or vicariance hypothesis could explain the present distribution of the extant autochthonous selenophorine and *Apenes* species. Resolution of this problem in zoogeographic explanation will be achieved in terms of the constraints imposed by knowledge of the geological history of the Caribbean Basin. Until this resolution is achieved, it seems best to accept the dispersalist hypothesis because such will explain the arrival in the islands of the exogenous stocks, and by extension, the different times of arrival of the ancestors of the autochthonous stocks.

30

Ventral surface rufo-piceous medially to piceous laterally. Antennae with antennomeres one to four piceous, antennomeres five to eleven dark rufous. Labrum piceous. Palpomeres rufo-brunneous. Legs rufo-piceous to piceous.

Macrosculpture Head with frons and vertex sparsely punctate; posterior portion of vertex transversely rugulose. Pronotum transversely sparsely rugulose medially, more densely so laterally. Elytra smooth.

Microsculpture Head with clypeus, frons and vertex with microlines nearly effaced, mesh pattern isodiametric. Labrum with microlines fine, mesh pattern isodiametric. Pronotum with disc with microlines fine, mesh pattern slightly transverse (sculpticells *ca.* two times wider than long); lateral areas with mesh pattern isodiametric. Elytral disc with microlines fine, mesh pattern isodiametric; outer intervals with microlines coarser, mesh pattern slightly transverse.

Lustre Dorsal surface generally shining, not iridescent, but elytra laterally slightly duller. Ventral surface iridescent.

Chaetotaxy Each elytron with interval three with two setae. Abdominal sternum VII of males with one pair setae.

Head Eyes large, but not protruded; subgenae narrow. Labium with mentum toothed.

Prothorax Pronotum trapezoidal, greatest width in apical third. Anterior margin moderately concave; posterior margin slightly lobed medially; lateral margins straight to slightly sinuate before antero-lateral angles. Antero-lateral angles broadly rounded; postero-lateral angles slightly projected, acute. Lateral grooves narrow; postero-lateral impressions absent. Disc sloped gradually and evenly each side, lateral areas not broadly flattened.

Pterothorax Metathorax normally developed, metepisterna markedly longer than wide.

Elytra Humeri broadly rounded. Apex with sutural angles acute. Interneurs shallowly impressed, very finely punctate. Intervals flat.

Hind wings Fully developed.

Geographical distribution This species is known from the island of Jamaica, only, at low elevations.

Chorological affinities The range of this species is overlapped by those of the related species *A. scobifera* Darlington.

Phylogenetic relationships *Apenes darlingtoni* and *A. scobifera* are probably closely related, but a more precise statement cannot be made now.

Material examined Type specimens, only.

Apenes iviei Ball and Shpeley, new species

Derivation of specific epithet Based on the surname of Michael A. Ivie, who sent us most of the type material of this species, and who organized and undertook a major survey of the hexapod fauna of the Virgin Islands.

Type material HOLOTYPE male, labelled: VIRGIN IS: St. John/ Est. Carolina/ N W of Coral Bay/ 24 May 1982, 250 ft.; at uv light/ W. B. Muchmore (USNM). ALLOTYPE female, labelled: VIRGIN IS: St. John/ Estate Carolina/ N W of Coral Bay/ 04 June 1982, 250 ft.; at uv light/ W. B. Muchmore (USNM). Fifty eight PARATYPES, sex and label data as follows. Three males: same as holotype, except 16 MAY 1982; (MAIC). Four males: same as holotype, except 18 MAY 1982; (MAIC). Two males: same as holotype, except 20 MAY 1982; (MAIC). Male, two females: same as holotype, except 21 MAY 1982; (MAIC). Male: same as holotype, except 22 MAY 1982; (MAIC). Two males: same as holotype, except 31 MAY 1982; (MAIC). Male: same as allotype (MAIC). Male: USVIs St John (*sic*) / Windberg/ WBM 75g27; (CDAE). Male: VIRGIN IS: St John/ nr.top Bordeaux Mt./ 27FEB1984, ground/ litter W. Muchmore; (MAIC). Male, female: VIRGIN IS: St John/ Catherineberg/ 14 MAY 1984,between/ lg. rocks, Muchmore; (MAIC). Male: VIRGIN IS:

St John/ Estate Browns Bay/ Brown Bay, shaded/ litter under low/ trees, 04 MAR 1984; (MAIC). Female: VIRGIN IS: St John/ Est. Caneel Bay/ Margaret Hill, top/ 05 MAR 1984, moist/ forest veg. Muchmore; (MAIC). Male: VIRGIN IS: St. John/ Est. Maho Bay/ Windberg ruins/ 20 & 31 MAY 1979/ W.B. Muchmore; along old/ walls; colr. W. B. Muchmore; (MAIC). Male: VIRGIN IS:/ St. John/ Lameshur Bay/ V.I.E.R.S./ 15 AUG 1980/ At UV Light; M.A. Ivie/ colr; (MAIC). Female: same as previous ex., except PN figured; (MAIC). Female: VIRGIN IS:St.John/ LameshurBay, VIERS/ MAR-MAY1984.pit-/ fall,W.B.Muchmore; (MAIC). Male: VIRGIN IS: St John/ Little Lameshur Bay/ 12 JAN 1986, litter at/ base of lg. tree/ W.B. Muchmore colr.; (MAIC). Four males, three females: VIRGIN IS: St John/ Catherineberg/ 14 MAR 1984/ W.B. Muchmore. colr./ lg. rocks und. mango; (MAIC). Male: VIRGIN IS: St John/ Lameshur Bay, VIERS/ 13-14 JAN 1986/ UV light/ W.B. Muchmore colr; (MAIC). Female: VIRGIN IS: St John/ Little Lameshur Bay; litter under/ large tree; 17 MAR 1984; W.B. Muchmore/ colr.; (MAIC). Male: VIRGIN IS: St John/ Salt Pond area/ 12 JAN 1986, litter/ W.B. Muchmore colr.; (MAIC). Female: VIRGIN IS: St Thomas/ Magens Bay/ Gut at S. End/ 01 AUG 1980/ At UV Light; M.A. Ivie &/ C.A. Jennings/ Colrs.; (MAIC). Male, female: BRITISH VIRGIN ISL./ Guana Island, 0-80m/ 13-26 July 1986/ S.E. Miller & M.G. Pogue; (USNM). Male, four females: BRIT. VIRGIN ISL./ Guana Island, 0-80m/ 5-23 July 1985/ S.E. & P.M. Miller; (USNM). Male: PUERTO RICO: Ciales/ V-1978, J. Micheli/ blacklight trap; (JMPR). Female: PUERTO RICO:/ Rd.10 Km.24/ 14- V -1977/ J. Micheli; on/ ground; (JMPR). Female: PUERTO RICO:/ Rd. 10, Km. 24; 10-VI-77/J. Micheli; black lite; (JMPR). Male: PUERTO RICO:/ Rd.10 Km.24; 12-VI-77/ J. Micheli; fluorescent/ lights; (JMPR). Male, female: PUERTO RICO:/ Rd. 10 Km 24/ 2-8-IX-1977/ J. Micheli; in black/ light trap; (JMPR). Male: PUERTO RICO:/ Rd. 10, Km 24; 19-26-XI-1977/ J.Micheli; in black/ light trap; (JMPR). Male: PUERTO RICO/ Rt.132 K.20/ V : 26.1972/ at light; Julio Micheli coll.; (USNM).Male: PUERTO RICO/ Rt.132 K.20/ V : 24,1972/ at lights; Julio Micheli coll.; (JMPR). Two females: Puerto Rico/ Rd. 132 K.20; 6-VI-75/ J.Micheli; (JMPR). Female: Puerto Rico/ Rd.132 K.20; 2-VIII-75/ J. Micheli; at lights; (JMPR). Male: Puerto Rico/ Rd.132 K.20; 6-VII-75; at lights; (JMPR).

Type locality Estate Carolina, northwest of Coral Bay, St John Island, Virgin Islands, U.S.A.

Recognition This is the only species in the Puerto Rico- Virgin Islands archipelago whose adults have three or more discal setae per elytron. Additionally, the elytra, though predominantly dark, have narrowly pale humeri, and small pale spots around the discal and marginal umbilical setigerous punctures. Specimens are small, with average SBL less than 5 mm. Adults of *A. prasina* resemble adults of *A. iviei*, but those of the former species have uniformly dark elytra, and only two discal setae per elytron.

Description Characteristics as recorded for genus, above, and as follows. Data about variation in SBL and in values for ratios HW/PW, PL/PW and PWB/PW in Table 5.2.

Colour Dorsal surface with head and pronotum cupreous to bright green; elytra of most specimens with faint cupreous sheen, bicoloured, dark piceous, each elytron with testaceous humeral fascia in intervals six to nine, testaceous spots around discal and umbilical setigerous punctures, lateral margin testaceous to rufo-piceous, and indistinct preapical fascia in intervals one, two, five, seven, and eight; epipleura testaceous to rufo-piceous. Ventral surface brunneous to rufo-piceous medially, piceous laterally. Antennae and palpomeres rufo-testaceous to rufo-piceous. Labrum rufous. Mandibles rufous basally, with apices piceous. Legs piceous.

Macrosculpture Head with frons and vertex sparsely, finely punctate. Pronotum medially rather sparsely rugulose, more densely so laterally, surface quite irregular. Elytra apparently rugulose, pattern of microsculpture irregular.

Microsculpture Head with clypeus, frons and vertex with microlines fine, mesh pattern isodiametric. Labrum with microlines fine, mesh pattern slightly transverse medially, isodiametric laterally; sculpticells convex, surface thus beaded. Pronotum with microlines

Chemical Ecology of Forest Insects
Pacific Southwest Research Station
Forest Service, U.S. Department of Agriculture
P.O. Box 245
Berkeley, California 94701

May 7, 1994

James D. Lazell, Ph.D.
The Conservation Agency
3930 D Marcom Street
Raleigh, North Carolina 27606

Dear Skip:

Here is a proposal for research activities by Barbara Thorne and me on Guana Island this October. Margaret's plans for Guana are still being formulated. She has indicated to me that she wants to go to Guana during Scientists' Month, provided she can get mosquito-free accommodations. She also indicated that she wanted to make additional collections of drywood termites in mangroves, but I will let her represent her goals and needs to you.

I hope our proposal isn't more reading than you want. In brief, we want to continue the studies we initiated over the past years. Last year we perfected some of our sampling techniques that will make our job much more efficient (and require less solvents and extractions!) this year. We should be able to get our field work done in short order and then leave. I plan to stay a few days more just to make sure everything gets done. If we're done on time, then I could start collections of other groups of termites to increase data base on the remainder of the termite fauna of Guana or other islands.

We realize that bed nights are likely to be a problem. However, if there is any chance that we can bring one or two assistants, we would like to give Nancy and Lori opportunities to participate in the field end of the research, not just handling samples brought into the lab. As I stated on the phone, we are in a position to pay for some bed nights or make other arrangements, such as those you worked out last year.

Either Barbara or I will be contacting you in a week or two to see that you received our proposal and to hear what you think of it. If we need to discuss anything more I can be reached @ 510-559-6471 (office), 510-283-5568 (home), or 510-559-6499 (FAX).

Sincerely,

Mike

MICHAEL I. HAVERTY
Chief Research Entomologist

Enclosure

cc Barbara L. Thorne, Margaret S. Collins

Research Proposal to the Conservation Agency

TERMITES OF GUANA ISLAND: 1994

Barbara L. Thorne, Michael I. Haverty,
and Margaret S. Collins

We have been studying the diversity of the termite fauna on Guana Island and the other islands in the British Virgin Islands since 1990 (Collins, Thorne, and Haverty, in preparation). The termite fauna of Guana Island is now well documented. To facilitate precise species discrimination and diagnoses, and to enable valid interpretation of biogeographic relationships of individual taxa, we have used the chemotaxonomic tool of hydrocarbon phenotypes in our studies of all termite taxa in the Caribbean Basin. Our present work is focused on use of hydrocarbons to better understand the chemical ecology and colony organization of select termite species on Guana Island.

The most conspicuous, and apparently abundant, species of termite on Guana Island is the arboreal nesting *Nasutitermes acajutlae*. We have recently focused our attention on developing methodology for characterizing and distinguishing individual nests on the basis of their cuticular hydrocarbons. Evaluation of comparative techniques has now yielded an efficient and reliable protocol for hydrocarbon sampling. In the course of sampling for intraspecific variation during the 1992 and 1993 field seasons, we discovered that individual nests or foraging groups of *N. acajutlae* can be uniquely characterized on the basis of cuticular hydrocarbons.

In the 1994 field season we will continue to assess seasonal or annual changes in hydrocarbon mixtures from our same study colonies of this species. Knowledge of this variability is crucial for using this technology to further study colony interactions and competition for resources, as well as the biogeography of termite species. Second, we will assess similarities in hydrocarbon mixtures of workers or soldiers within each nest with these same castes found in foraging tubes associated with the same nest. If there

is a quantitative similarity between nestlings and foragers, we will then be able to associate distant foraging populations with a given nest. This knowledge could advance the study the foraging biology of this ecologically important species of termite without resorting to difficult marking techniques. Third, we will initiate studies of the heritability of cuticular hydrocarbon mixtures. By studying inbred and outbred groups we will be able to determine whether incipient colonies inherit the phenotypes of their parents, or establish their own "colony identity/phenotype" even if highly related. The ultimate goal of this research is to use analytical chemistry techniques to study biogeographic patterns, population ecology, and structure of termite colonies in the Caribbean.

Specific Objectives of 1994 Program

I. Annual Variation of Hydrocarbon Phenotypes Among Nests of *N. acajutlae*.

The hydrocarbon mixture in the epicuticle of termites is presumed to be under genetic control and is known to be affected only slightly by exogenous factors such as temperature and relative humidity. The relative proportions of the components in the hydrocarbon mixture vary among individuals and castes within a colony, among colonies within a geographic area or "population," and among populations of a species. Quantitative differences are greatest among populations within a species and least within a colony.

We propose to characterize cuticular hydrocarbon mixtures for all nests of *Nasutitermes acajutlae* within 50 m of White Bay Beach. We will also locate, mark, and sample *N. acajutlae* nests at several other distant locations on Guana Island. To provide out groups, or colonies that are certain to not be connected with the nests we are studying, we will sample *N. acajutlae* nests on Tortola and Puerto Rico.

To standardize nest comparisons and to eliminate within nest variability, cuticular hydrocarbons will be collected from soldiers and workers. Furthermore, to eliminate any variability due to time of year, all samples will be collected during the month of October. Soldiers will be collected by

shaving off a 15-cm tangential slice of the nest on the uppermost surface. As the soldiers pour out of the "wound" water will be sprayed on the cut surface and two pieces of corrugated paper will be affixed to the open breach. When the soldiers cover the paper, it will be removed, soldiers tapped into a dish. The process will be repeated until about 1000 soldiers have been collected. To collect large quantities of workers, the procedure is similar, except the paper will be left on the "wound" for 30 to 60 minutes. After this much time has passed, most of the soldiers will have retreated back into the nest and workers will congregate on the paper to begin repair activities. Workers will then be tapped into a dish and separated from any soldiers until a sample of at least 400 mature workers has been collected.

Soldiers will be separated into four subsamples of 200 and workers into subsamples of 100. Samples will be dried for extraction later in the Forest Service laboratory in Albany, CA. Collections from the outer islands will be made simple by excising large sections of nest material, placing these in plastic bags and returning them to the Guana Island Termite Lab for processing, as above. Cuticular hydrocarbons will be processed and quantitatively characterized by established, standard procedures at the Pacific Southwest Forest Sciences Laboratory in Albany, CA, during the winter and spring. Comparisons of hydrocarbon mixtures among nests will involve discriminant and canonical analyses of relative proportions of the hydrocarbon components. Similarities of nests will be determined by calculation of Mahalanobis distances.

II. Association of Foraging Groups with Nest.

To study the ecology of foraging of *N. acajutlae*, it is important to be able to associate a distant foraging party with its nest. We will determine whether the hydrocarbon profiles of workers and soldiers in foraging tunnels positively associated with a nest are the same. Concurrent with sampling hydrocarbons of workers and soldiers in nests (see above) we will gather foraging parties from tubes that are directly associated with the nest. We will endeavor to collect enough individuals to have several replicates of 100 workers and 200 soldiers for statistical comparisons (MANOVA) between nest termites and foragers.

We will also collect foraging parties from tubes and wood that is not readily assignable to a given nest. These parties will be plotted in relation to known nests in hopes of eventually associating the group with a given nest. Hydrocarbon mixtures will be compared with mixtures of sampled nests or foraging groups associated with a nest with MANOVA. From these data we hope to be able to use characterization of hydrocarbon profiles to associate foragers with their nest.

III. Heritability of Hydrocarbon Phenotypes.

During the 1994 field season, we propose an important extension of this study: to assess heritability of hydrocarbon phenotypes by breeding *Nasutitermes* alates within and among the White Beach colonies. In October 1993 we set up a pilot series of alate breeding experiments to determine feasibility and survivorship. Mature, winged alates were collected from field colonies. Individual males and females were tethered and allowed to fly for a minimum of 60 seconds to simulate their dispersal flight. After flight the wings were disengaged along the basal suture and reproductive pairs were placed in individual 4.5 cm diameter Petri dishes lined with moist corrugated paper. Small pieces of sea grape were added as substrate for food and nesting material. Both inbred and outbred series of alate pairs were established.

Within days after pairing the termite pairs were transferred to Thorne's laboratory in Maryland as authorized by a U.S.D.A. permit to import live pests. Growth and survivorship of these incipient termite colonies have been monitored for the past six months. As of April 1994, we have had over 80% survivorship of incipient colonies established on Guana Island during October 1993.

When these young colonies yield sufficient numbers of worker progeny, we will characterize their hydrocarbon phenotypes, which can then be interpreted in light of their known parentage and the phenotypes characterizing the colony of origin of each parent. The prospect of this study represents an exciting extension of any work to date on hydrocarbons in termites. Breeding alate termites and rearing incipient colonies is a challenge and has been accomplished only rarely for any purpose, but we have

established a successful protocol and anticipate that this will be a novel and highly productive line of research.

Mature alates are present in *N. acajutlae* colonies on Guana Island during the month of October. In 1994 we plan to set up a minimum of 300 incipient colonies which will be hand-carried to the United States, where they will be reared for hydrocarbon analysis.

Requested Time on Guana Island, October 1994:

We are requesting research time on Guana Island as part of Scientists' Month to provide continuity with the studies of previous years. The previous years cannot be interpreted as completely without continued study. Our specific request is: B.L. Thorne, 7 bed nights; M.I. Haverty, 10 bed nights. The field work proposed here will be done by Thorne and Haverty within 7 to 10 days. However, if space is available, we would like to bring one or two assistants for up to 7 days each to assist the principal investigators and to participate in other research activities on Guana.

Any time in October would be satisfactory, but the latter part of October would be best for the maturity of *N. acajutlae* alates. We found that Fallen Jerusalem provided the best laboratory conditions. If at all possible, we would like to be assigned there. Please call to coordinate.

Our Contributions:

1. Salaries of Thorne (10%), Haverty (10%), Assistants (15 to 30%)
2. Round trips to Tortola, supplies and mass spec and computer time.
3. Cost of one Boat Trip to outer island ($350)
4. Publications:

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UNIVERSITY OF MARYLAND AT COLLEGE PARK

COLLEGE OF LIFE SCIENCES • DEPARTMENT OF ENTOMOLOGY

23 March 1995

Dr. James D. Lazell
The Conservation Agency
1140 Monroe Street
Jackson, Mississippi 39202

Dear Skip,

Enclosed are a couple of pieces of progress. The nodule paper is finally launched. Of more interest to you, we have finally untangled the taxonomic mess surrounding *N. acahootlae* (correct pronounciation). It turned out to be a fun detective story with a remarkable twist that salvages the name *acajutlae*. I have run this by 2 hot-shot taxonomists already and they think it is clean, but I'll await your opinion, and I plan to confirm it yet again with people at the Smithsonian.

I hope that you are beginning to dig out from under the mountains of neglected accounting details - you've certainly got my compassion on that one!

With best wishes,

Barbara

Barbara L. Thorne
Assistant Professor

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AN ANTILLEAN TERMITE NAMED FOR A LOCALITY IN CENTRAL AMERICA: TAXONOMIC MEMORIAL TO A PERPETUATED ERROR

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Following publication of our paper on the taxonomy and biogeography of the Neotropical termites *Nasutitermes acajutlae* (Holmgren) and *N. nigriceps* (Haldeman) (Thorne *et al.* 1994), we received letters from two colleagues who independently noticed a potential problem with the nomenclature assignment of *N. acajutlae*. Our paper stated that *N. acajutlae* was originally described (as *Eutermes*) by Holmgren (1910) based on specimens from Acajutla, San Salvador, Bahamas (alate) and from St. Thomas, U.S. Virgin Islands (one soldier, one worker, and one alate). Both sets of specimens are labeled as COTYPES, and both reside in the collection of the American Museum of Natural History (AMNH). We presented evidence that Snyder's (1949) synonomy of *N.*

acajutlae as *N. nigriceps* was not justified. We resurrected the name *N. acajutlae* (Holmgren) for the species occurring in the Lesser Antilles from Puerto Rico east and south to Trinidad (not recorded from all intervening islands), into Guyana and possibly into other locations in South America.

This stated distribution did not include the Bahamas, and thus the San Salvador COTYPE of *N. acajutlae* was left dangling. As our correspondents noted, if the San Salvador alate specimen was not *N. acajutlae*, then we should at a minimum designate the St. Thomas COTYPE as the LECTOTYPE, awkward as that might be given that the species name was derived from a different locality. The name could be invalidated for the Lesser Antillean species if the San Salvador species had not been named previously and if someone identified precedence (or designated the LECTOTYPE) for that insect.

We were aware of those potential problems, but we had elected to postpone decisive action because Holmgren's specimen from San Salvador was perplexing to us, and we had not resolved its identity to our satisfaction. Banks (1919) commented that Holmgren's San Salvador alate is probably *N. rippertii* (Rambur) (originally described by Rambur (1842) and redescribed by Silvestri (1903)). Emerson (1925) continued to list San Salvador as the type location and as part of the range of *N. acajutlae*. Based on examination of the single alate specimen from San Salvador we found that it fit within the description of the *N. acajutlae / N. nigriceps* complex (we have not identified alate characters that discriminate the two species). However, it was odd because neither species has ever been collected from the Bahamas in a published record or in any museum collection that we examined. Furthermore, the Bahamas location was inconsistent with the rest of the biogeographic pattern that reflected species

distributions that were otherwise consistent with the historical geography of the region (Thorne *et al.* 1994). The conservative approach to indecision seemed to be inaction. However, primed by the two letters we focused on the problem yet again. This time we have resolved it with decisive and surprising results.

Holmgren's (1910 pp. 262-3) original description lists the type material and locations as follows: "Fundort. (locality) San Salvador: Acajutla - Mus. Hamburg. St. Thomas - WASSMANNs Sammlung (collection)." From that point on, nearly all reports assume that Acajutla, San Salvador referred to the island of San Salvador in the Bahamas (Emerson 1925; Snyder 1949, 1956). Banks (1919 p.484) appears to be the single exception. He comments, "(Holmgren's) adult from Central America is different, and different from what I consider the adult of *N. creolina.* The adult *E. acajutlae* of Holmgren from San Salvador is extremely close to and probably identical with the *E. rippertii.*"

Banks' conclusion is odd because *N. rippertii* has been found only from the Bahamas, Cuba, and Jamaica (Snyder 1949, Araujo 1977). Nevertheless, his casual comment regarding locality spurred us to track the location of Acajutla more aggressively than we had during our previous study. At that time we had tried to locate Acajutla on the island of San Salvador, but we failed to find the location. We dismissed that as a frequent idiosyncrasy of collection records: a small town may not persist, and often collectors list a relatively ephemeral campsite or a colloquial name for a locality. We had not been too concerned because the island of San Salvador is small, so issues such as altitude and exact location were not so important.

Upon launching a broader search for Acajutla, our suspicions after reading Banks were confirmed, and this unexpected breakthrough finally made the

whole puzzle fit. Acajutla is a small town nestled on the edge of San Salvador, the capital city of the country of El Salvador in Central America. El Salvador was founded as an independent country in 1840. Holmgren never listed the country in which his specimen was collected (possibly sent to him by some third party - Wassmann???), and no other authors ever identified El Salvador as a type location or in distribution lists. In fact, Holmgren's alate from Acajutla, El Salvador, is probably *N. nigriceps*, the close relative of *N. acajutlae*. Alates of these two species are at this point indistinguishable.

N. nigriceps was described by Haldeman (1853) from a specimen collected in western Mexico. Because that 1853 description precedes Holmgren's COTYPE designated in 1910, that alate from Acajutla, El Salvador, is dismissed as a COTYPE, leaving the series from St. Thomas, U.S. Virgin Islands as the valid and unique TYPE specimens for *Nasutitermes acajutlae* (Holmgren). (Roy - do you think that we should make this pristine and designate the soldier from the St. Thomas series as the lectotype? I always consider that inappropriate in social insect taxonomy because a complete caste series is so much more useful and should be encouraged...)

This concludes the saga of why a numerically and ecologically conspicuous termite in the eastern Caribbean is named for a locality on the Pacific Coast of Central America.

Sincere thanks to Drs. James D. Lazell and Roy R. Snelling for encouraging further investigation into this taxonomic puzzle.

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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 shows 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

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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 fluids (Light 1933), and occasionally other materials such as sand (Thorne and Haverty, pers. obs.). 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 U.S. and British Virgin Islands, and Trinidad (Araujo 1977, Thorne *et al.* 1994). There are isolated reports of members of the *N. nigriceps* "complex" from 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 any species. 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 normal 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 sheathes (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*. 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 and 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 British Virgin Islands. Observations of the nodule inclusions are reported. Nutrient analyses of nests without nodules and comparative chemical analyses of nodule material versus the surrounding "normal" carton matrix 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 Penninsula 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, B.V.I.) was analyzed in 1989-1990. Two of the nests (the *N. nigriceps* nest from Panama and a 1988 *N. acajutlae* nest from the B.V.I.) contained nodules. Chemical composition of the nodules and a sample of "normal" dark carton material was examined from those two nests, and samples of normal carton matrix from different parts of three

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 and Terry (1963) method as modified by Moore and 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 and 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 and 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). Replicates of all analyses were acceptable within 1% relative error.

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 diameter 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 younger nests the royal cell is a distinctly thicker sphere or ellipsoid of layered carton (generally up to 12-15 cm in diameter) surrounding the royal chamber. In huge nests the royal chamber is 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. At present we know of no distinguishing characters to differentiate nests of *N. acajutlae* from *N. nigriceps*.

The carton-covered foraging trails built by large *N. acajutlae* and *N. nigriceps* colonies are larger and less regular than in *N. corniger, N. costalis*, or *N. ephratae*. Small *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 of 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 report on discovery of the 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, B.V.I., and a nest dissected on the island of Tortola, B.V.I. in October, 1994.

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 diameter which placed it in a medium size category for conspecific nests in that area. Twenty nodule formations, most measuring 3.0-4.8 cm in diameter with some as small as 1 cm diameter, 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 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 diameter 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, showing evidence of consumption by termites. As with the *N. nigriceps* nest, immature termites occupied the interior of hollowed-out nodules.

A *N. acajutlae* nest collected on the island of Tortola, B.V.I. 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 diameter, 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 large 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, normal 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 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. 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 sample of nest or nodule material.

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 normal carton will enhance 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, B.V.I. 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 B.V.I. samples. This probably reflects a difference in diet among the two populations (*N. acajutlae* sampled from Guana Island were feeding substantially on sea grape, *Coccoloba uvibfera* , 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 portions of both *N. acajutlae* and *N. nigriceps* nodules offered to workers of *N. acajutlae, N. nigriceps, N. costalis, Reticulitermes flavipes* (Kollar) and *Zootermopsis nevadensis* (Hagen) were rapidly consumed in the laboratory.

Termite nest material can be used as nutritional food reserves in some species. Hegh (1922) commented that 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 British Virgin Islands 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 immatures 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 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, contributes to the survival of these two *Nasutitermes* species in arid or otherwise marginal habitats not colonized by other members of the genus.

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FIGURE LEGENDS.

Figure 1. Photographs of nodules embedded within normal nest carton matrix in the *Nasutitermes nigriceps* colony collected in Panama in April 1981.

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

FIGURE 1.

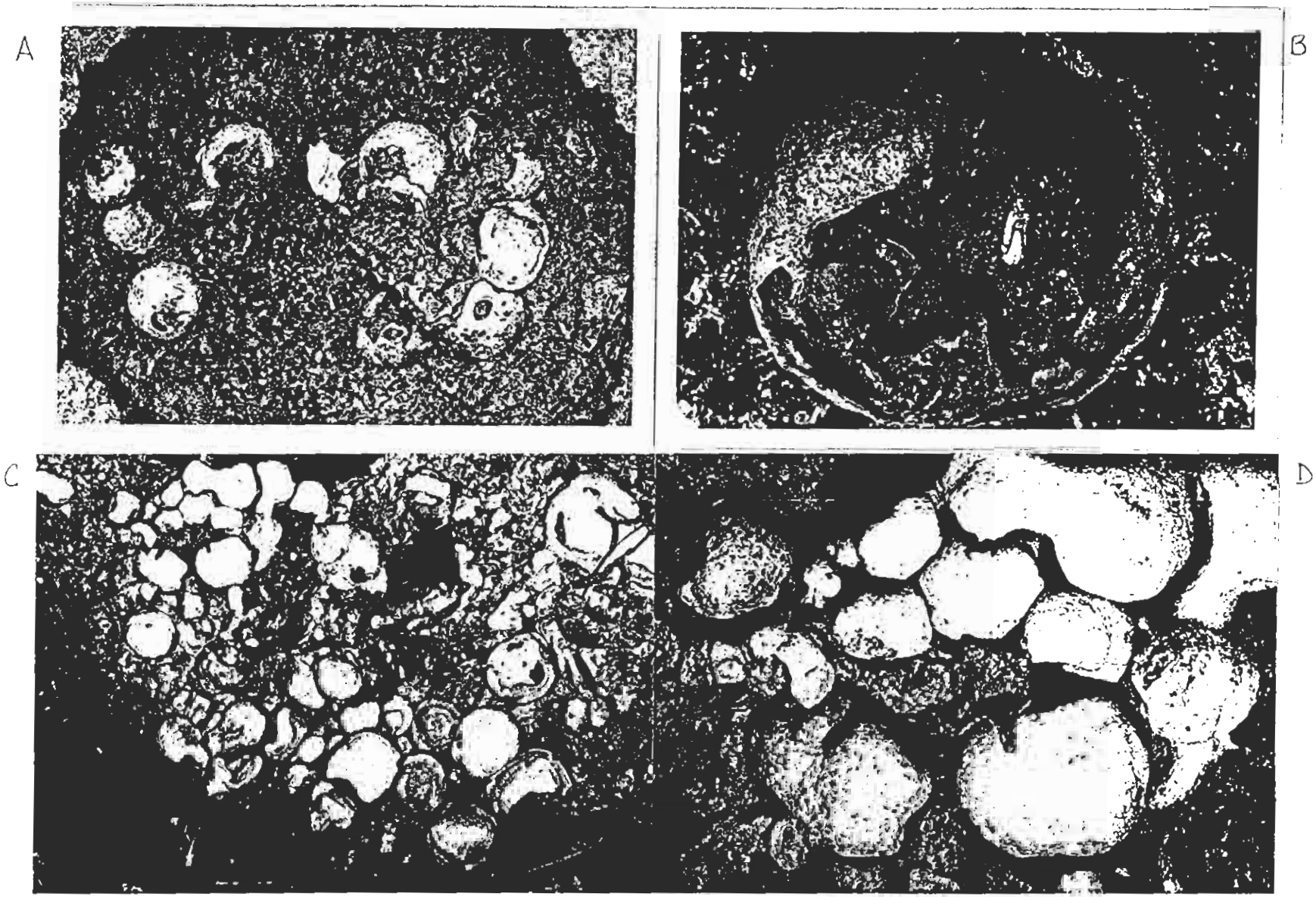

FIGURE 2.

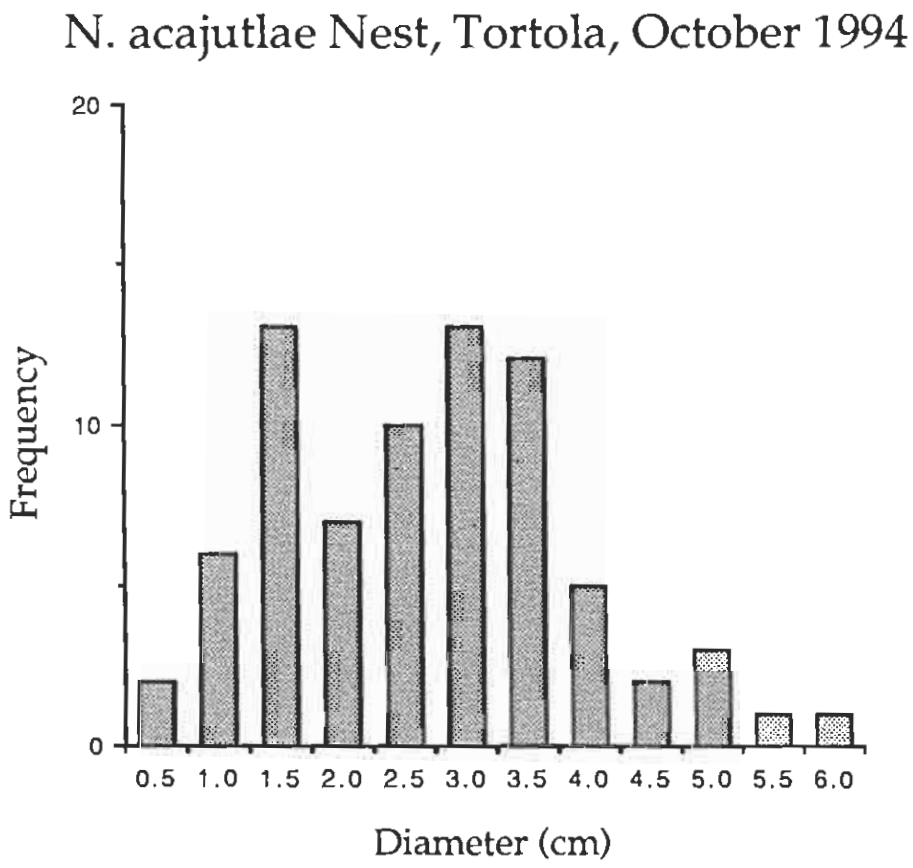
N. acajutlae Nest, Tortola, October 1994
Frequency
20
10
0
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Diameter (cm)

Table 1. Nutrient composition of nodule and normal nest carton samples. All values are presented as percent dry matter except IVOMD is expressed as percent organic matter and energy is expressed as kJ/g dry matter. OM is organic matter; N is nitrogen; P is phosphorus; IVOMD is in vitro organic matter digestibility.

	OM	Cellulose	Lignin	Cutin	Lipids	N	P	IVOMD	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- 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- 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	19.7
Dry nest carton	88.3	15.3	35.1	30.6	1.4	0.80	0.08	2.5	19.6

Chemical Ecology of Forest Insects
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March 28, 1995

James D. Lazell, Ph.D.
The Conservation Agency
3930 D Marcom Street
Raleigh, North Carolina 27606

Dear Skip:

As you have been painfully aware, my colleagues and I have been conducting chemosystematic studies of the termites of the Caribbean Basin since the late 1980s. Our studies have focused on utilizing cuticular hydrocarbons as taxonomic characters. Over the years we have been "perfecting" our techniques to characterize and quantify cuticular hydrocarbons of Caribbean termites. The enclosed manuscript summarizes a portion of our work, particularly with *Nasutitermes acajutlae*.

My co-authors and I would appreciate your opinion of this paper. We would be interested in any technical comments you care to make. We hope to submit this paper before the beginning of the summer. If you can review our paper within the next 3 weeks, we would be very grateful (especially if you have good things to say about it!). If you do not have the time, just let me know by fax @ 510-559-6499 and simply recycle the manuscript.

This manuscript will set the stage for others. Next I will begin the paper on the cuticular hydrocarbons of the termites of the Britsh Virgin Islands. This will be a more detailed treatment of the material that was once in the paper Margaret is currently finishing. After the overview paper, I will start on the colony differences paper. Just want you to know that we are working!

Sincerely,

Mike

MICHAEL I. HAVERTY
Chief Research Entomologist

cc Barbara Thorne and Lori Nelson

Enclosure

Journal of Chemical Ecology

Send Galley Proofs to:

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

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Abstract -- In the present study we describe standard or acceptable methodologies for collecting and extracting termites for characterization of cuticular hydrocarbons under field conditions in the tropics. We chose the arboreal nesting *Nasutitermes acajutlae* (Holmgren) as our subject. This methodology described here should be applicable to most termites in the 2.6 mg to 7.1 mg (wet weight) range. Treatments were compared to a standard of extracting live workers in 10 ml of hexane for 10 minutes Specifically, we evaluate (1) the effect of the duration and the number of extractions; (2) the effect of the condition of the termite specimens (live or dried); (3) the effect of group/sample size; and (4) the effect of solvents (ethanol vs. hexane) on the similarity or reproducibility of cuticular hydrocarbon profiles.

We identified 34 hydrocarbons from workers and 38 from soldiers of *N. acajutlae*. The hydrocarbons consist of two distinct groups: early eluting components, primarily normal alkanes and methyl-branched alkanes, and late-eluting compounds, which consist almost exclusively of unsaturated components with one to five double bonds. Soldiers have considerably greater quantities of the early-eluting compounds than do workers. Olefins, comprising greater than 70 percent of the total hydrocarbon component, are the predominant class of hydrocarbons. Nests of this species from the same island produced qualitatively similar (not identical), but quantitatively dissimilar hydrocarbon mixtures.

Extraction of the same 300 workers a second time, resulted in hydrocarbon mixtures that were qualitatively and quantitatively quite similar to the mixture from the first extraction. The amount of hydrocarbon in the second extract was about half of what was extracted during the first wash. A third extraction recovered only the trivial amounts of cuticular hydrocarbons that remain and was superfluous.

Very brief rinses (in 10 ml of hexane for only 20 seconds) of 300 live workers produced chromatograms qualitatively and quantitatively equivalent to a 10-minute extraction. Furthermore, the 20-second rinse removed the same amount of

hydrocarbon as a 10-minute extraction. Holding a sample of 300 workers in hexane for a period of 2 years provided qualitatively and quantitatively different mixtures of hydrocarbons than did standard 10-minute extractions. Mixtures of cuticular hydrocarbons extracted from live or dried workers were quantitatively different from one another; drying workers tended to enhance extraction of the less abundant unsaturated compounds such as C41:4+C41:5. We found no evidence that any abundant hydrocarbons were unique to dried samples.

The less abundant compounds, or those that were present only in trace amounts from extractions of the standard group size (100), were either missing or infrequently recorded in samples of 25 or 50 workers. The most abundant compounds, such as C39:1 and C41:1, had a lower mean value in groups of 100 or 200 workers of *N. acajutlae*. Storage in ethanol caused numerous unidentified, non-hydrocarbon compounds to be extracted either from the cuticle or from internal tissues.

Extractions using a minimum 100 workers (live or dried), with a hexane wash of 20 seconds to 10 minutes will be acceptable for characterizing cuticular hydrocarbons. 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. For quality of chromatograms and for several logistical reasons, we obtain the best results come by drying at least 100 termites then extracting them by the standard technique (10 minutes in 10 ml hexane). For our purposes, storage of termites in ethanol before extraction is not acceptable.

Key Words -- Chemotaxonomy, Isoptera, Termitidae, Nasutitermitinae, Neotropical termites, *Naustitermes acajutlae* (Holmgren), gas chromatography

INTRODUCTION

Termites play an essential role in the ecological dynamics of many tropical ecosystems, recycling nutrients and aerating soils in forests, mangroves 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 have been found to be useful for discriminating termite species in both tropical and temperate 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 both taxonomic and/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 humidity problems, 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 may also extract internal hydrocarbons and confound the characterization of cuticular hydrocarbons.

We have been collecting termites from the islands in the Caribbean, and have also been receiving 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 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 beetles from the termites and froze them at -20°C before extraction. Haverty et al. (1988) froze *Zootermopsis* spp. individuals, then subsequently thawed them to room temperature before extraction. The termites for the study of *Zootermopsis* were brought from the field to the laboratory alive. However, after extracting a few live termites one of us (LJN) noticed that the termites convulsed and emptied their gut contents into the hexane during the process. Freezing prevents this possible contamination of the samples.

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*], 1991, unpublished [for *Reticulitermes*]), or termites dried in the field and later extracted in the laboratory (Haverty et al., 1990a, 1996b [for *Coptotermes*], 1991, 1992 [for *Reticulitermes* and *Coptotermes*]). Our preliminary results comparing extraction of live vs. dried *Nasutitermes acajutlae* (Holmgren) indicated that resulting chromatograms were not equivalent.

Many of our colleagues are not willing to dry termites in the field while working in the tropics. Drying of termite under field conditions does present some logistical problems. Ovens, heating lamps, or even electricity are not always available. Also, nocturnal insects, lizards, and other vermin are attracted to the

lamps used to dry specimens. They may eat or otherwise contaminate the sample. Since drying is often impractical, many researchers would rather collect termites directly into alcohol or hexane. Detailed comparisons of cuticular hydrocarbon patterns derived from extractions of live or dried termites or termites stored for extended periods in alcohol or hexane is thus warranted and is one focus of this study.

Another factor affecting the quality of cuticular hydrocarbon assessments is the number of insects that are extracted, or quantity of "wax" extracted. In some studies a variable number of individuals (and mixture of castes) was included. Haverty et al. (1991) used anywhere 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 is extracted: individual *Zootermopsis* spp. pseudergates, nymphs, soldiers or alates (Haverty et al., 1988); 100 *Coptotermes formosanus* Shiraki workers or soldiers (Haverty et al., 1990a); 200 *C. formosanus* 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. Characterization of hydrocarbons from dilute extracts would likely underestimate or eliminate the less abundant compounds and over-estimate the proportions and importance of 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.

Preliminary observations of Seybold and Nelson (pers. comm.) indicate that ethanol (EtOH) will extract cuticular hydrocarbons of adult *Ips pini* (Say)(family Scolytidae). Also, the longer beetles are in EtOH, the greater the extraction efficiency. This work shows (1) that the EtOH-extracted insects can be further extracted with hexane, (2) that the EtOH extract can be dried and the lipids re-eluted in hexane, and

(3) that the EtOH extract and the EtOH-extracted-insect extract can be recombined to provide a reconstituted extract for characterization. It is not clear whether the resulting chromatogram is comparable to an extract gathered by the standard 10-minute extraction of live or dead insects with hexane.

In the present study we attempt to define standard or acceptable methodologies for collecting and extracting termites under "field" conditions. Our field work was based at our research site on Guana Island, British Virgin Islands (Thorne et al., 1994; Collins, 1996 or Scheffrahn et al., 1994; Haverty et al. 1996a). 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 *Nasutitermes acajutlae* (Holmgren) (Thorne et al., 1994; Collins, 1996 or 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* will be important to our understanding of the taxonomy, ecology, and biogeography of this and other *Naustitermes* species. We anticipate that the methodology we develop for sampling and extracting cuticular hydrocarbons for this species should be applicable to most of the termites in the 2.6 mg to 7.1 mg (wet weight) range.

In this paper we report the results of a sequence of studies, conducted in 1989 and 1993, to compare and improve our sampling and extraction techniques for the characterization of cuticular hydrocarbons. In 1993 we tried to design studies that would ultimately provide us with the "best" field method(s) for characterizing both the composition and relative abundance of the cuticular hydrocarbons of *N. acajutlae*. Each year we included a standard treatment of extracting live workers in 10 ml of hexane for 10 minutes (Haverty et al., 1988, 1990b). 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 the conditions of the termite specimens (live or dried); (3) the effect of group/sample size; and (4) the effect of solvents (ethanol vs. hexane) on the quality and similarity or reproducibility of cuticular hydrocarbon profiles.

METHODS AND MATERIALS

Collecting and Processing Termite Specimens. We collected workers and soldiers of *Nasutitermes acajutlae* during two separate trips to Guana Island, British Virgin Islands. During each trip different collection techniques were used. In 1989 two nests were sampled from the plantation area 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 index cards (cardboard) 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.

In 1993 we selected 13 colonies from the plantation area. This year 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 soldiers with the least effect on nest structure. A tangential slice, 2 to 3 cm deep and 15 to 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 X 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 the 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 seconds the squares were removed and a collection of nearly pure soldiers was tapped into a collection pan. To readily obtain a nearly clean sample, 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 minutes. 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 is gently removed a dense sample of workers adheres to the surface. Contrary to the technique for collecting soldiers, the squares of corrugated paper 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.

Since 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 to 8 ml of soldiers in 20-ml scintillation vials 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.

Additional termites from each of the sampled colonies (with soldiers and alates, when available, as diagnostic castes) were placed in 80 percent ethanol 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.

Duration and Number of Extractions. One of the primary reasons we began our 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., 1998). 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*.

In 1989 we evaluated the following extraction regimes using *N. acajutlae* from Guana Island, British Virgin Islands, to determine whether we could improve upon rinsing 100 live workers in 10 ml of hexane for 10 minutes:

A. Rinse 300 workers in 10 ml hexane for 10 minutes (standard technique with an increased sample size).

B. Sequentially rinse 300 workers three times in 10 ml of hexane for 10 minutes, keeping each extract separate (to determine if the standard technique left significant quantities of hydrocarbon on the sample).

C. Rinse 300 workers in 10 ml of hexane for 20 seconds followed by a 10 minute rinse in 10 ml of hexane, keeping each extract separate (to determine whether most cuticular hydrocarbons are on the surface).

D. Rinse 300 workers in 10 ml of hexane for 20 seconds followed by soaking for 24 hours in 10 ml hexane, keeping each extract separate (same as "b" with an extended post-rinse extraction) to determine whether an extended soaking would remove additional hydrocarbons from the cuticle or from other tissues.

E. Soak 300 workers in 10 ml of hexane for 24 hours (to allow for a less stringent extraction schedule).

F. Soak 300 workers in 10 ml of hexane for 2 years (to allow field collection with subsequent laboratory storage for an extended period).

G. Isolate 300 workers (alive) from soldiers for 24 hours, then rinse in hexane for

10 minutes (to allow time for defense secretions that might have contaminated the sample to evaporate or be groomed off one another by the nestmates in the sample).

H. Rinse 350 soldiers in 10 ml of hexane for 10 minutes (to contrast worker and soldier hydrocarbon patterns).

Condition of the Termites: Live vs. Dried. We used three samples of 100 workers from each of 13 nests. Live termites were extracted at the field laboratory on Guana Island. Live termites were placed directly into 20-ml scintillation vials and extracted in 10 ml of hexane for 10 minutes. The hexane was then decanted into a separate 20-ml scintillation vial and was subsequently returned to our laboratory in California for characterization of cuticular hydrocarbons.

N. acajutlae workers were dried by placing 20-ml scintillation vials, each containing 100 individuals, in a wire box over a single 75-watt, 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. Once termites were completely dried, we kept them dry by tightly sealing the vials. The vials were returned to our laboratory in California for extraction and characterization of cuticular hydrocarbons.

Effects of Group/Sample Size. Samples of 25, 50, 100, or 200 *N. acajutlae* workers from 5 different colonies were extracted either live or dried as described above. Each combination (sample size X live vs. dried X colony) was replicated 3 times.

Effects of Solvent. For each of 5 colonies, 3 replicates of 200 workers were placed in either 10 ml of hexane or 20 ml of 100% ethanol and left in the solvent for 60 days. The ethanol extract was removed, dried under nitrogen and re-eluted with 10 ml of hexane. After the ethanol was drained from the termites, they were extracted with hexane as described above.

Extraction Procedure and Characterization of Cuticular Hydrocarbons. Detailed descriptions of extraction procedures for specific termite species have been published elsewhere (Blomquist et al., 1979; Howard et al., 1978, 1982a,b, 1988; Haverty et al., 1988, 1990a,b). In this study cuticular lipids were extracted by immersing termites, as a group, in 10 ml of *n*-hexane. After extraction, hydrocarbons were separated from other components by pipetting the extract through 4 cm of activated BioSil-A in Pasteur pipette mini-columns. 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.

GC-MS analyses were performed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5970B Mass Selective Detector interfaced with a Hewlett Packard Chemstation computer. The GC-MS was equipped with an HP-1, fused silica capillary column (30 m x 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/minute with a final hold of 16 minutes. Electron impact (EI) mass spectra were obtained at 70 eV. *n*-Alkanes were identified by their mass spectra and by comparing their retention times with external standards (*n*-C22, *n*-C24, *n*-C28 and *n*-C32). Methyl-branched alkanes were tentatively identified by comparing their retention times to those of the *n*-alkanes. Mass spectra of methylalkanes were interpreted as described by Blomquist et al. (1987) to identify methyl branch locations. Alkenes were tentatively identified by their mass spectra and retention times. 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 by the data analysis software (HP59974J Rev. 3.1.2) in the Hewlett-Packard Chemstation. GC-MS peak areas were converted to percentage of the total hydrocarbon fraction. These percentages for each hydrocarbon were the response variables used to make statistical comparisons among extraction/collection techniques.

Statistical Analyses. The response variables for all statistical tests were the percentages of each cuticular hydrocarbon. The effects of the condition of the termites before extraction (live vs. dead and dried) were assessed by a t-test of the differences between the means (3 replicates or subsamples from each nest or colony) of each of two conditions for each of 5 colonies. The significance of the calculated t-value was tested at $\alpha = 0.05/34$ or 38, the number of hydrocarbons for workers and soldiers, respectively. Our null hypothesis was that the 10-minute extraction of 100 dried termites was not significantly different from the standard 10-minute extraction of 100 live termites.

The effect of group size was tested with an analysis of variance for each hydrocarbon. The significance of the F-statistic was tested with $\alpha = 0.05/34$, the number of hydrocarbons from workers. The model used was a completely randomized design, with colonies being the blocking factor. Each treatment combination (group size X dry vs. live X colony) was replicated 3 times. The four sample sizes were compared separately for termites extracted live or dried. Our null hypothesis was that all group sizes provide chromatograms with the same quantities of each hydrocarbon. We were looking for the smallest group size that results in a hydrocarbon profile equivalent in resolution to those of the next greatest group size. Group size was considered inadequate if they yield hydrocarbon mixtures that are significantly different in quantity from those of a larger size.

RESULTS AND DISCUSSION

Cuticular Hydrocarbon Mixtures of N. acajutlae. Over the years, we have been improving our techniques for extracting and then characterizing the cuticular hydrocarbons of *N. acajutlae*. We identified 34 hydrocarbons from workers and 38 from soldiers (Table 1, Figure 1). The hydrocarbons found in these chromatograms can be seen in two distinct groups. The early eluting components (peaks 1-21) are primarily normal alkanes, methyl-branched alkanes, and a few normal alkenes. The second group of later-eluting compounds (peaks 22-40) consist almost exclusively of unsaturated components with one to five double bonds and a two monomethyl alkanes in trace amounts. Soldiers have considerably greater quantities of the earlier-eluting compounds than do workers (Table 1, Figure 1). By far, the predominant class of hydrocarbons is the alkenes, comprising greater than 70 percent of the total hydrocarbon component in workers.

We are confident in our identification of the late-eluting monounsaturated alkenes. The spectra for nonatriacontene (C39:1) and hentetracontene (C41:1) display the parent ion (547/7 and 574/5, respectively) as well as the characteristic fragmentation pattern with the predominant peaks (fragments) (83 and 97) being 2 mass units less than would be expected from *n*-alkanes and exceeding fragment 67 in quantity (Figure 2). The polyunsaturated components, hentetracontatetraene (C41:4) and hentetracontapentaene (C41:5) are identified by the presence of a parent ion (568/9 and 566/7, 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,

nonatriacontatetraene. Similarly, we tentatively identified high molecular weight dienes, trienes, and tetraenes from the cuticular lipids of other species of *Nasutitermes* from the Caribbean Basin (Haverty et al., 1992). Thus far we have only recovered high molecular weight polyunsaturated olefins from the cuticular hydrocarbons of tropical termites.

Duration and Number of Extractions. Early in our research on the chemotaxonomy of termites we extracted live or recently frozen individuals. The standard 10-minute rinse of 300 live workers of *N. acajutlae* allowed us to resolve and characterize most of the components later identified for this species (Figure 4A). We also discovered that different colonies (nests of this species from the same island) produced qualitatively similar (not identical), but quantitatively dissimilar hydrocarbon mixtures (Figure 4A). Furthermore, these colony-specific profiles are repeatable; when a second group of 300 live workers was extracted the chromatograms for each colony were qualitatively identical and quantitatively quite similar (Figure 4A,B1).

A second extraction of the same 300 workers resulted in a hydrocarbon mixture that was qualitatively and quantitatively quite similar to the mixture from the first extraction. The amount of hydrocarbon in the sample, as represented by the plot of the total ion chromatogram, was about half of what was extracted (total abundance of $2.4X10^6$ vs. $1.1X10^6$ and $4.0X10^6$ vs. $2.4X10^6$) during the first wash (Figure 4B1,B2). A third 10-minute extraction resulted in chromatograms that did not resemble those from either of the first two extractions. Because the amount of hydrocarbon in this sample was significantly reduced (total abundance of $0.3X10^6$ for both colonies), only the predominant peaks (C25, C27, C39:1, and C41:1) were detected.

We conclude that subsequent extractions did not improve the quality of the chromatograms of cuticular hydrocarbons of *N. acajutlae* (Figure 4). The first extraction removed about 2/3 of the hydrocarbons. The second extraction removed

another 1/3 of the hydrocarbon, but the mixture was proportional to the first extraction. A third extraction recovered only the trivial amounts of cuticular hydrocarbons that remain and was superfluous.

Very brief rinses (in 10 ml of hexane for only 20 seconds) of 300 live workers produced chromatograms qualitatively and quantitatively equivalent to a 10-minute extraction (Figure 5A,C1,D1). Furthermore, the 20-second rinse produced the same amount of hydrocarbon as a 10-minute extraction and was repeatable (see abundances in Figure 5A,C1,D1). A subsequent 10-minute extraction produced a qualitatively and quantitatively similar chromatogram, but the amount of hydrocarbon removed was about half of the first rinse (total abundance of $3.6X10^6$ vs. $1.2X10^6$ and $3.6X10^6$ vs. $1.6X10^6$) (Figure 5C1,C2).

300 workers soaked for 24 hours following a 20-second rinse resulted in a chromatogram that was both qualitatively and quantitatively different from the standard extraction (10 ml for 10 minutes) or a 20-second rinse. The amount of hydrocarbon was about one half that of the first extraction (Figure 5A,D1,D2). Proportional relationships change dramatically: C25 and C27 were much more prominent as was C41:4 and C41:5 for colony 1.

Extraction of 300 workers for 24 hours produced equivocal results. For colony 1, the chromatogram appears quantitatively different from the standard 10-minute extraction; for colony 2 the resulting chromatogram appears almost identical to the standard 10-minute extraction (Figure 6A,E). Holding a sample of 300 workers in hexane for a period of 2 years provided a radically different mixture of hydrocarbons, both qualitatively and quantitatively, than the standard 10-minute extraction (Figure 6A,F). Many hydrocarbons that we rarely see in *N. acajutlae* workers (such as C22, C23:1, C24:1, C25:1 [an additional isomer], C26:1, 11,13-MeC25, C27:2, 2-MeC26, C29:2, 5-MeC29, C31:1, C43:5, C43:4, and C43:2) were present in quantities exceeding trace amounts. Furthermore, some compounds (C25, C27, C41:5, and C41:4) were present

in much greater proportions. As a result of these qualitative and quantitative differences, we do not consider such an extraction regime to provide results comparable to the standard 10-minute extraction.

When a nest is breached or a large group of termites in a pan is disturbed, soldiers congregate at the site of the disturbance and discharge large quantities of defense secretions or glue. The effect of these defense secretions on extraction of worker hydrocarbons was unknown. We isolated 300 workers from each of two colonies for 24 hours to allow time for these secretions to degrade or for the workers to groom one another to remove these compounds. The resulting chromatograms did not appear to differ from the standard (Figure 7A,G).

Duration and number of extractions is summarized as follows:

1) Extractions of very short (20 seconds) or short duration (10 minutes) produce equivalent chromatograms.

2) Extractions of extremely long duration (2 years) result in a mixture of hydrocarbons that is qualitatively and quantitatively different than those from extractions of short to moderate duration (24 hour).

3) The majority (about 2/3) of the hydrocarbons are extracted within the first 20 seconds. Nothing is gained (or lost!) by extracting for as long as 10 minutes. Likewise, there is no benefit to sequential extractions.

Condition of the Termites: Live vs. Dried. Mixtures of cuticular hydrocarbons extracted from live or dried workers were quantitatively different from one another. Of the 34 hydrocarbons, 20 were significantly different in quantity (Table 2). The most striking differences were exhibited in the late-eluting alkenes (Figure 8). Drying the workers before extraction resulted in highly significant increases in the relative amounts of C41:4+C41:5 (peak 30). Related to the increased efficiency of the extraction of these compounds was the apparent decrease in the relative amounts of the most abundant compounds, C39:1, C41:1, and C43:1 (peaks 27, 32, and 39). In

general, drying workers first tended to enhance extraction of the less abundant unsaturated compounds. Extraction of 100 dried workers did not result in quantitatively equivalent mixtures of hydrocarbons when compared to extraction of 100 live workers, and may not be comparable for taxonomic purposes. However, either technique would suffice for characterization of cuticular hydrocarbons for ecological studies.

There is the possibility that these differences are due to the extraction of hydrocarbons from internal tissues. By definition, these hydrocarbons are not cuticular lipids, though the components may be the same (deRenobales et al., 1991). Dried termites are more fragile and often lose legs, antennae, or even heads during shipment. Also, the cuticle can become cracked. These conditions 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 (with the possible exception of C41:2, C43:5 or C43:2) were unique to dried samples.

After examining hundreds of samples of termites extracted live or after drying it appears to us that the chromatograms from dried individuals are sharper and have a flatter base line than those from live, field-extracted individuals. Three possible reasons are: (1) live termites void gut contents when placed in hexane, (2) hexane in a vial extracts contaminants from the vial lids during transit from the field to the laboratory, or (3) live insects have a higher water content in the cuticle and less hydrocarbon is extracted because hexane is hydrophobic. Further study of this phenomenon is warranted.

Effects of Group/Sample Size. Hydrocarbon mixtures from different sized groups of workers of *N. acajutlae* were significantly different in quantities of

components from one another. The less abundant compounds, or those that are present only in trace amounts from extractions of the standard group size (100), are either missing or infrequently recorded (with a lower mean value resulting) in samples of 25 or 50 workers (Tables 3 and 4, Figure 9). The most abundant compounds, such as C39:1 and C41:1, have a lower mean value in groups of 100 or 200 workers (Tables 3 and 4; Figure 9). 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 groups of 25 or 50 workers and they do not add to the total hydrocarbon. Thus, for workers of *N. acajutlae*, 100 appears to be the minimum acceptable sample size for adequately characterizing the cuticular hydrocarbons for quantitative comparisons.

It is likely that our earlier problem with quantifying hydrocarbons from *N. costalis* and *N. ephratae* (Haverty et al., 1990b) resulted from three 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, only 100 workers were used in the extractions; therefore, 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* (~~wet~~ dry weight = 1.31 mg) (Thorne, 1985); 100 large workers of the former species may not have had sufficient surface lipids to provide an adequate chromatogram. Third, in our earlier studies of *N. costalis* and *N. ephratae* we used a flame ionization detector to quantify hydrocarbon peaks. Even though we used the same type of column, the resolution of that earlier chromatographic scheme (Haverty et al., 1990b) did not resolve peaks as well as the equipment we currently use.

Effects of Solvent. For field entomologists working in the tropics it would be convenient if storage of termites in 100 percent ethanol allowed for equivalent extraction of hydrocarbons. Unfortunately, the resulting chromatograms for both

workers and soldiers were not comparable to those where the standard technique was utilized. Storage in ethanol caused numerous unidentified, non-hydrocarbon compounds to be extracted either from the cuticle or from internal tissues (Figure 10). These compounds were not removed after evaporating the ethanol under nitrogen, re-dissolving in hexane, and pipetting through activated BioSil-A. Furthermore, enormous quantities of nitrogen were required to dry the samples. 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 are reproducible; multiple chromatograms from separate samples of each colony are qualitatively identical and quantitatively quite similar. Only one extraction of a group of workers is necessary. Subsequent extractions of the same insects do not improve the quality of the chromatograms of cuticular hydrocarbons of *N. acajutlae*. The first extraction removes about 2/3 of the hydrocarbons; a third extraction recovers only the trivial amounts of cuticular hydrocarbons that remain and is superfluous.

Very brief rinses (in 10 ml of hexane for only 20 seconds) of live workers produces chromatograms qualitatively and quantitatively equivalent to a 10-minute extraction. Holding a sample of workers (or soldiers)in hexane for a period of 2 years will provide a radically different mixture of hydrocarbons, both qualitatively and quantitatively, than the standard 10-minute extraction, and is not recommended.

Drying workers of *N. acajutlae* before extraction results in highly significant increases in the relative amounts of C41:4+C41:5 and an apparent decrease in the relative amounts of the most abundant compounds, C39:1, C41:1, and C43:1. In

general, drying workers first tends to enhance extraction of the less abundant unsaturated compounds. Extraction of dried workers does not result in quantitatively equivalent mixtures of hydrocarbons when compared to extraction of live workers, and may not be comparable for taxonomic purposes. However, either technique would suffice for characterization of cuticular hydrocarbons for ecological studies.

Storage in 100 percent ethanol caused numerous non-hydrocarbon compounds to be extracted either from the cuticle or from internal tissues. These compounds are not removed after evaporating the ethanol under nitrogen, re-dissolving in hexane, and pipetting through activated BioSil-A. Furthermore, enormous quantities of nitrogen are required to dry the samples. Therefore, unless a different cleanup procedure is developed, we feel storage in ethanol is unacceptable for characterizing the hydrocarbons from *Nasutitermes* or any other termites.

Extracting a minimum of 100 workers (live or dried) with hexane for 20 seconds to 10 minutes seems to be the best method for characterizing cuticular hydrocarbons of *N. acajutlae*. For smaller species, groups of 200 would 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. For quality of chromatograms and for several logistical reasons, we obtain the best results by drying at least 100 termites then extracting them by the standard technique (10 minutes in 10 ml hexane). Extraction of live termites in the field requires twice the number of vials and transportation of (highly?) flammable liquids. Even with the potential logistical difficulties involved with drying termites in the tropics, we recommend this as the optimal technique to use.

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Table 1. Relative abundance of cuticular hydrocarbons of workers and soldiers of *Nasutitermes acajutlae* (Holmgren) from Guana Island, British Virgin Islands.[a]

Peak[b]	Hydrocarbon[c]	Relative Amounts[d]	
		Workers	Soldiers
1	C23:1	tr	tr
2	C23	+	++
3	11,9 MEC23	0	+
4	C24:1+3 MEC23	0	tr
5	C24	tr	+
6	11 MEC24	0	tr
7	C25:1	+	++
8	C25	++	+++
9	13,11 MEC25	tr	++
10	C26:1+3 MEC25	0	+
11	C26	tr	+
12	13,12,11 MEC26	0	tr
13	C27:1	+	++
14	C27	++	++
15	13,11 MEC27	tr	++
16	2 MEC27	tr	tr
17	C28	tr	tr
18	C29:1	0	tr
19	C29	+	+
20	C31	tr	tr
21	C33	tr	tr
22	C37:1	tr	+
23	C38:1	+	+
24	C39:5	tr	tr
25	C39:4	++	++
26	C39:2	tr	++
27	C39:1	+++	+++
28	15 MEC39	tr	tr

29	C40:1	++	++
30	C41:4+C41:5	+++	+++
31	C41:2	++	++
32	C41:1	+++	+++
33	15 MEC41	tr	tr
34	C42:1	++	++
35	C43:6?	tr	0
36	C43:5	tr	0
37	C43:4	++	++
38	C43:2	+	++
39	C43:1	+++	+++
40	C45:1	++	++

[a] 200 workers and 5 to 8 ml of soldiers were dried with heat from an incadescent light before extraction with 10 ml of hexane for 10 minutes.

[b] Peak numbers refer to peaks identified in Figure 1.

[c] This shorthand uses a descriptor for the location of the methyl group (X-Me), the total number of carbons (CXX) in the hydrocarbon component, excluding the methyl branch(es), and the number of double bonds following the colon (CXX:Y).

[d] The relative amounts of hydrocarbon are coded as follows: +++ $\geq$ 5.0 % of the total, ++ from 1.0 to 5.0% of the total, and + from 0.5 to 1.0% of the total hydrocarbon component. Some trace (tr) components appear infrequently or consistently in very small quantities ($<$ 0.5% of the total). A zero indicates the hydrocarbon was never identified for this caste.

Table 2. Quantities of cuticular hydrocarbons (mean and standard deviation) from samples of workers of *Nasutitermes acajutlae* (Holmgren) from Guana Island, British Virgin Islands, extracted alive or after drying.

	Extracted Live[c]		Extracted Dry[c]		
Hydrocarbon[a,b]	Mean	Std. Dev.	Mean	Std. Dev.	*t* value[d]
C23:1	0.41	0.32	0.32	0.30	0.819
C23	0.80	0.29	0.97	0.33	-3.029
C24	0.45	0.27	0.22	0.22	3.348
C25:1	0.36	0.35	0.68	0.27	-4.445
C25	2.64	0.95	3.05	1.14	-2.618
13,11 MEC25	0.04	0.10	0.16	0.17	-4.361
C26	0.53	0.32	0.26	0.27	4.453
C27:1	0.34	0.33	0.86	0.42	-6.610
C27	1.40	0.95	1.90	1.60	-2.706
13,11 MEC27	0.03	0.09	0.07	0.11	-1.747
2MEC27	0.00	0.00	0.15	0.13	-4.944
C28	0.37	0.22	0.15	0.19	3.251
C29	0.53	0.34	0.73	0.56	-2.438
C31	0.16	0.20	0.06	0.15	2.394
C33	0.01	0.04	0.00	0.00	1.000
C37:1	0.45	0.22	0.40	0.17	1.724
C38:1	0.63	0.25	0.53	0.20	3.537
C39:5	0.03	0.09	0.36	0.33	-4.439
C39:4	0.54	0.40	2.00	0.73	-7.526
C39:2	0.08	0.36	0.43	0.48	-3.272
C39:1	28.12	2.49	20.01	2.20	17.314
15 MEC39	0.03	0.09	0.04	0.12	-0.380
C40:1	2.84	0.27	2.51	0.42	3.928
C41:4+C41:5	6.23	3.61	18.77	3.81	-16.929
C41:2	0.01	0.05	1.21	0.63	-8.168
C41:1	33.77	2.73	24.43	2.74	15.720
15 MEC41	0.07	0.17	0.13	0.23	-1.302

C42:1	1.46	0.50	1.60	0.65	-1.650
C43:6?	0.03	0.07	0.00	0.00	1.000
C43:5	0.00	0.00	0.34	1.23	-1.863
C43:4	0.99	1.14	4.00	1.79	-7.865
C43:2	0.04	0.12	0.83	0.52	-5.876
C43:1	13.15	1.41	10.40	1.61	11.519
C45:1	3.44	0.66	2.41	0.82	6.233

[a] Peak numbers refer to peaks identified in Figure 1.

[b] This shorthand uses a descriptor for the location of the methyl group (X-Me), the total number of carbons (CXX) in the hydrocarbon component, excluding the methyl branch(es), and the number of double bonds following the colon (CXX:Y).

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

[d] The critical $t_{12,.995} = 3.055$.

Table 3. Quantities of cuticular hydrocarbons (mean and standard deviation) from four sizes of groups of workers from five nests of *Nasutitermes acajutlae* (Holmgren) from Guana Island, British Virgin Islands, extracted live.[a]

	25 workers		50 workers		100 workers		200 workers	
Hydrocarbon	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
C23:1	0.00	0.00	0.00	0.00	0.33	0.31	0.00	0.00
C23	1.33	0.50	1.26	0.40	0.85	0.20	0.74	0.14
C24	1.18	0.59	0.99	0.25	0.41	0.18	0.41	0.09
C25:1	0.00	0.00	0.01	0.05	0.33	0.30	0.25	0.13
C25	4.73	1.31	4.05	1.15	2.84	0.48	2.78	0.44
13,11 MEC25	0.00	0.00	0.02	0.05	0.05	0.14	0.16	0.10
C26:1+3 MEC25	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03
C26	1.49	0.53	1.01	0.24	0.49	0.18	0.45	0.13
C27:1	0.14	0.44	0.11	0.21	0.42	0.31	0.36	0.15
C27	2.48	1.23	1.97	0.73	1.59	0.65	1.44	0.66
13,11 MEC27	0.00	0.00	0.00	0.00	0.05	0.14	0.11	0.08
2 MEC27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C28	1.17	0.41	0.75	0.19	0.32	0.21	0.25	0.10
C29	1.18	0.39	0.73	0.15	0.46	0.26	0.35	0.10
C31	0.45	0.34	0.31	0.14	0.08	0.14	0.13	0.06
C33	0.02	0.09	0.02	0.05	0.00	0.00	0.02	0.04
C37:1	0.07	0.18	0.14	0.22	0.50	0.18	0.48	0.15
C38:1	0.21	0.38	0.33	0.27	0.68	0.23	0.80	0.16
C39:5	0.00	0.00	0.03	0.09	0.04	0.10	0.27	0.26
C39:4	0.10	0.22	0.15	0.27	0.73	0.34	0.81	0.38
C39:2	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.16
C39:1	29.19	2.47	28.68	1.62	27.85	2.70	26.39	1.32
15 MEC39	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.11
C40:1	2.01	0.85	2.47	0.40	2.87	0.13	3.23	0.52
C41:4+C41:5	5.47	2.51	6.43	2.44	7.92	4.12	9.27	2.52
C41:2	0.00	0.00	2.34	8.96	0.00	0.00	0.70	1.15
C41:1	35.55	2.79	32.23	8.88	32.41	2.90	30.38	1.98

15 MEC41	0.00	0.00	0.01	0.04	0.00	0.00	0.26	0.16
C42:1	0.99	0.78	1.50	0.77	1.54	0.58	2.12	0.66
C43:4	0.23	0.50	0.94	0.97	1.49	1.44	2.20	0.71
C43:2	0.00	0.00	0.02	0.08	0.03	0.11	0.33	0.11
C43:1	10.73	1.26	11.54	0.87	12.51	1.48	12.37	1.11
C45:1	1.29	1.10	1.96	0.84	3.21	0.59	2.70	0.98

[a] Means are from 3 subsamples from each of 5 separate nests.

Table 4. Quantities of cuticular hydrocarbons (mean and standard deviation) from four sizes of groups of workers from five nests of *Nasutitermes acajutlae* (Holmgren) from Guana Island, British Virgin Islands, extracted after drying.[a]

	25 workers		50 workers		100 workers		200 workers	
Hydrocarbon	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
C23:1	0.00	0.00	0.00	0.00	0.24	0.28	0.05	0.06
C23	1.20	0.38	1.08	0.28	0.93	0.20	1.12	0.26
C24	0.34	0.34	0.39	0.28	0.24	0.23	0.36	0.20
C25:1	0.24	0.41	0.40	0.27	0.64	0.21	0.59	0.19
C25	5.25	2.44	3.83	1.09	3.04	0.77	3.69	0.81
13,11 MEC25	0.13	0.23	0.20	0.15	0.17	0.16	0.29	0.10
C26:1+3 MEC25	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.06
C26	0.41	0.35	0.41	0.31	0.35	0.29	0.41	0.26
C27:1	0.33	0.43	0.58	0.22	0.89	0.56	0.74	0.25
C27	2.75	1.62	2.49	1.47	2.13	1.44	2.65	1.46
13,11 MEC27	0.03	0.13	0.09	0.11	0.09	0.11	0.20	0.07
2 MEC27	0.00	0.00	0.15	0.11	0.13	0.11	0.23	0.08
C28	0.05	0.13	0.25	0.20	0.26	0.22	0.29	0.17
C29	0.62	0.50	0.69	0.42	0.84	0.52	0.85	0.50
C31	0.00	0.00	0.03	0.07	0.04	0.09	0.13	0.09
C33	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04
C37:1	0.12	0.22	0.22	0.15	0.47	0.07	0.43	0.15
C38:1	0.26	0.35	0.45	0.16	0.57	0.10	0.63	0.21
C39:5	0.04	0.16	0.19	0.14	0.36	0.20	0.36	0.13
C39:4	0.97	0.75	1.30	0.43	2.00	0.68	1.91	0.92
C39:2	0.00	0.00	0.00	0.00	0.67	0.46	0.69	0.28
C39:1	22.58	1.99	21.27	1.57	19.65	1.97	19.57	1.79
15 MEC39	0.00	0.00	0.00	0.00	0.06	0.16	0.22	0.17
C40:1	1.90	0.29	2.05	0.33	2.41	0.19	2.27	0.55
C41:4+C41:5	18.61	4.60	18.88	3.05	19.41	3.39	17.44	2.43
C41:2	0.05	0.20	0.61	0.51	1.21	0.59	1.58	0.54
C41:1	28.65	2.20	26.30	1.85	23.14	1.77	23.42	3.16

15 MEC41	0.00	0.00	0.01	0.05	0.11	0.21	0.34	0.17
C42:1	1.33	1.02	1.75	0.80	1.72	0.52	1.44	0.49
C43:4	3.10	1.58	4.17	0.98	4.25	1.92	4.40	1.09
C43:2	0.12	0.47	0.48	0.32	0.91	0.63	0.94	0.37
C43:1	9.58	1.01	9.84	0.76	10.00	1.77	10.27	1.30
C45:1	1.35	0.72	1.89	0.54	2.47	0.55	2.26	0.83

[a] Means are from 3 subsamples from each of 5 separate nests.

Figure Legends

Fig. 1. Total ion chromatogram of the cuticular hydrocarbons of workers (A) and soldiers (B) of *Nasutitermes acajutlae* (Holmgren) from two colonies from Guana Island, British Virgin Islands. These chromatograms were derived from dried samples of 100 workers and ca. 8 ml of soldiers collected in October 1993. Numbers identify peaks listed in Tables 1.

Fig. 2. Mass spectra of *n*-alkenes nonatriacontene and hentetracontene.

Fig. 3. Mass spectra of hentetracontatetraene and hentetracontapentaene.

Fig. 4. Chromatograms of cuticular hydrocarbons extracted from 300 live workers from colonies 1 and 2 of *N. acajutlae* from Guana Island, B.V.I. A = Extraction with 10 ml hexane for 10 minutes (standard); B1 = First extraction a separate group with 10 ml hexane for 10 minutes (equivalent to the standard); B2 = Second extraction of the same termites as B1 with 10 ml hexane for 10 minutes; and B3 = Third extraction of the same termites as B1 with 10 ml hexane for 10 minutes.

Fig. 5. Chromatograms of cuticular hydrocarbons extracted from 300 live workers from colonies 1 and 2 of *N. acajutlae* from Guana Island, B.V.I. A = Extraction with 10 ml hexane for 10 minutes (standard); C1 = First extraction of a different group with 10 ml hexane for 20 seconds; C2 = Second extraction of the same termites as C1 with 10 ml hexane for 10 minutes; and D1 = First extraction a third group with 10 ml hexane for 20 seconds (equivalent to C1, but with a different group of 300 workers); and D2 = Second extraction of the same termites as D2 with 10 ml hexane for 24 hours.

Fig. 6. Chromatograms of cuticular hydrocarbons extracted from 300 live workers from colonies 1 and 2 of *N. acajutlae* from Guana Island, B.V.I. A = 10 ml hexane for 10 minutes (standard); E = Extraction of a second group of termites with 10 ml hexane for 24 hours; F = Extraction of a third group of termites

with 10 ml hexane for 2 years.

Fig. 7. Chromatograms of cuticular hydrocarbons extracted from 300 live workers (A) from colonies 1 and 2 of *N. acajutlae* from Guana Island, B.V.I. G = A second group of 300 workers that were isolated from other colony mates for 24 hours before extraction with 10 ml hexane for 10 minutes (equivalent to the standard extraction technique).

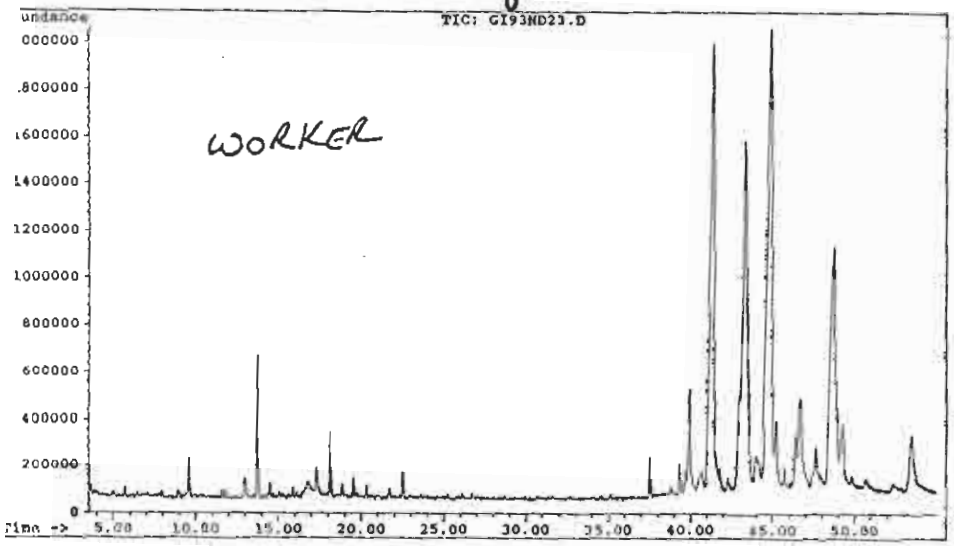
Fig. 8. Chromatograms of cuticular hydrocarbons from 100 workers from two colonies of *N. acajutlae* from Guana Island, British Virgin Islands. Workers were extracted with 10 ml of hexane for 10 minutes either live or after drying.

Fig. 9. Chromatograms of cuticular hydrocarbons from four sizes of groups (25, 50, 100, and 200) workers from one colony of *N. acajutlae* from Guana Island, British Virgin Islands, extracted live with 10 ml hexane for 10 minutes.

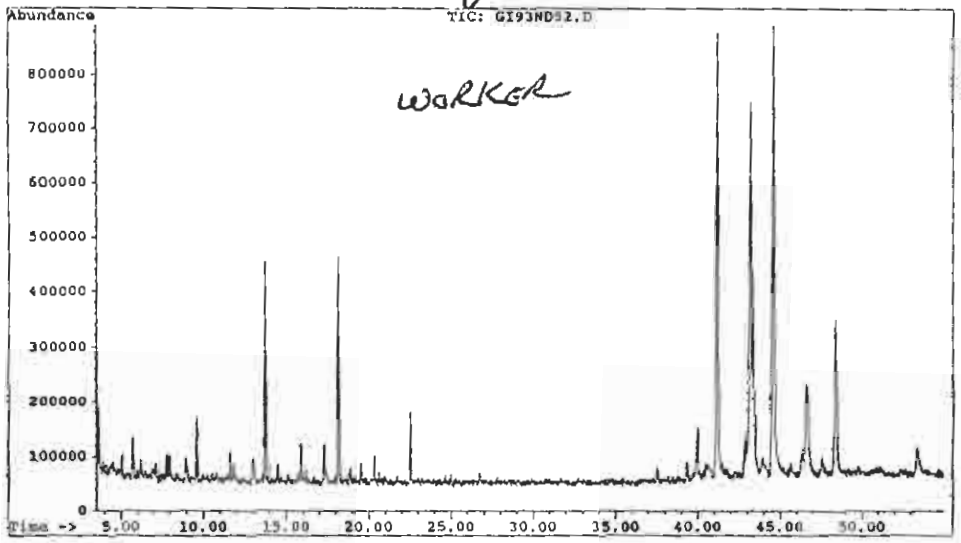
Fig. 10. Chromatograms of cuticular hydrocarbons (and other compounds) from 200 workers (A) or 200 soldiers (B) collected into 100 percent ethanol. For each caste the first chromatogram (A1 or B1) represents the pattern of compounds that were extracted by the ethanol. The second chromatogram for each 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 minutes.

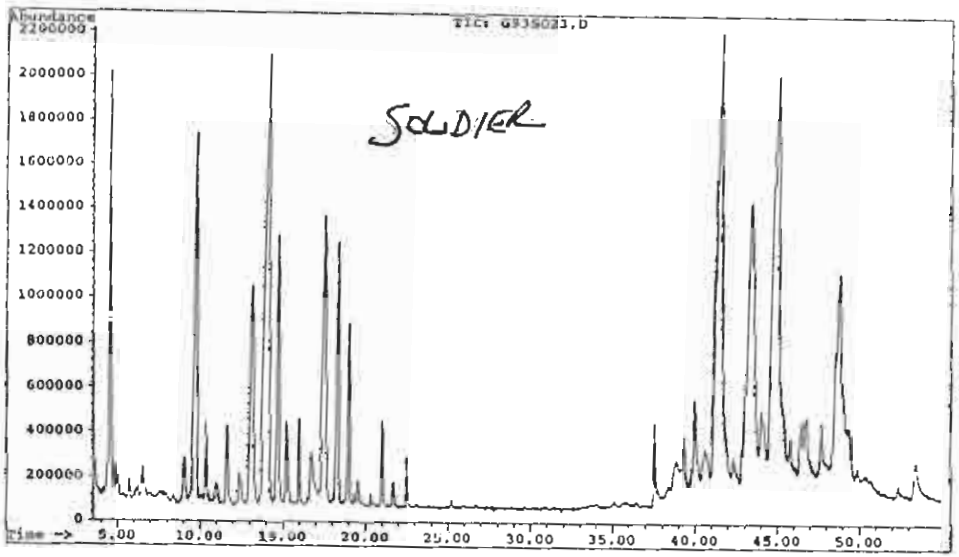
Figure 1

colony 2



colony 5





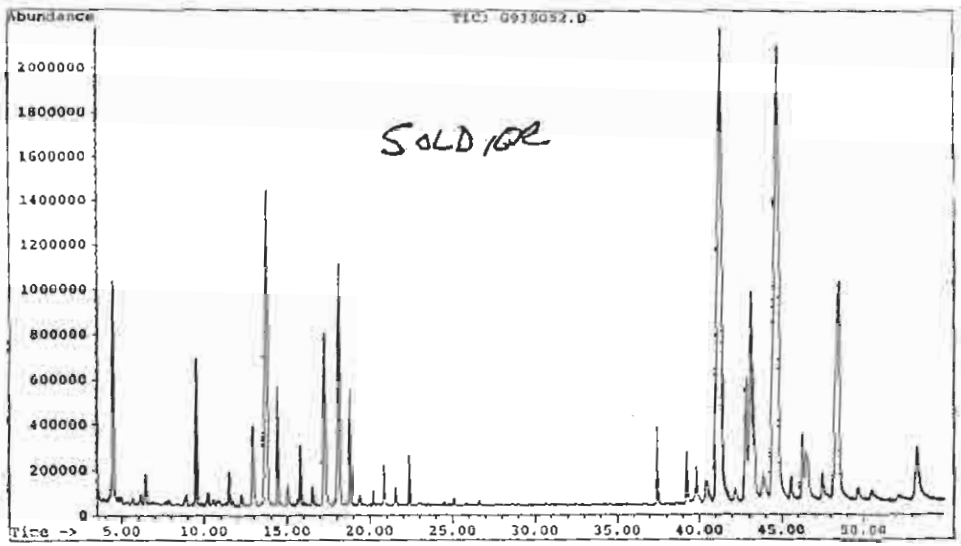
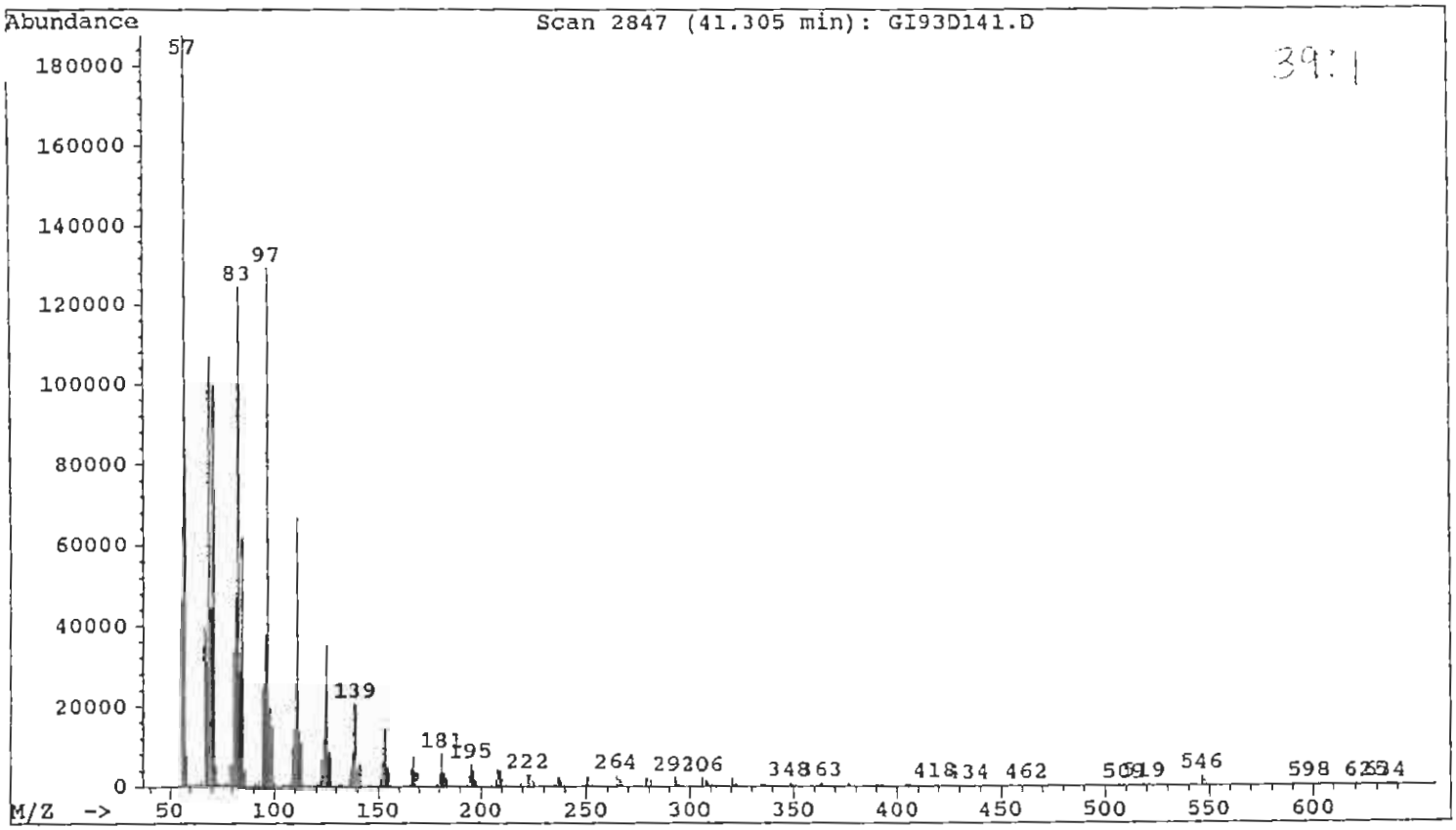


Figure 2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97
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181
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150
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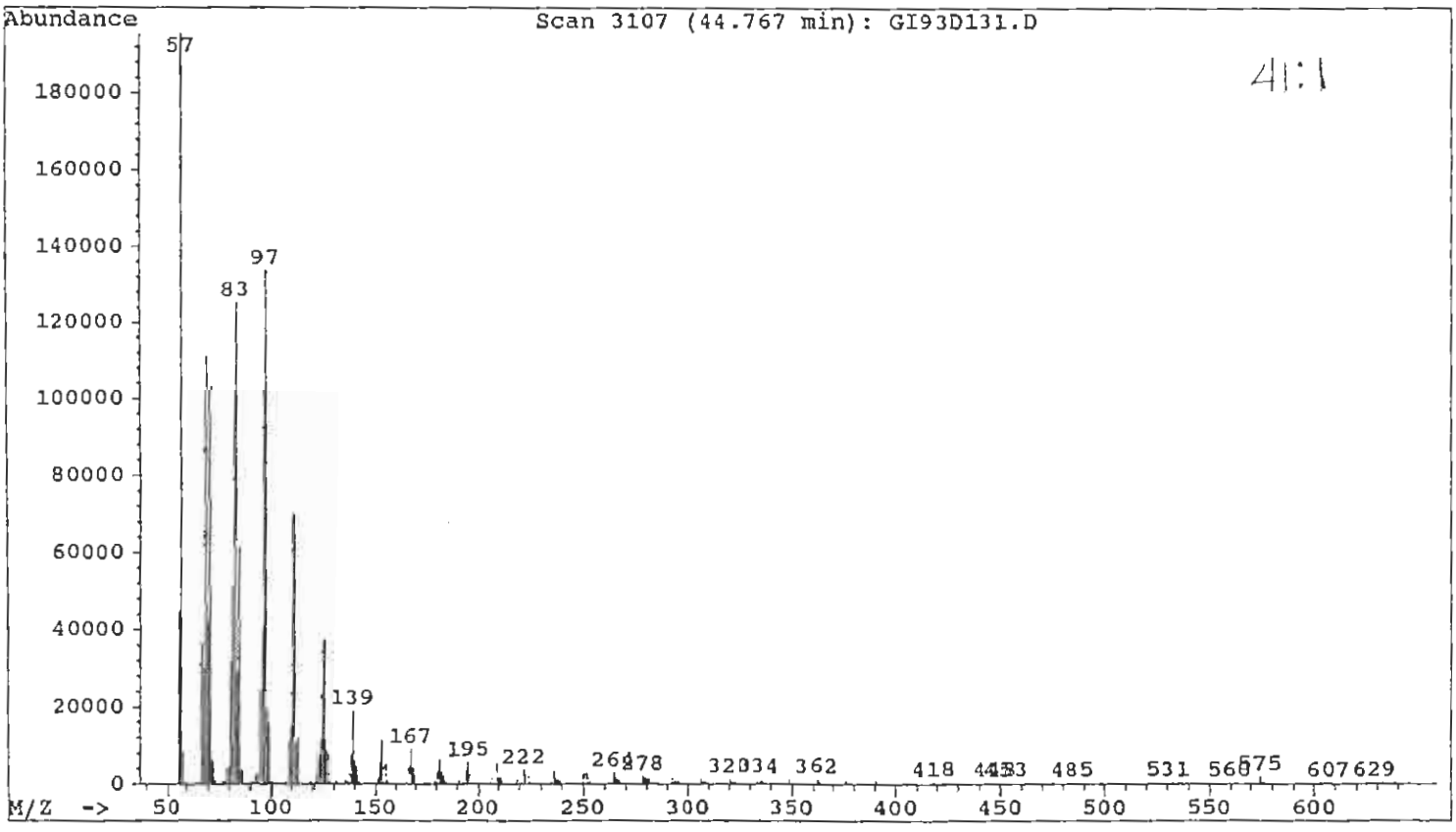
250
300
350
400
450
500
550
600


Abundance
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97
83
139
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195
222
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150
200
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300
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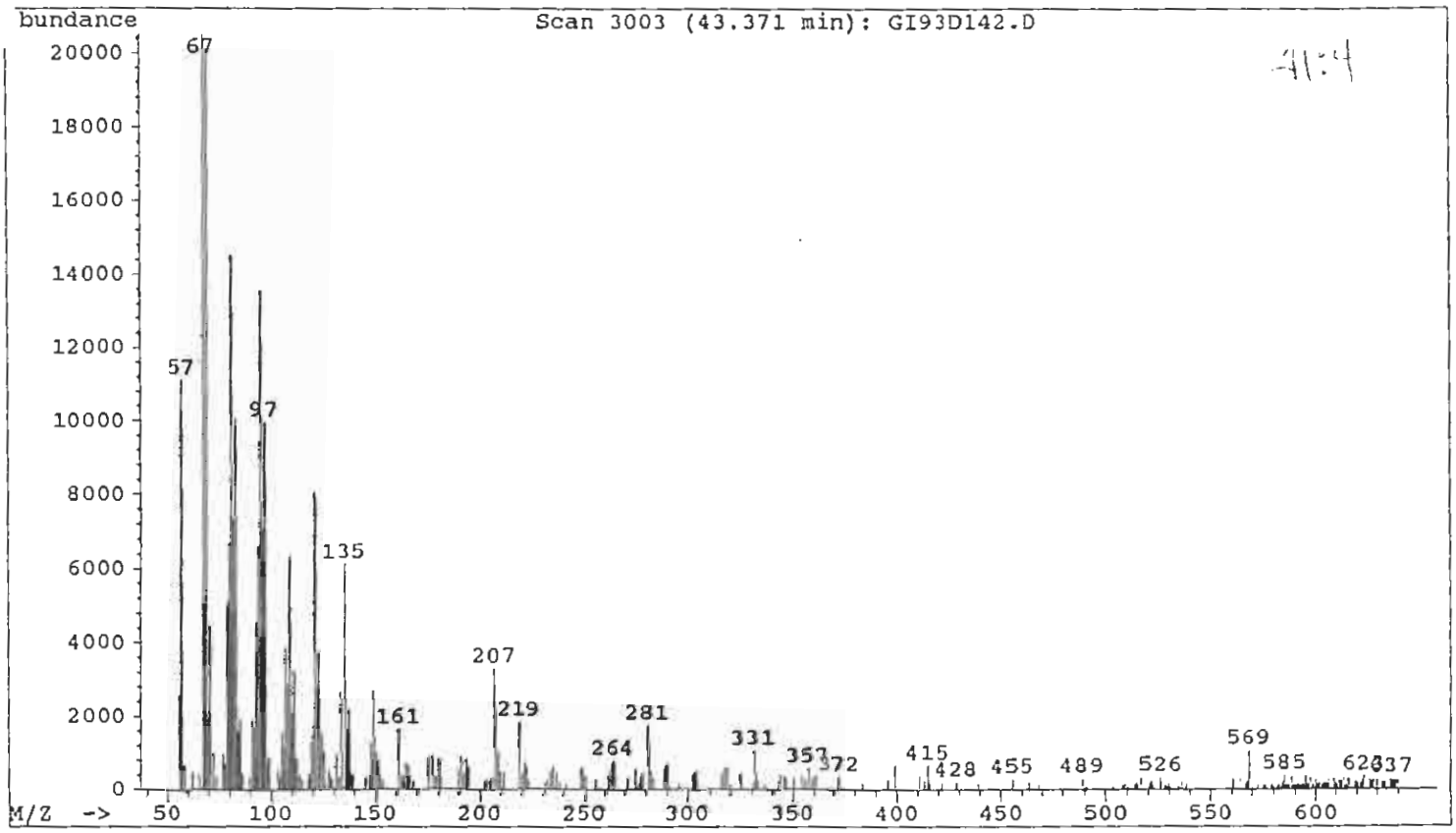
400
450
500
550
600

Figure 3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2000
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67
57
97
135
207
161
219
281
264
331
357
372
415
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585
M/Z ->
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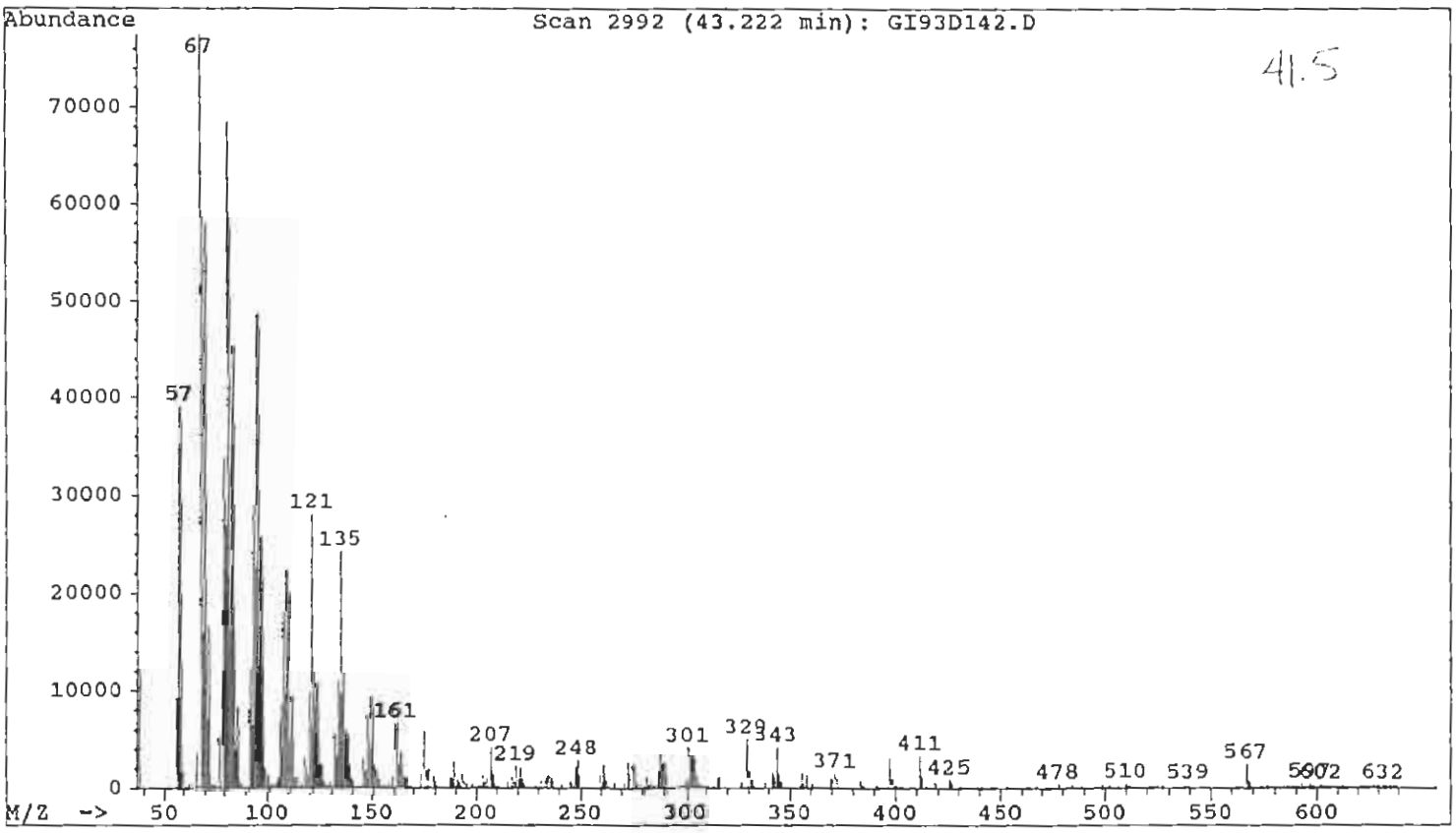
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121
135
161
207
219
248
301
329
343
371
411
425
478
510
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M/Z ->
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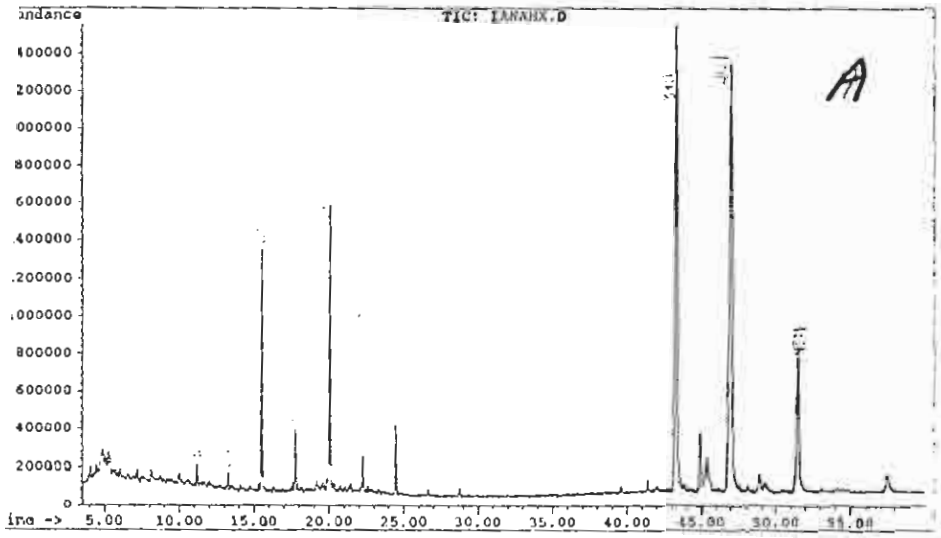
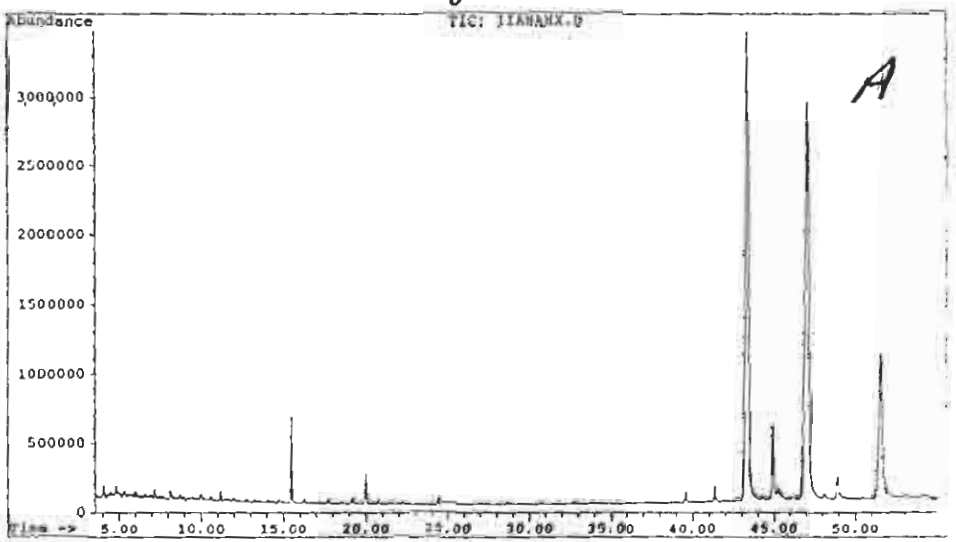
300
350
400
450
500
550
600

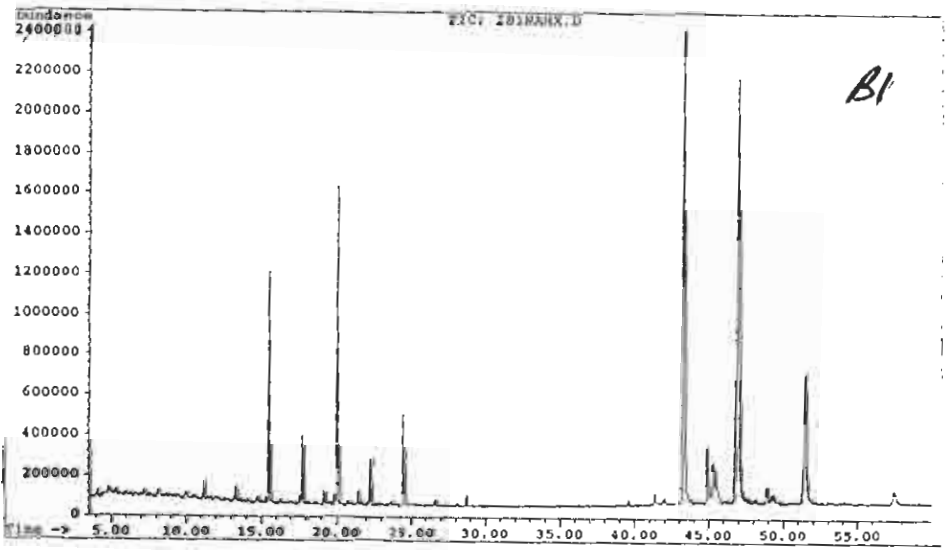
Figure 4

Colony 1



Colony 2





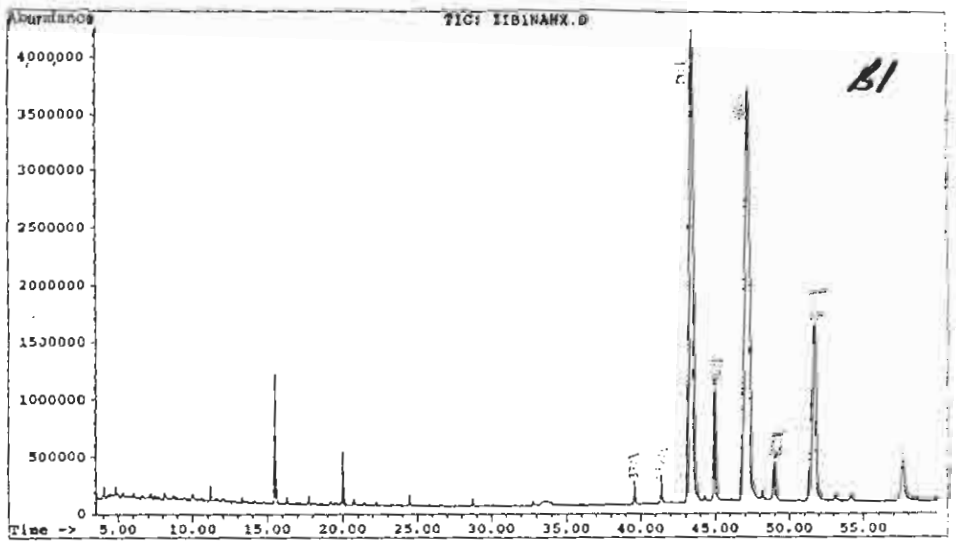
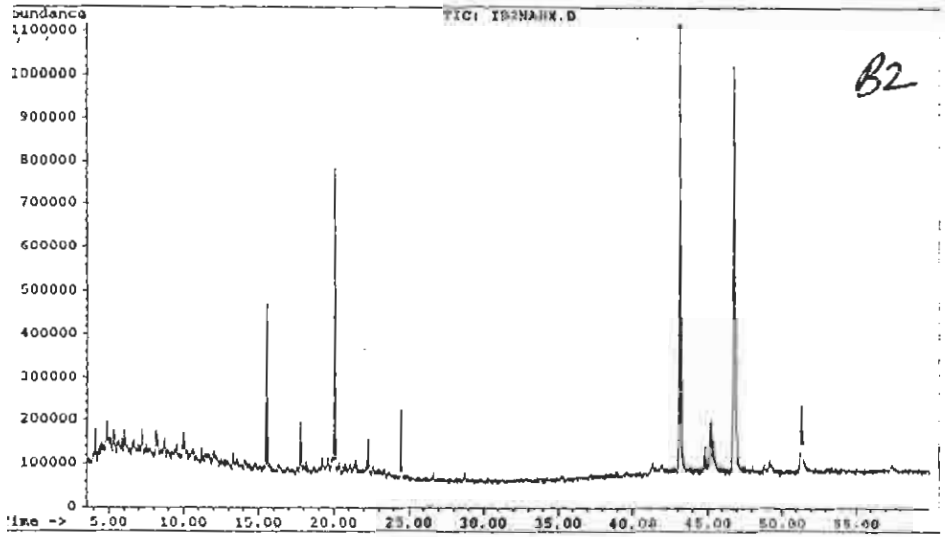


Fig. 4, cont'd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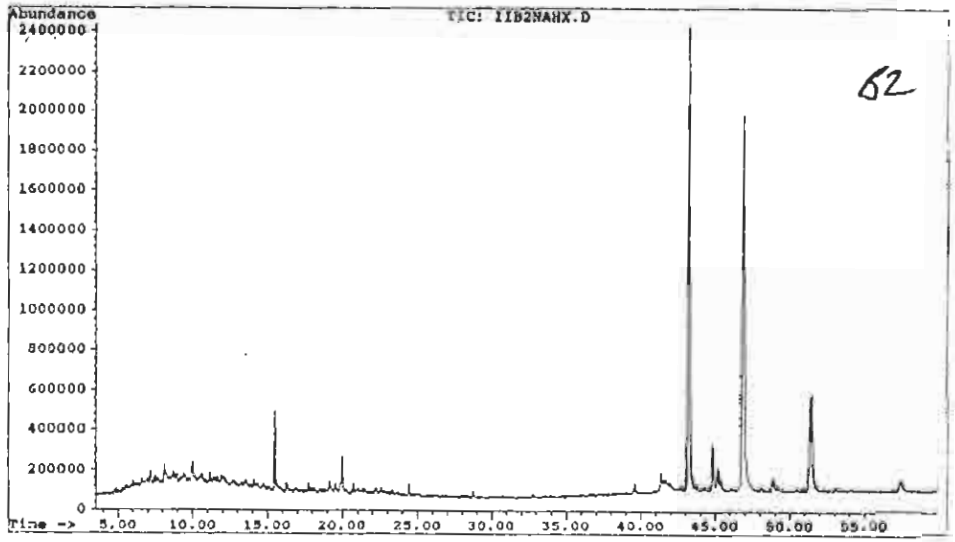
B2
Abundance
Time ->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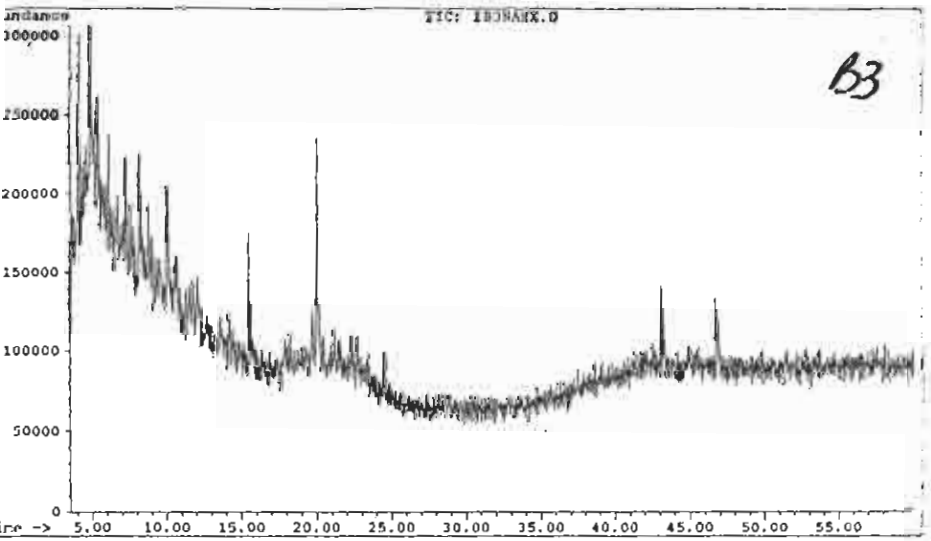
Time ->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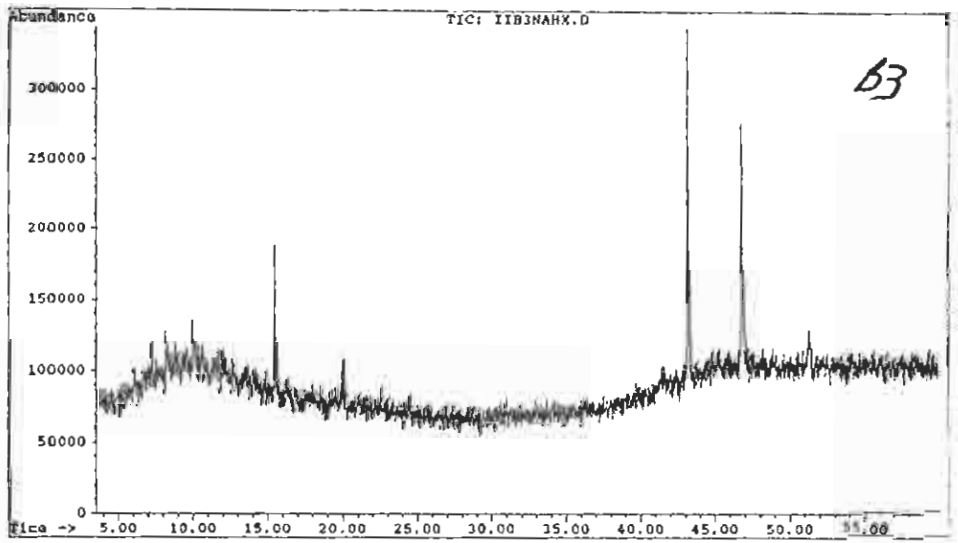
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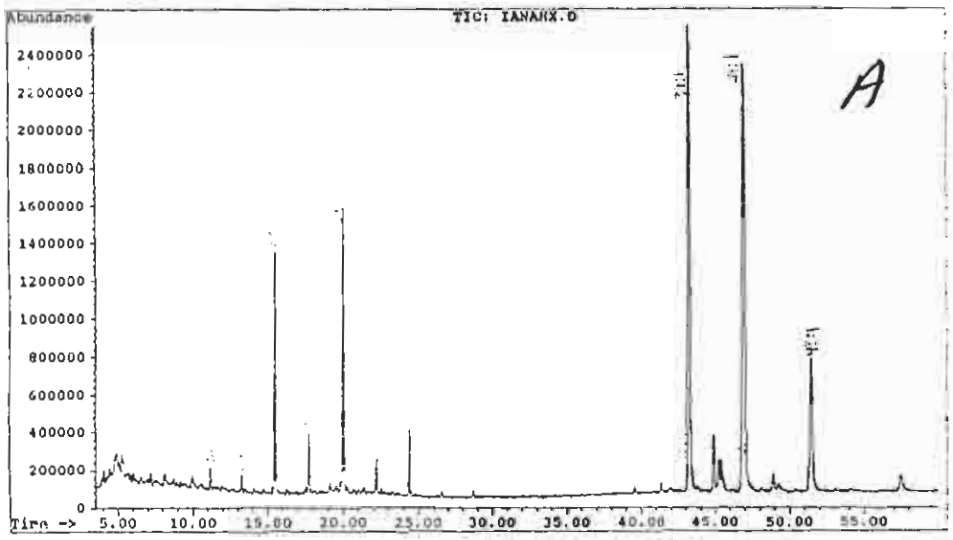
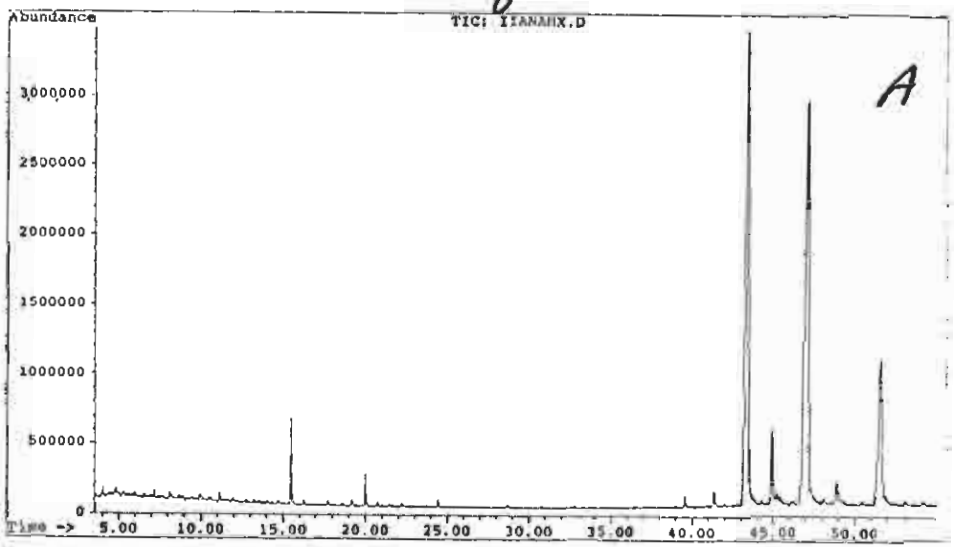
B3
Abundance

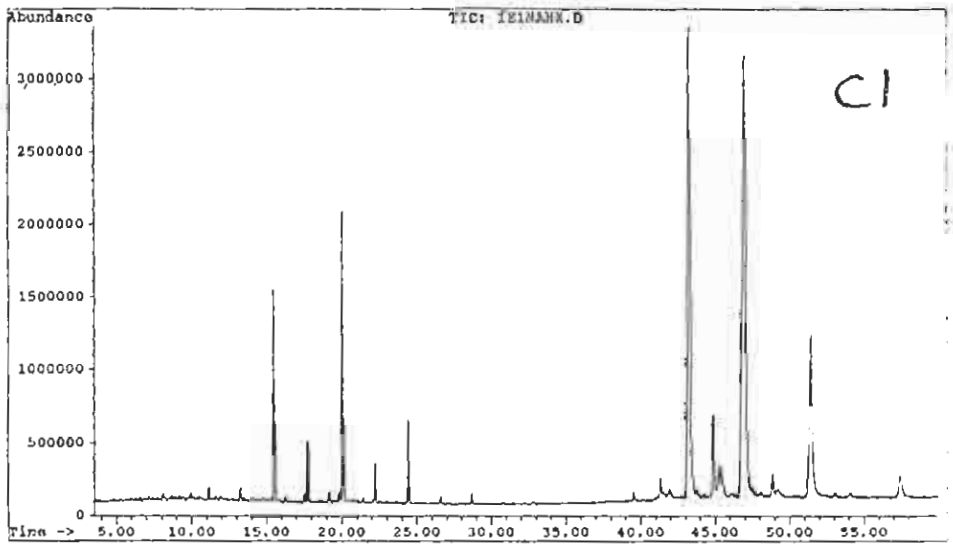
Fig 5

Colony 1



Colony 2





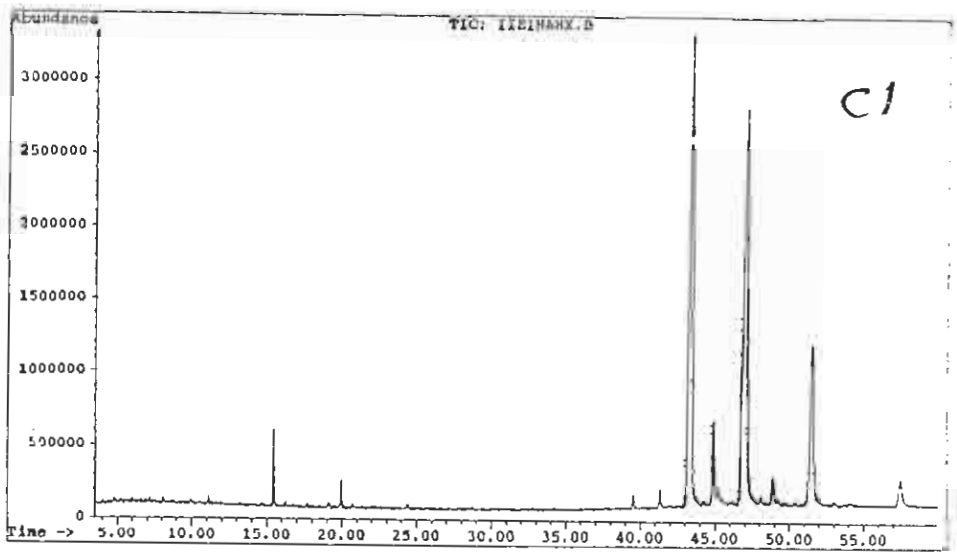
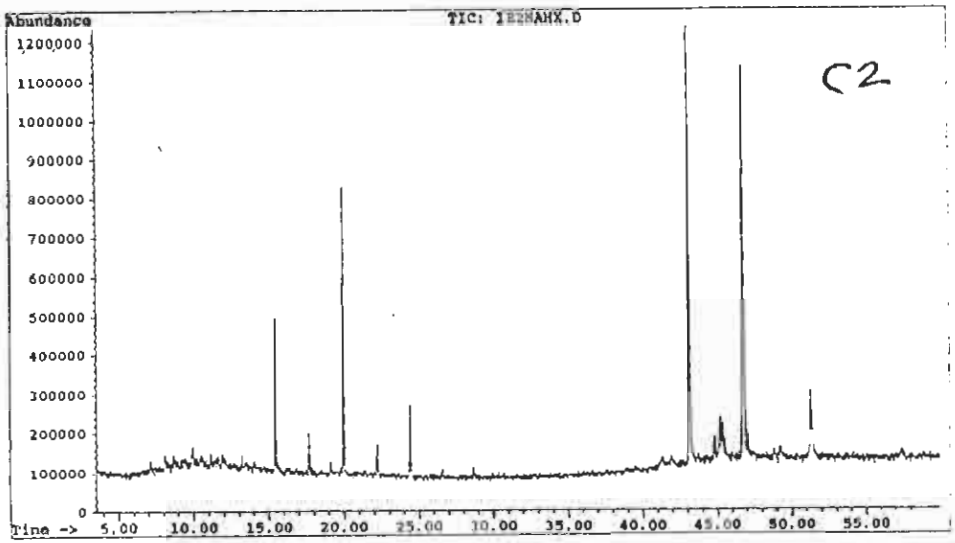


Fig. 5 cont'd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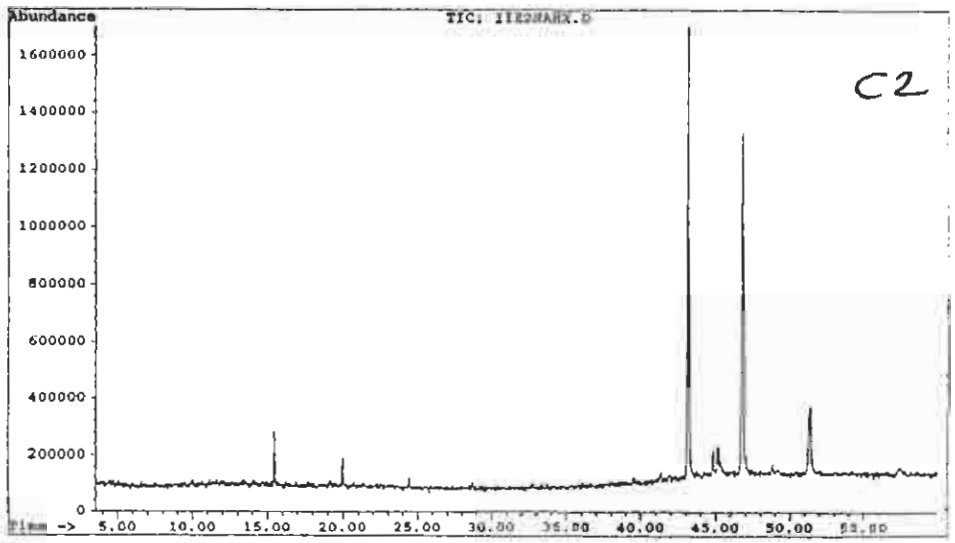
45.00
50.00
55.00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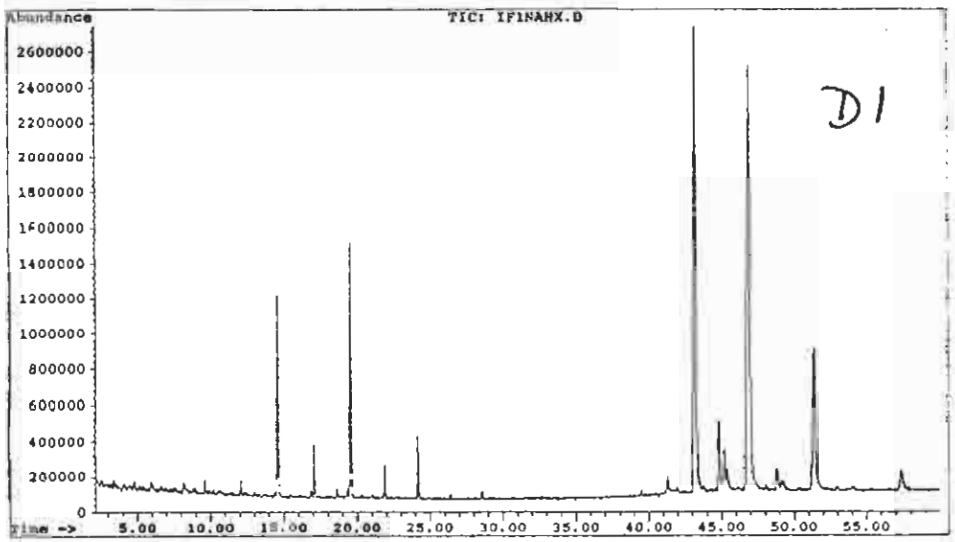
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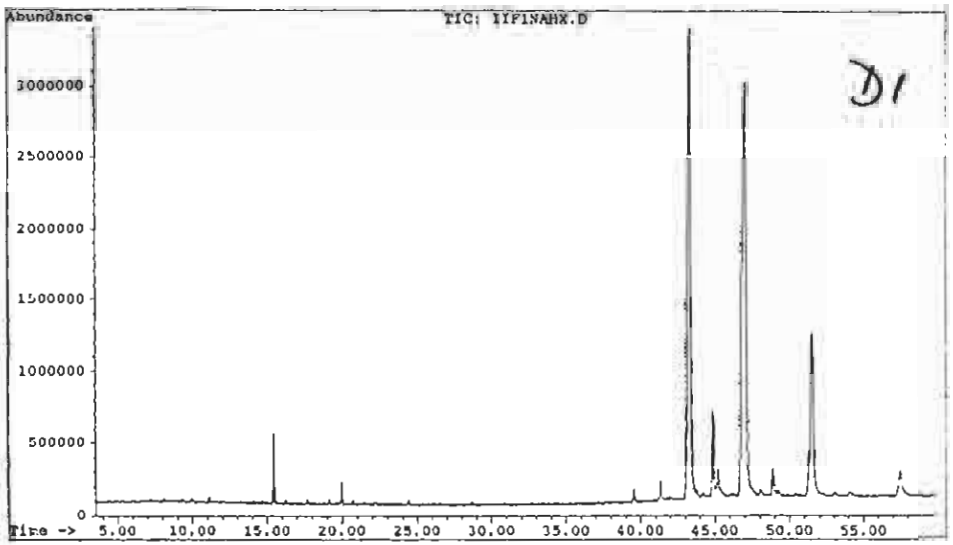
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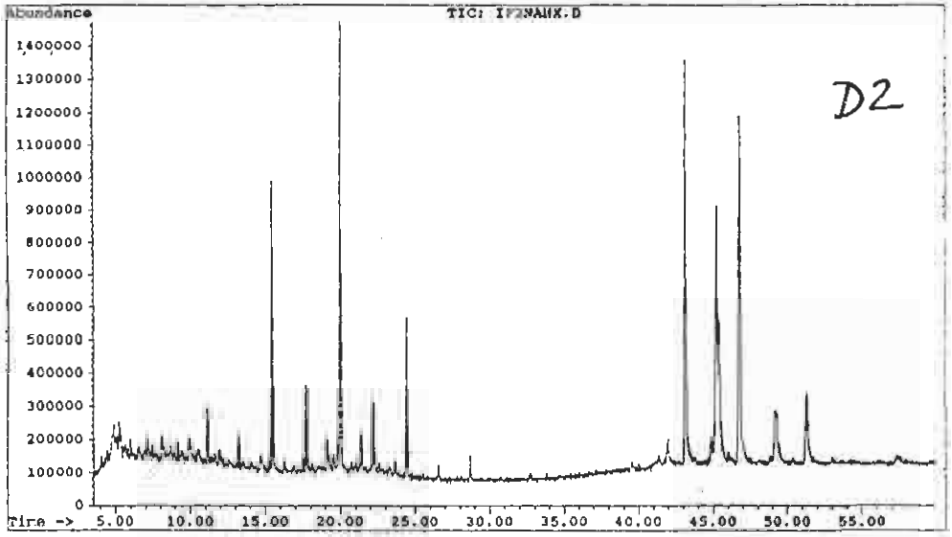
Fig 5, cont'd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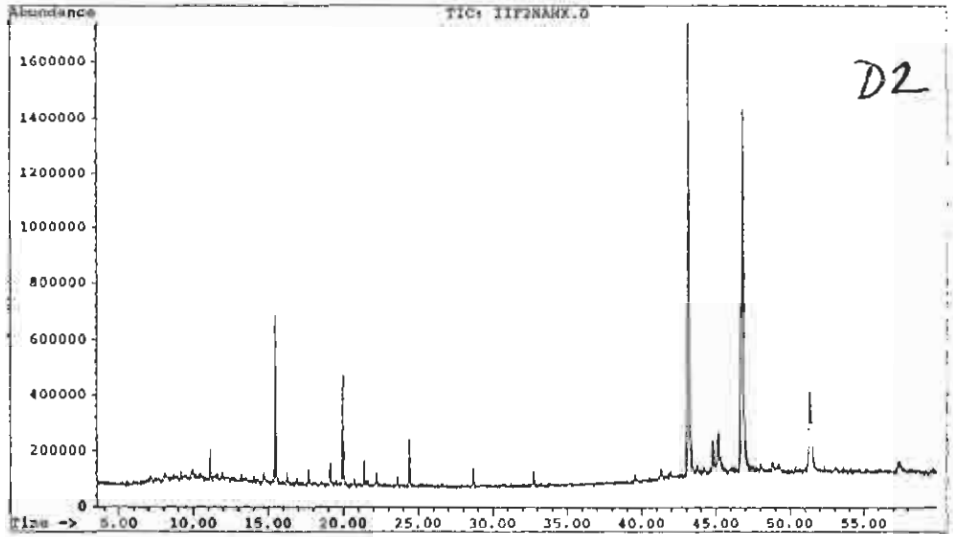
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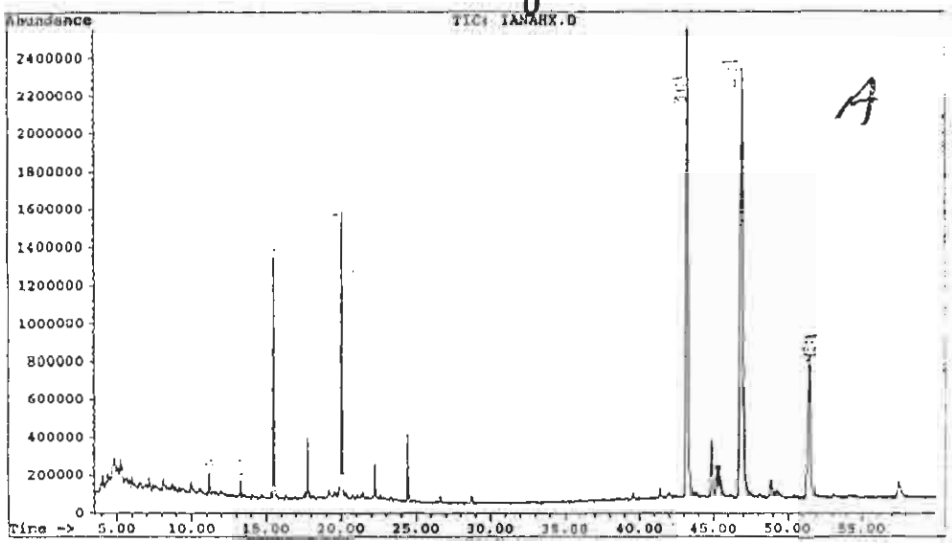
Fig 6

Colony 1



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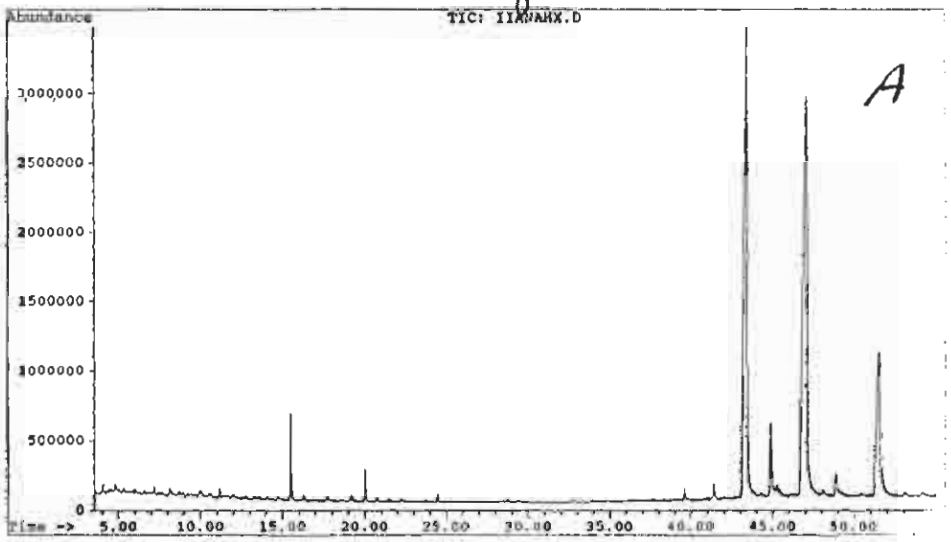
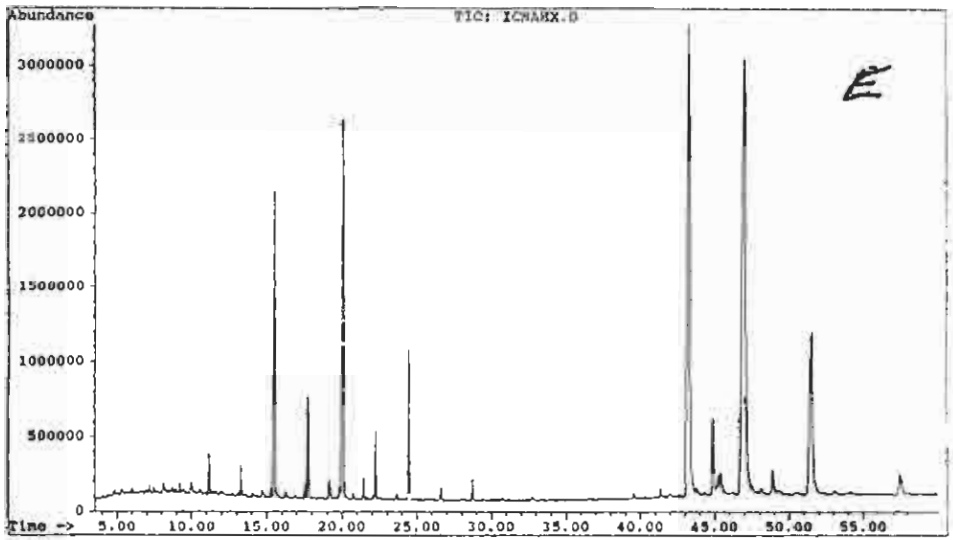




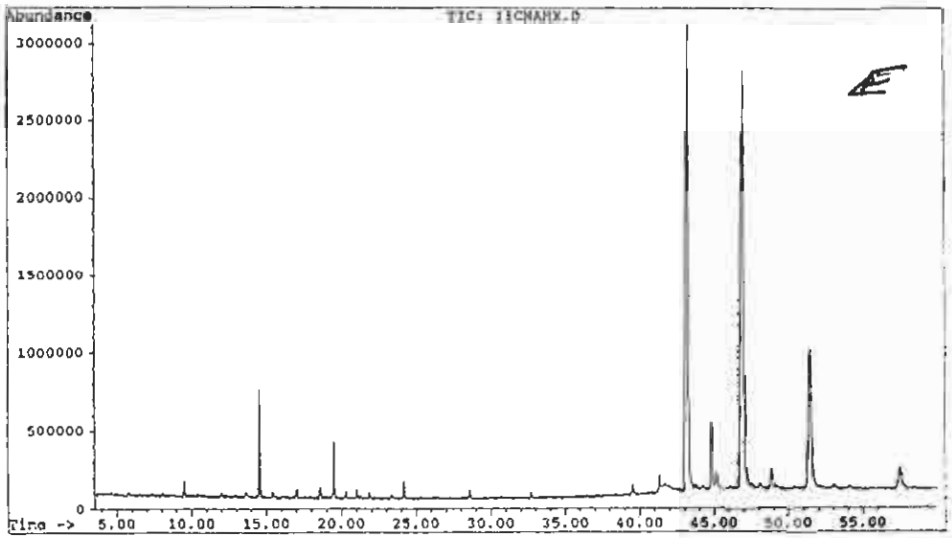
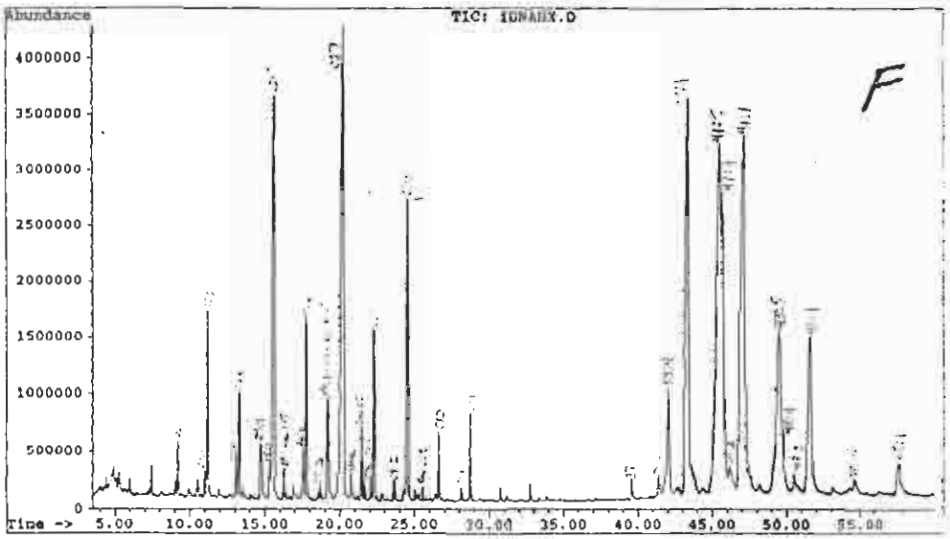


Fig 6, cont'd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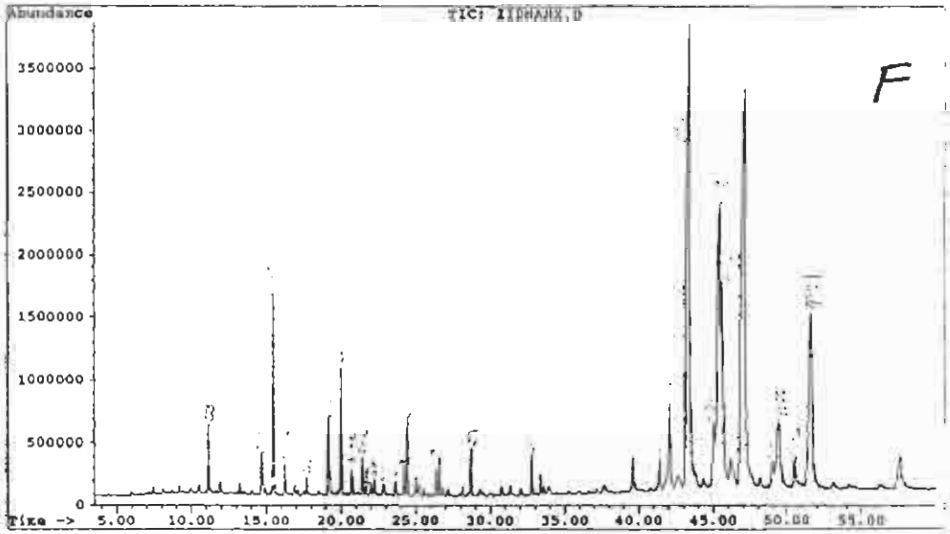
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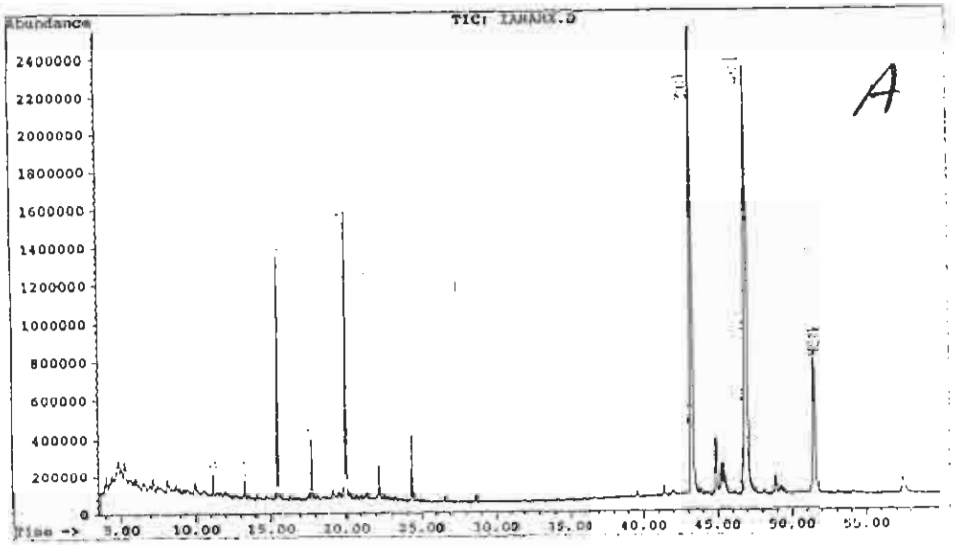
Figure 7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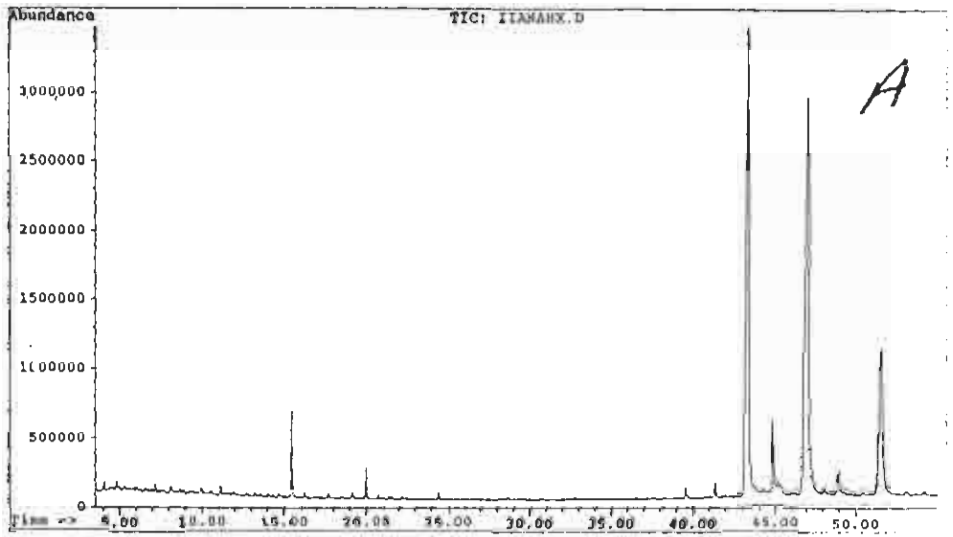
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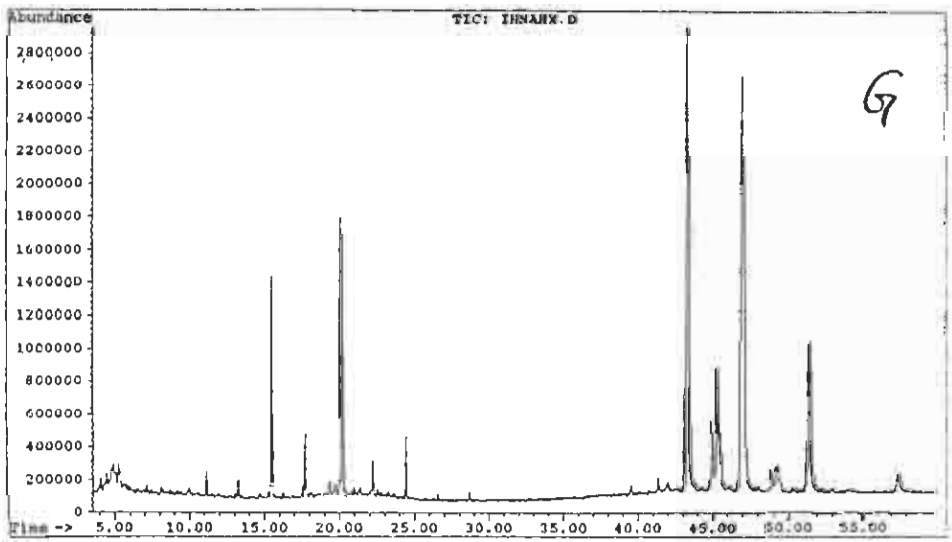
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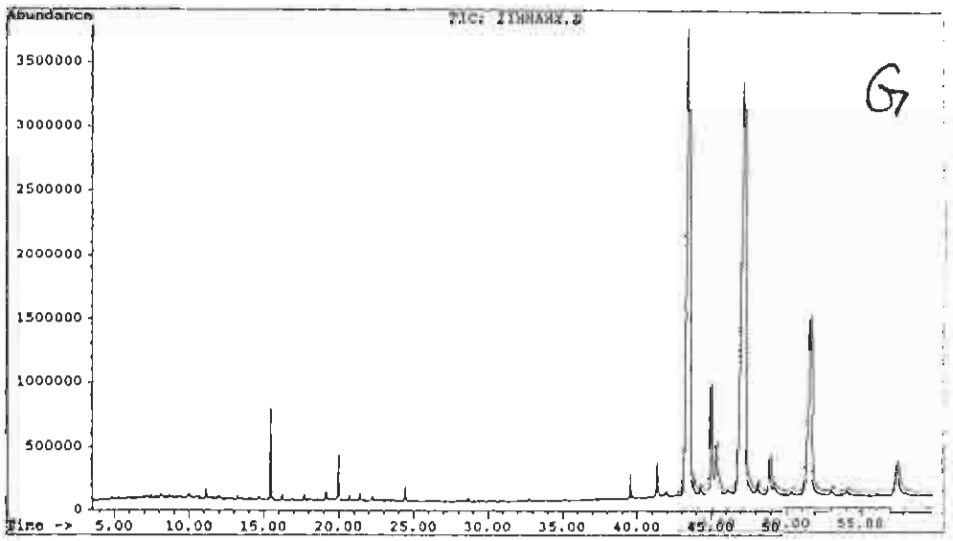
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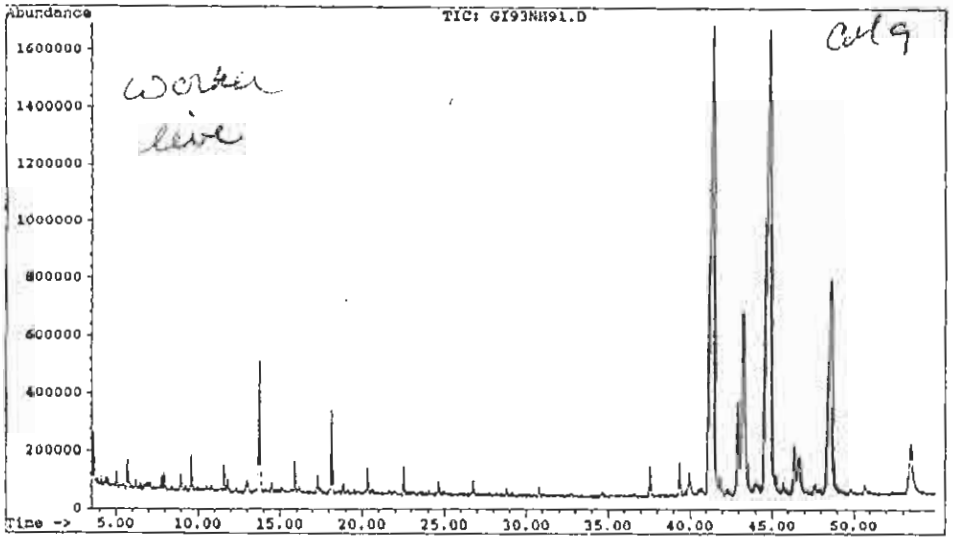
Figure 8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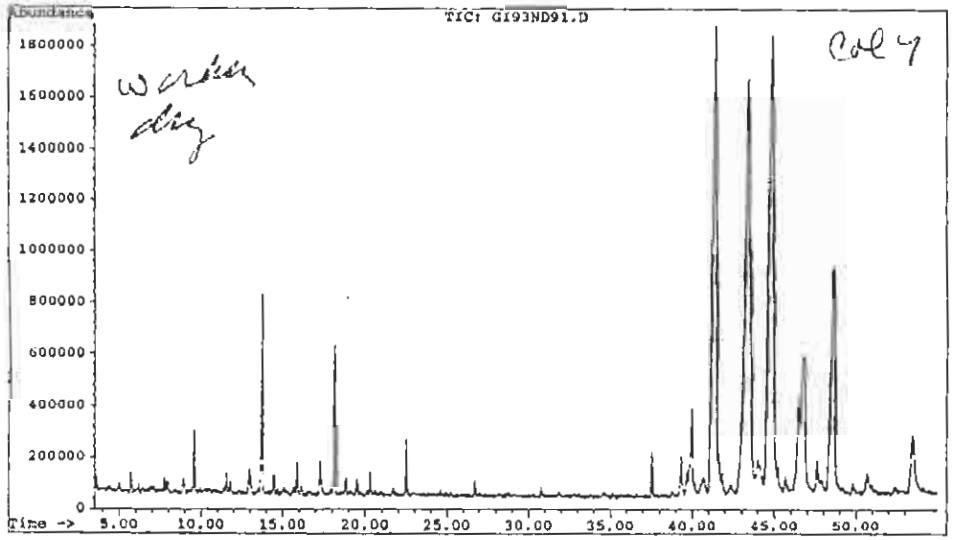
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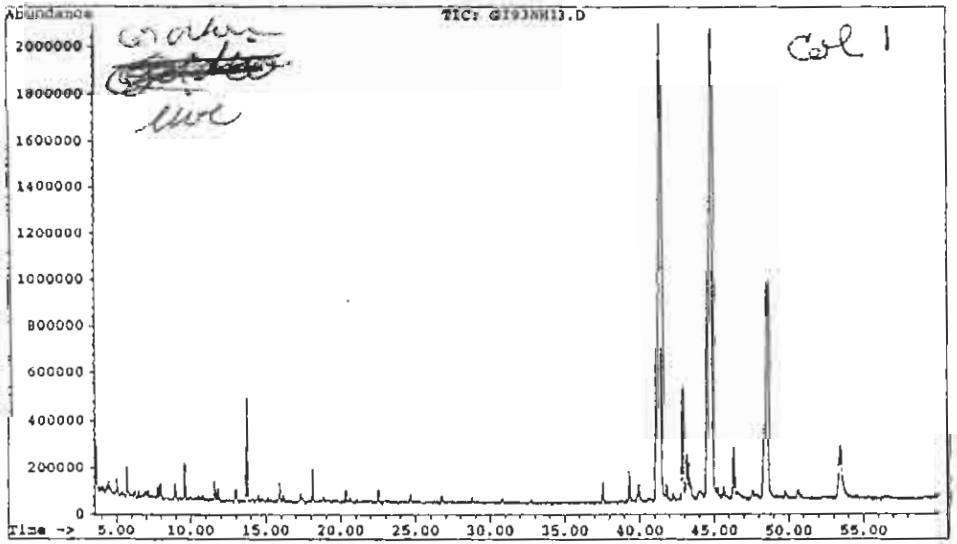
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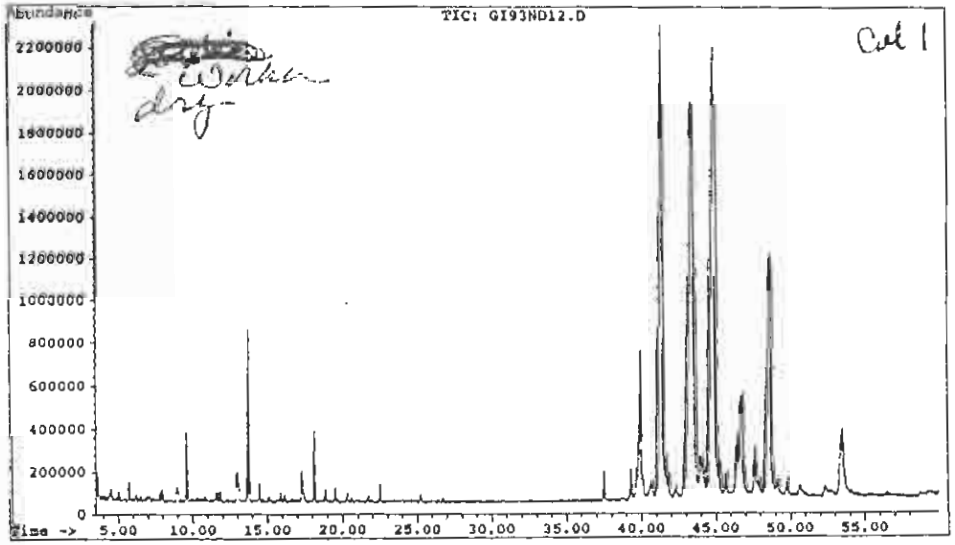
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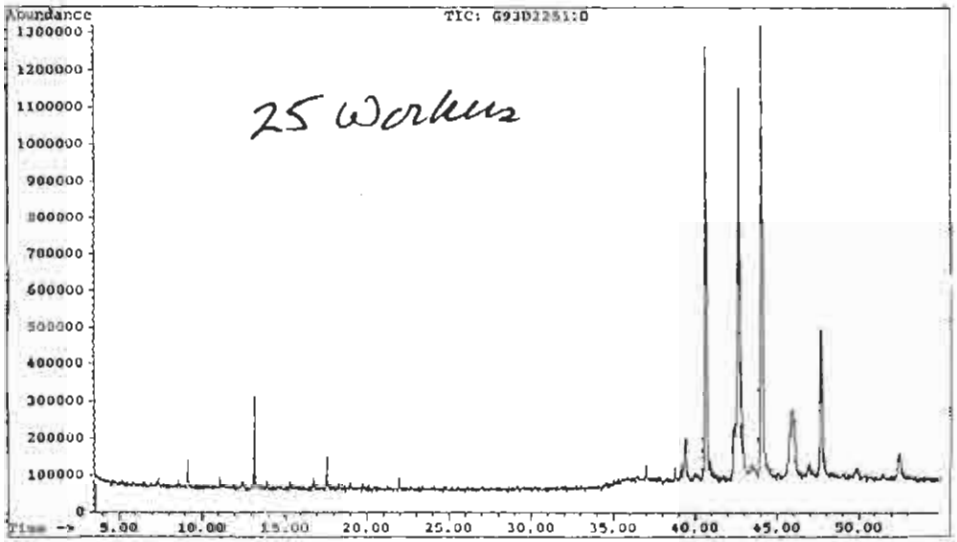
Figure 9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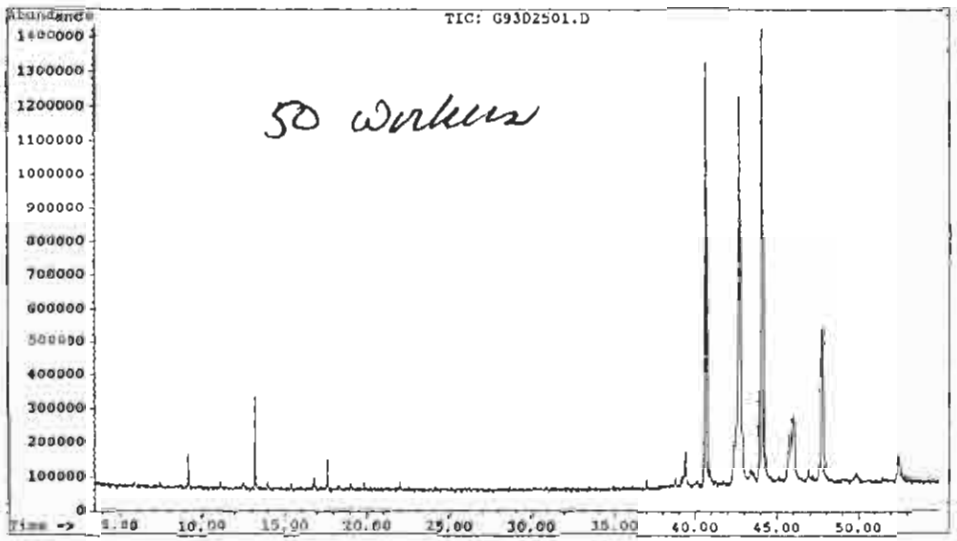
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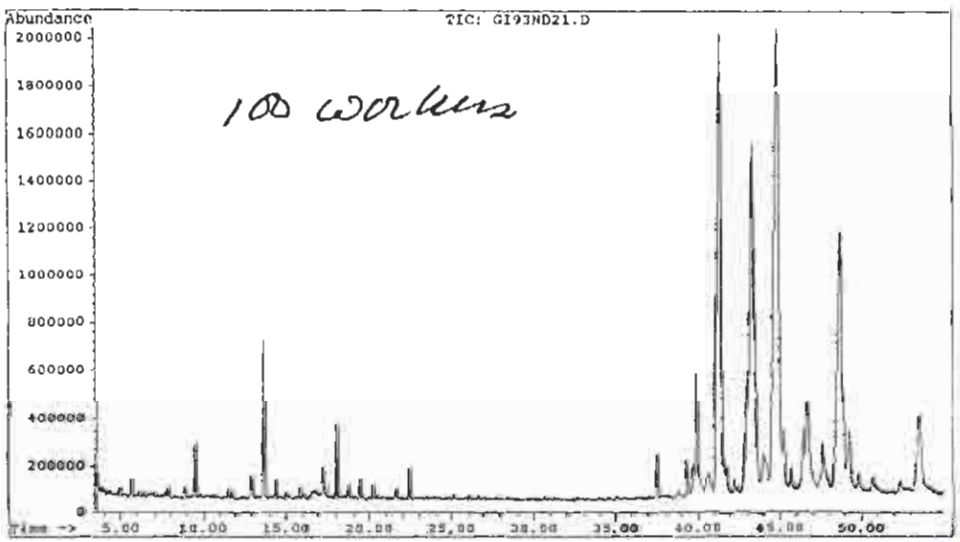
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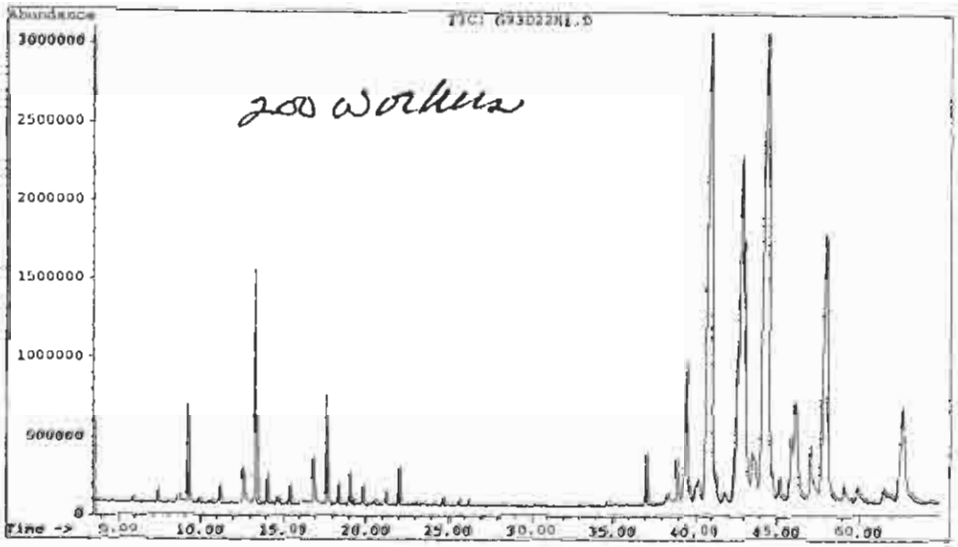
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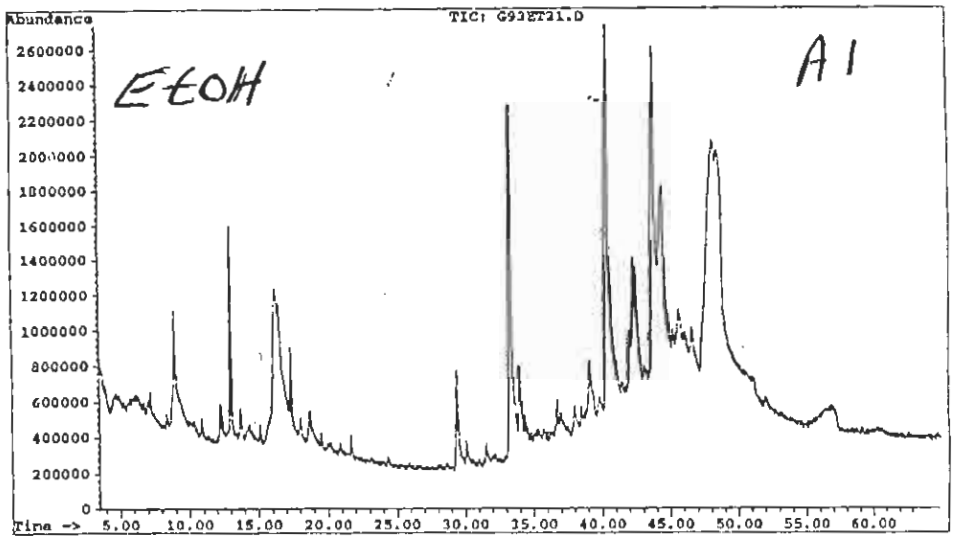
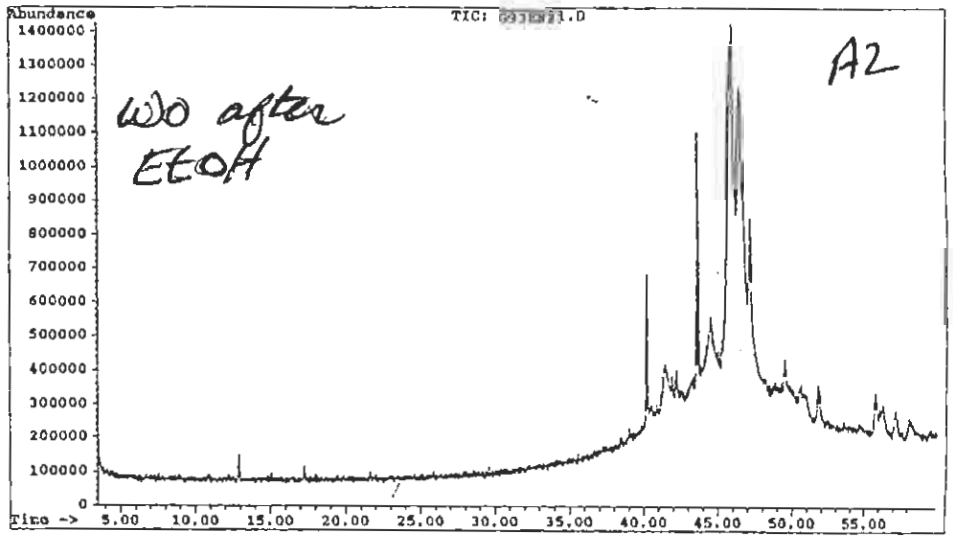
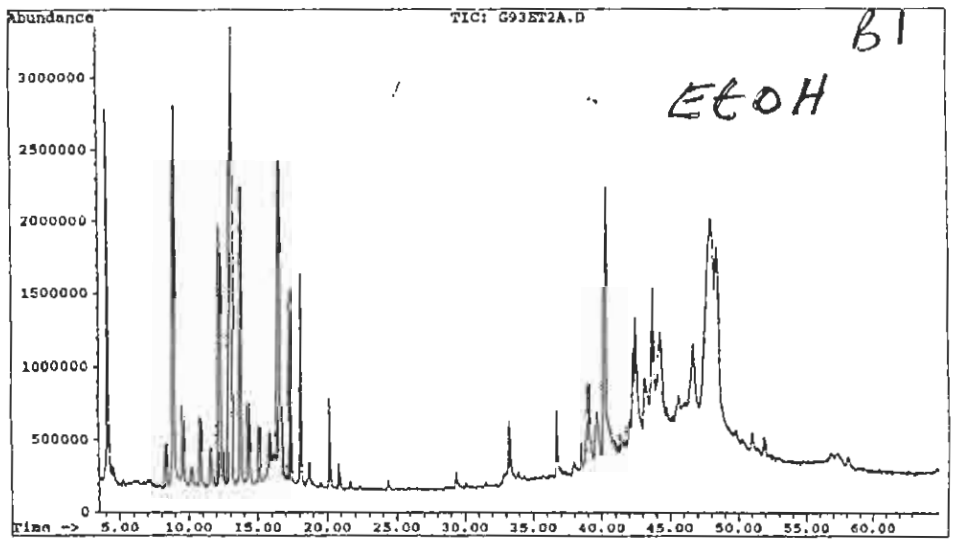
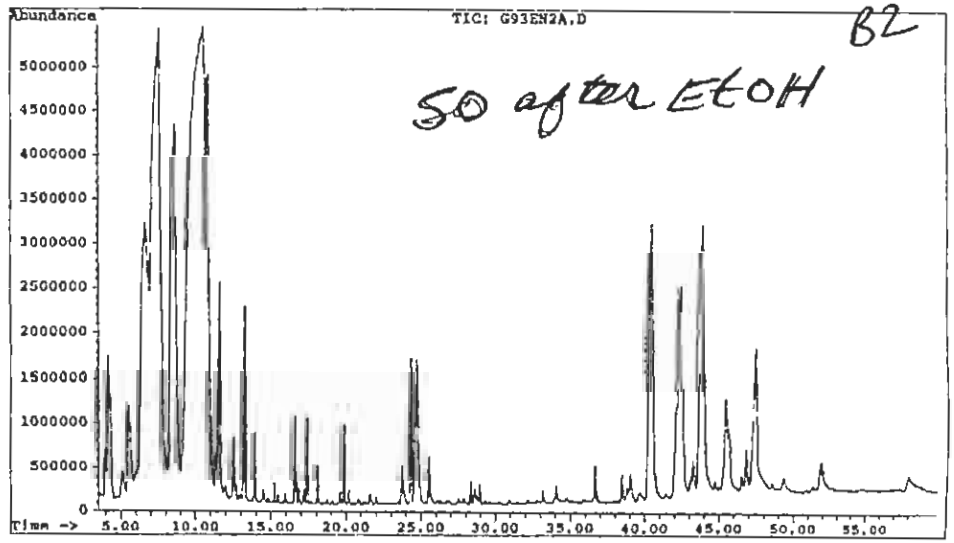
Figure 10

EtOH









Taxonomy and Biogeography of *Nasutitermes acajutlae* and *N. nigriceps* (Isoptera: Termitidae) in the Caribbean and Central America

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ABSTRACT The Neotropical termites *Nasutitermes acajutlae* (Holmgren) and *N. nigriceps* (Haldeman) are resurrected as discrete morphological species based on three diagnostic characters of the soldier caste. No consistent differences in alate morphology or in nest architecture have been identified. Based on current sampling, separation of the two species on the basis of cuticular hydrocarbon profiles of workers is equivocal. The known distributions of *N. acajutlae* and *N. nigriceps* are allopatric and consistent with current understandings of the biogeography of the Caribbean islands and Central America.

KEY WORDS *Nasutitermes*, nest architecture, cuticular hydrocarbons

THE *Nasutitermes nigriceps* complex comprises one of the most conspicuous groups of termites in Central America and much of the Caribbean, reportedly ranging into northern South America and Brazil (Emerson 1925, Snyder 1949, Araujo 1977, Mathews 1977). These termites build arboreal nests that are among the largest built by *Nasutitermes* in the Neotropics (maximal recorded measurements close to 2 m in height and >1 m in girth [B.L.T., personal observation]). The termites inhabit lowland and montane forests, mangroves, and occur commonly, but not exclusively, in relatively drier or exposed areas. In their habitats, colonies of these species are numerically and ecologically conspicuous. Their ability to withstand dry conditions coupled with facultative food storage (in the form of nest nodules) may explain the success of these termites even in exposed or marginal habitats.

Morphological variation within this group has long been a source of confusion, but it has been difficult to resolve whether the variation reflected a geographic cline or true species differences. Examination of museum specimens and new collections from the Caribbean and Central America has enabled us to expose a biogeographical pattern within the *N. nigriceps* complex and to divide the group into two species, *N. nigriceps* (Haldeman) and *N. acajutlae* (Holmgren), based on consistent morphological characters.

Taxonomic History. Haldeman's (1853) original description of *Termes nigriceps* from western Mexico is brief and vague. He expressed uncertainty about whether or not he examined a soldier within the sample. The species description was elaborated extensively by Light (1933) based on specimens from three localities in Mexico. The type material for this species has not been located. Light did not refer to seeing the type specimens. Snyder (1949) does not give the locatiou of the type material. Araujo (1977) states that the type is in the collection of Franklin & Marshall College (presumably the North Museum), Lancaster, PA, but, if so, the curators cannot locate it.

Nasutitermes acajutlae was originally described (as Eutermes) by Holmgren (1910) based on specimens from Acajutla, San Salvador; Bahamas (alate); and from St. Thomas, U.S. Virgin Islands (one soldier, one worker, and one alate). Both groups of specimens are labeled as COTYPES; both are in the American Museum of Natural History (AMNH) collection.

Holmgren's assignments regarding *N. acajutlae* have been questioned, and much material from the Caribbean has been grouped with *N. nigriceps*. Banks (1919) commented that the *N. acajutlae* alate, which Holmgren described from San Salvador, is probably *N. rippertii* (Rambur) (originally described by Rambur ([1842]; redescribed by Silvestri [1903]). Banks (1919) described a new species, *N. creolina*, based on alates from the island of Montserrat, now in the Museum of Comparative Zoology (MCZ TYPE 10078) that he considered to represent the adult of *N. creolina*, and soldiers from Trinidad (labeled "paratype", but also containing an MCZ TYPE 10078 label). Emerson (1925) notes that Banks' Montserrat alate does not belong with the

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List 1. Collection locations of material examined for this study

Type material

Eutermes acajutlae COTYPE Acajutla, San Salvador (AMNH)
Eutermes acajutlae COTYPE St. Thomas, U.S. Virgin Islands (AMNH)
Eutermes guatemalae COTYPE San Jose, Guatemala (AMNH)
Eutermes pacificus COTYPE W. coast of South or Central America (AMNH)
Eutermes pilifrons COTYPE Bogota, Colombia (AMNH)
Eutermes pilifrons COTYPE Curacao, Dutch West Indies (AMNH)
Nasutitermes creolina TYPE Montserrat, West Indies (MCZ TYPE 10078)
Nasutitermes creolina PARATYPE Trinidad (MCZ TYPE 10078)

Other specimens[a]

Belize: Boca del Rio on Ambergris Caye
Brazil: Recife, Pernambuco
British Virgin Islands: Anegada, Beef Island (2), Cooper, Eustatia, George Dog (3), Great Camino, Great Thatch, Great Tobago, Guana Island (17), Jost van Dyke (2), Little Camino, Necker, Peter, Scrub, Thatch, Tortola (6), Virgin Gorda
Costa Rica: Guanacaste (6)
Grand Cayman Island: Boddentown, Booby Cay, Canaan Land (2)
Jamaica: Dromilly (4), nr. Constant Springs, Kingston, Priory (9), St. Amis
Mexico: Acapulco (3), Campache, Colima, Guerro, Jalisco (3)
Nicaragua: Leon (4)
Panama: Barro Colorado Island (4), Frijoles, Galeta (7)
Puerto Rico: Isla Magueyes (2), Guanica, La Parguera (3)
Trinidad: Valencia
Turks and Caicos Islands: Providenciales Island (2)
United States Virgin Islands: St. Croix (5), St. Johns (14), St. Thomas

[a] If material was available from more than one colony per location, the number of colony samples examined is shown in parentheses.

soldier; Snyder (1949) and Araujo (1977) identify the Montserrat alate as *N. ephratae* (Holmgren). Emerson (1925) synonomized *N. creolina* Banks (1919) with *N. acajutlae* based on identity of soldier morphology. *N. creolina* Banks (Trinidad soldiers only) was synonomized with *N. nigriceps* by Snyder (1949).

Emerson (1925) expanded Holmgren's description of *E. (Nasutitermes) acajutlae* based on specimens from three locations in British Guiana (now Guyana) (one queen, no alates, many soldiers) and from St. Croix and St. Thomas, U.S. Virgin Islands (three queens, no alates, many soldiers). Emerson (1925) does not address Banks' (1919) note that Holmgren's *N. acajutlae* alate from San Salvador may be *N. rippertii*. In his listing of the termites of the world, Snyder (1949) synonomized *N. acajutlae* (Holmgren) with *N. nigriceps* (Haldeman).

In this article, we present evidence that Snyder's synonomy of *N. acajutlae* as *N. nigriceps* was not justified. We resurrect the name *N. acajutlae* (Holmgren) for the species occurring in the Lesser Antilles from Puerto Rico east and south to Trinidad (not recorded from all intervening islands), into Guyana and possibly other locations in South America. *N. nigriceps sens. str.* occurs on Jamaica and the Cayman Islands, as well as Mexico south to at least Panama. A discussion of Caribbean biogeography renders this pattern of distribution consistent with the historical geography of the region.

Materials and Methods

Morphological Separation of *N. acajutlae* and *N. nigriceps*. Collection locations of material examined for this report are listed in List 1. All or most of the nontype material from each country was relatively fresh (collected by ourselves or colleagues within the past 6 yr), except for the material from Brazil and Mexico (collected >20 yr ago). All specimens were examined with a light microscope. Structures on a series of freeze-dried specimens from Panama, Mexico, Jamaica, St. Croix, St. Johns, Puerto Rico, and Guana Island were examined using scanning electron microscopy.

Voucher specimens are deposited at the United States National Museum, Smithsonian Institution, Washington, DC.

Nest Architecture. Arboreal carton nests of both *N. acajutlae* and *N. nigriceps* were examined externally when collections of termites were taken. In Panama, eight *N. nigriceps* nests were completely or substantially dissected by B.L.T. In large nests of both species the central region can be extremely hard, preventing complete dissection of those galleries and the royal cell. Nests on Jamaica, the Cayman Islands, Puerto Rico, the U.S. Virgin Islands, and the British Virgin Islands were examined externally, and often the outer layers of carton were shaved with a saw or a machete to expose a portion of the gallery system. Three *N. acajutlae* nests on Guana Island were completely dissected.

Cutieular Hydrocarbon Phenotypes of *N. acajutlae* and *N. nigriceps*. Samples of *N. acajutlae* and *N. nigriceps* were gathered along with collections of many other species of termites during an ongoing study of the chemotaxonomy of tropical termites. Occasionally, samples were collected by associates who encountered one of

these two species while conducting field work for other projects.

All collections reported in this article were taken from arboreal nests. The usual method was to make a tangential slice in the nest, then return the nest material and termites to a laboratory to remove the termites. Termites were separated from carton and other debris either directly with forceps or indirectly by placing wet paper towels over the carton to attract the termites. Samples of at least 100 workers were placed in vials and dried over an incandescent light. Termites were dried while alive and for an additional 8–10 h after they died. Voucher specimens from each colony, consisting of each caste present, were preserved in 85% ethanol; samples are kept at the University of Maryland, the Smithsonian Institution, or the Pacific Southwest Research Station in Albany, CA. Dried samples were shipped to the Pacific Southwest Research Station in Albany for characterization of the cuticular hydrocarbons. Dried samples were held at ambient temperature until they were extracted.

Detailed descriptions of extraction procedures for specific termite species have been published elsewhere (Haverty et al. 1988, 1990a, b). Dried termite samples were transferred to clean 20-ml vials and immersed in 10 ml of *n*-hexane for 10 min. The resulting extract of cuticular lipids was pipetted through 4 cm of activated Biosil A in a Pasteur pipet minicolumn to isolate the hydrocarbon components. The hydrocarbon extract was then evaporated to dryness under nitrogen and redissolved in 60 μl of hexane.

Gas chromatography-mass spectrometry (GC-MS) analyses of the hydrocarbons were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a capillary column (25 m by 0.2 mm i.d., HP-1) and a Hewlett-Packard 5970B mass selective detector. The system was interfaced with HP Chemstation software for data acquisition and analysis. Helium was the carrier gas, column flow was 2 ml/min, and the GC-MS was operated in the split mode with a ratio of 7:1 and a temperature program from 200 to 320°C at 3°C/min with a final isothermal hold of 16 min. Electron impact mass spectra were obtained at 70 eV, and scanning was done from 55 to 640 atomic mass units every 1.0 s. *n*-Alkanes, alkenes, and methyl-branched alkanes were identified by their mass spectral fragmentation patterns (Pomonis et al. 1980, Blomquist et al. 1987) and corroborated by calculating equivalent chain lengths (Jackson & Blomquist 1976).

For this study, comparisons between colonies of the same species, between localities of the same species, or between species was empirical and based on qualitative differences in hydrocarbon components. A more detailed assessment of intra- and interspecific variability of the cuticular hydrocarbons of the *Nasutitermes* from the Caribbean Basin is in progress.

Results and Discussion

Morphological Separation of *N. acajutlae* and *N. nigriceps*. The following are three key morphological characters that distinguish soldiers of *N. acajutlae* from *N. nigriceps:* (1) Pilosity on the soldier head capsule is much denser in *N. acajutlae* (Fig. 1A) than in *N. nigriceps* (Fig. 1B). Bristles are present on the head capsule and nasus of *N. acajutlae* soldiers, whereas they are normally absent from the nasus of *N. nigriceps* soldiers except at the tip. Ventrally, bristles can be seen cresting from the posterior margin in *N. acajutlae*, whereas that margin appears nearly or totally glabrous on *N. nigriceps* soldiers. (2) The anterior margin of the pronotum has a distinct medial indentation in *N. acajutlae* (Fig. 1C) but is more evenly convex with only a minor medial indentation in *N. nigriceps* (Fig. 1D). In some *N. acajutlae* specimens, the pronotum has a more gradually convex anterior margin with a rather weaker median incision but still with a more distinct medial indentation than in *N. nigriceps*. R. Scheffrahn (personal communication) has noted that the pronotum shape of *N. acajutlae* soldier specimens he has examined from Puerto Rico are more variable and often intermediate between the two examples illustrated in Fig. 1 C and D. Pilosity of the pronotum is more conspicuous in both bristle size and number on both the anterior and posterior margins of the pronotum in *N. acajutlae* than in *N. nigriceps*. (3) Bristles are present on the ventral portion of the head capsule of *N. acajutlae* on the regions flanking each side of the postmentum (Fig. 2). These areas are glabrous on *N. nigriceps* soldiers.

Measurements of *N. acajutlae* and *N. nigriceps* soldiers are given in Table 1. Although representative, morphometric ranges should not be considered definitive because *Nasutitermes* soldier size is known to vary within and among colonies and locations. For that reason, no statistical comparisons of morphometric figures are made between the two species based on this small sample size. We consider it risky to use morphometrics as diagnostic characters for soldiers of these two species of *Nasutitermes*.

We have not yet discovered characters that unambiguously separate alates of *N. acajutlae* from *N. nigriceps*, although relatively few specimens were available. Alate measurements (Table 2) should be interpreted with the same caveat as above regarding measurements on soldiers.

Nest Architecture. Nests of *N. acajutlae* and *N. nigriceps* are indistinguishable based on sampling to date. The arboreal nests are usually spherical to ellipsoidal, with the long axis occasionally >2.0 m. The outer envelope is irregularly textured with bumps and drip formations. A thin carton layer covers interior galleries, whose walls gradually increase in thickness from the periphery to the center. The center portion of a

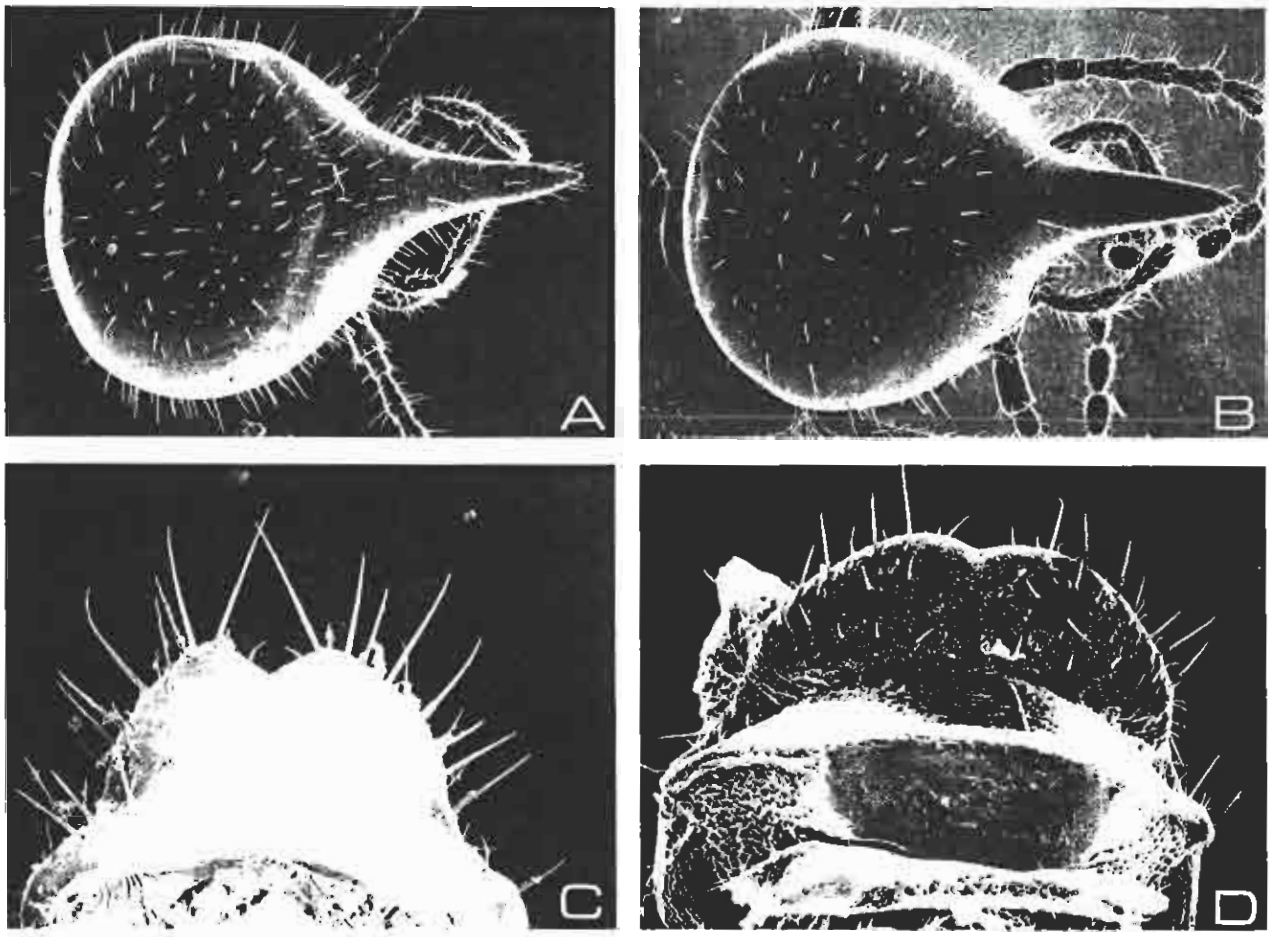

Fig. 1. (A) Soldier head capsule, *N. acajutlae*. (57×; coll. S. C. Briscoe, St. Johns, U.S. Virgin Islands). (B) Soldier head capsule, *N. nigriceps* (61×; Acapulco, Mexico, collection of C. H. Seevers, det. A. E. Emerson, AMNH collection). (C) Soldier pronotum, *N. acajutlae*. (166×, coll. B.L.T., St. Croix, U.S. Virgin Islands. (D) Soldier pronotum, *N. nigriceps*. (168×, coll. B.L.T., Priory, Jamaica).

mature nest is typically built of very dense carton, with walls of 1–2 cm between galleries. The royal chamber is positioned in this hard central nest core, often adjacent to the origin of forking branches on the host tree.

On Virgin Gorda, British Virgin Islands, *N. acajutlae* has been observed nesting under large rocks in areas where real estate development has reduced available trees and where aggressive control measures are directed at exposed nests.

A unique and distinctive feature of some *N. acajutlae* and *N. nigriceps* nests is the inclusion of spherical *nodules*, first described from nests of *N. nigriceps* in Jamaica by Hubbard (1877). The nodules differ in color and composition from the other nest carton and are embedded in the normal carton matrix within the nest. They apparently function as food reserves, and frequently house clusters of immature termites (Hubbard 1877, Andrews 1911). These nodules will be considered in further detail in a subsequent article.

In general, nests of *N. acajutlae* and *N. nigriceps* resemble each other closely and are readily distinguished from mature nests of other common arboreal *Nasutitermes* species inhabiting the same locality. Mature *N. acajutlae* and *N. nigriceps* nests are larger than any sympatric congenerics and also differ in external appearance. *N. rippertii* builds very large nests (>2 m in height) in the Bahamas (R. Scheffrahn, personal communication), but this species is not known to be sympatric with either *N. acajutlae* or *N. nigriceps*. *N. ephratae* builds regularly spherical or ellipsoidal nests with a smooth shell covering a hard outer gallery layer (Thorne 1980). *N. costalis* and *N. corniger* nest exteriors have a regular texture of small bumps (Thorne 1980), contrasted with the frequently irregular or more exaggerated bumps of an *N. acajutlae* or *N. nigriceps* nest shell.

Cuticular Hydrocarbon Phenotypes of *N. acajutlae* and *N. nigriceps*. We characterized the cuticular hydrocarbons of collections of *N. acajutlae* and *N. nigriceps* (Fig. 3) to see if chemotaxonomic characters, which have proved useful in resolving taxonomic problems of other termite groups, might be helpful in this case. Cuticular hydrocarbons effectively separate at least some other species of *Nasutitermes* from the Caribbean Basin (*N. costalis* and *N. ephratae* [Haverty et al. 1990b, 1992]), as well as subspecies (or sibling species) of the Pacific dampwood ter-

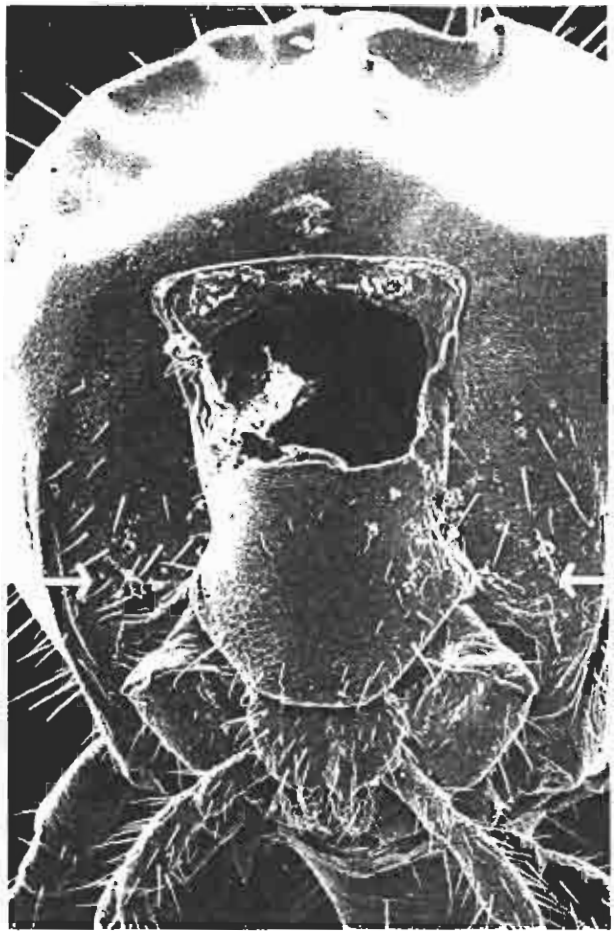

Fig. 2. Ventral view of soldier head capsule, *N. acajutlae* (100×; coll. by S. C. Briscoe, St. John, U.S. Virgin Islands). Note bristles on each side of the postmentum (arrows). These are absent in *N. nigriceps*.

mites, *Zootermopsis* (Haverty et al. 1988, Thorne & Haverty 1989, Thorne et al. 1993).

Our preliminary data suggest that species determinations on the basis of cuticular hydrocarbons would be equivocal within the *N. nigriceps* complex. The intraspecific variation in cuticular hydrocarbon mixtures within *N. acajutlae*, even among adjacent colonies on Guana Island, British Virgin Islands, is difficult to explain at this time (Fig. 3 A and B). We have examined specimens of *N. acajutlae* from many islands in the British Virgin Islands (Fig. 3 C and D), Puerto Rico, and Vieques, and *N. nigriceps* from Panama, Belize, and the Cayman Islands (Fig. 3 E and F). The predominant or abundant hydrocarbons from both species are long-chain alkenes (C39:1, C41:1, and C43:1) (Figs. 3 A–F). Other hydrocarbons, especially those with 23 to 29 carbons, occur sporadically in both species, as do occasional long-chained alkenes (C38:1, C40:1, C42:1, C44:1, C45:1, and C47:1), alkadienes (C39:2, C41:2, C43:2, and C45:2), alkatetraenes (C41:4 and C43:4), and alkapentaenes (C41:5). We found little qualitative variation. Most of the variation between colonies on Guana Island and between colonies from different islands lies in the relative proportions of the predominant peaks. We do not yet know how to interpret this variation.

The cuticular hydrocarbon profile of *N. costalis* (Holmgren), which occurs on many Caribbean islands with either *N. acajutlae* or *N. nigriceps*, is quite different from these latter two species (morphological differences between *N. costalis* and *N. acajutlae*/*N. nigriceps* are distinct as well). Comparison of the hydrocarbon profiles of *N. costalis* (Fig. 3 G and H) with those of *N. acajutlae* and *N. nigriceps* (Fig. 3 A–F) demonstrate a species specificity of these chemotaxonomic characters.

Cuticular lipids affect water conservation in termites (Collins 1969). Both *N. acajutlae* and *N. nigriceps* can live in dry, exposed habitats that are largely inhospitable to other arboreal nasutes. The similarity in the hydrocarbon profiles of *N. acajutlae* and *N. nigriceps* may, at least in part, represent a common adaptation to water retention. Clearly, additional research on the factors affecting cuticular hydrocarbon variation in *N. acajutlae* and *N. nigriceps* is warranted.

Geographic Distribution of *N. acajutlae* and *N. nigriceps*. When soldier morphology is used as the basis for species diagnosis, known records of *N. acajutlae* and *N. nigriceps* show distinct allo-

Table 1. Measurements of *N. acajutlae* and *N. nigriceps* soldiers

Measurement	*N. acajutlae* soldiers[a]		*N. nigriceps* soldiers[b]	
	Mean ± SD	Range	Mean ± SD	Range
Length of head capsule including nasus, mm	1.74 ± 0.05	1.66–1.89	1.65 ± 0.05	1.48–1.74
Length from tip of nasus to anterior margin of postclypeus, mm	0.59 ± 0.07	0.52–0.67	0.60 ± 0.04	0.50–0.67
Width of head capsule, mm	1.19 ± 0.07	1.02–1.42	1.07 ± 0.05	0.97–1.16
Length of foretibia, mm	1.06 ± 0.02	0.93–1.14	0.96 ± 0.04	0.87–1.08
Length of hindtibia, mm	1.47 ± 0.06	1.33–1.64	1.37 ± 0.05	1.27–1.48

[a] Specimens were from the following localities and 10 individuals were measured from each colony (number of colonies from each location given in parentheses): Guana Island, British Virgin Islands (6); Guanica, Puerto Rico (1); Isla Migueyes, Puerto Rico (1); La Parguera, Puerto Rico (1); St. Croix, U.S. Virgin Islands (3); St. John, U.S. Virgin Islands (3). In total, 150 soldier termites were measured.

[b] Specimens were from the following localities and 10 individuals were measured from each colony (number of colonies from each location given in parentheses): Acapulco, Mexico (1); Barro Colorado Island, Panama (2); Canaan Land, Grand Cayman (1); Colima, Mexico (1); Galeta, Panama (3); Jalisco, Mexico (2); Leon, Nicaragua (2); Priory, Jamaica (2); Woodstock, Jamaica (1). In total, 150 soldier termites were measured.

Table 2. Measurements of *N. acajutlae* and *N. nigriceps* female and male alates

Measurement	Female			Male		
	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*
			N. acajutlae alates[a]			
Head width, including eyes, mm	1.63 ± 0.06	1.50–1.71	25	1.68 ± 0.03	1.63–1.72	25
Diameter compound eye, mm	0.63 ± 0.02	0.60–0.65	25	0.63 ± 0.04	0.60–0.68	25
Maximum ocellus dimension, mm	0.21 ± 0.02	0.20–0.24	25	0.21 ± 0.02	0.18–0.22	25
Length of foretibia, mm	1.10 ± 0.04	1.10–1.21	25	1.15 ± 0.16	0.83–1.32	25
Length of hindtibia, mm	1.75 ± 0.05	1.70–1.86	25	1.72 ± 0.18	1.30–1.90	25
			N. nigriceps alates[b]			
Head width, including eyes, mm	1.68 ± 0.08	1.56–1.82	9	1.72 ± 0.10	1.56–1.80	7
Diameter compound eye, mm	0.64 ± 0.04	0.57–0.72	9	0.65 ± 0.04	0.60–0.73	7
Maximum ocellus dimension, mm	0.17 ± 0.02	0.15–0.20	9	0.17 ± 0.02	0.15–0.20	7
Length of foretibia, mm	1.22 ± 0.06	1.14–1.30	9	1.23 ± 0.06	1.14–1.30	6
Length of hindtibia, mm	1.78 ± 0.13	1.62–1.96	9	1.94 ± 0.05	1.74–2.00	7

[a] Specimens from St. Thomas, U.S. Virgin Islands (COTYPE); Guana Island, British Virgin Islands.
[b] Specimens from Barro Colorado Island, Panama; Jalisco and Acapulco, Mexico; and Woodstock, Jamaica.

patric distribntions. *N. acajutlae* occurs on Pnerto Rico, Vieques Island (near Puerto Rico), all major U.S. Virgin Islands (St. Croix, St. John, and St. Thomas), and on all the major British Virgin Islands (Tortola, Virgin Gorda, Gnana, and Anegada). Of the smaller islands in the British Virgin Islands, *N. acajutlae* has been found on George Dog, Scrub, Thatch and such others as have enough soil to snpport a scrub forest (see List 1). Collections from Trinidad (including soldiers labeled as *N. creolina* Banks, paratype, MCZ Type 10078 collected by W. Beebe in Trinidad [see *Taxonomic History*] and recent collections from Valencia Long Stretch, Trinidad by J.P.E.C. Darlington) also sort as *N. acajutlae*. Collections from the Lesser Antilles islands between the British Virgin Islands and Trinidad are scarce. Surveys of the islands of Guadeloupe, Montserrat, Grenada, and Tobago (J.P.E.C. Darlington 1992, personal communication) revealed no specimens of *N. acajutlae* or *N. nigriceps*. The termite fauna of the Lesser Antilles should be examined further for presence of *N. acajutlae*. Furthermore, material from northern South America should be examined to determine the full southern range of *N. acajutlae*.

N. nigriceps ranges from Panama (and possibly into South America) north through Central America into Mexico. It also occurs on the Cayman Islands and Jamaica. The termite fauna of both Cuba and Hispaniola has been aggressively collected (most recently by J. Křeček in Cuba and R. H. Scheffrahn in the Dominican Republic). Neither *N. acajutlae* nor *N. nigriceps* has been recorded from either of these islands. Specimens from Providenciales Island, Turks and Caicos Islands, British West Indies, seem close, but not identical, to either *N. acajutlae* or *N. nigriceps* (Scheffrahn et al. 1990; R. H. Scheffrahn, personal communication; B.L.T., personal observation). Further sampling and evaluation of material from the British West Indies will be required to substantiate a diagnosis.

The distributions of *N. acajutlae* and *N. nigriceps* thus fit logically with current theories regarding the biogeography of the Caribbean Islands. The geology of the Caribbean land masses is not firmly established, but there is a strong concensus that the Greater Antilles and Lesser Antilles island groups had independent origins (Donnelly 1988). Their present juxtaposition in an apparently regular arc is one of coincidence associated with continental plate movements; it does not reflect historical derivation from a single land mass. The Anegada Passage separating the Greater Antilles, Puerto Rico, and the Virgin Islands from the Lesser Antilles is extremely deep, with a sill depth approaching 2,000 m and basins with ocean depths exceeding 4,000 m (Donnelly 1988).

The relatively young Lesser Antilles developed as a volcanic chain along the Caribbean plate. There is little probability that land connections existed between these volcanos from the Miocene period onward (Donnelly 1988). The origin of the Greater Antilles was earlier and is subject to greater debate. These islands probably arose as volcanoes as well, although the island of Cuba remains a geologic enigma because of the complexity of its structural fragments, which may reflect a continental origin (Donnelly 1988).

For the purposes of this article, the precise geological origin of the Greater Antilles is of less interest than the recent dynamics of sea level and, thus, land bridges associated with the islands. A clear and concise review of this phenomenon, and its relevance to Caribbean biogeography, is presented in Lazell (1989) and summarized below.

During the Sangamon interglacial period (100,000 yr ago, preceding the last great ice age), all of southern Florida, nearly all of the Bahamas, and much of the current land area of the Caribbean islands was underwater (Fig. 4). Sea level was ≈21 m above present level. The Würm glaciation period began ≈80,000 yr ago, ice accu-

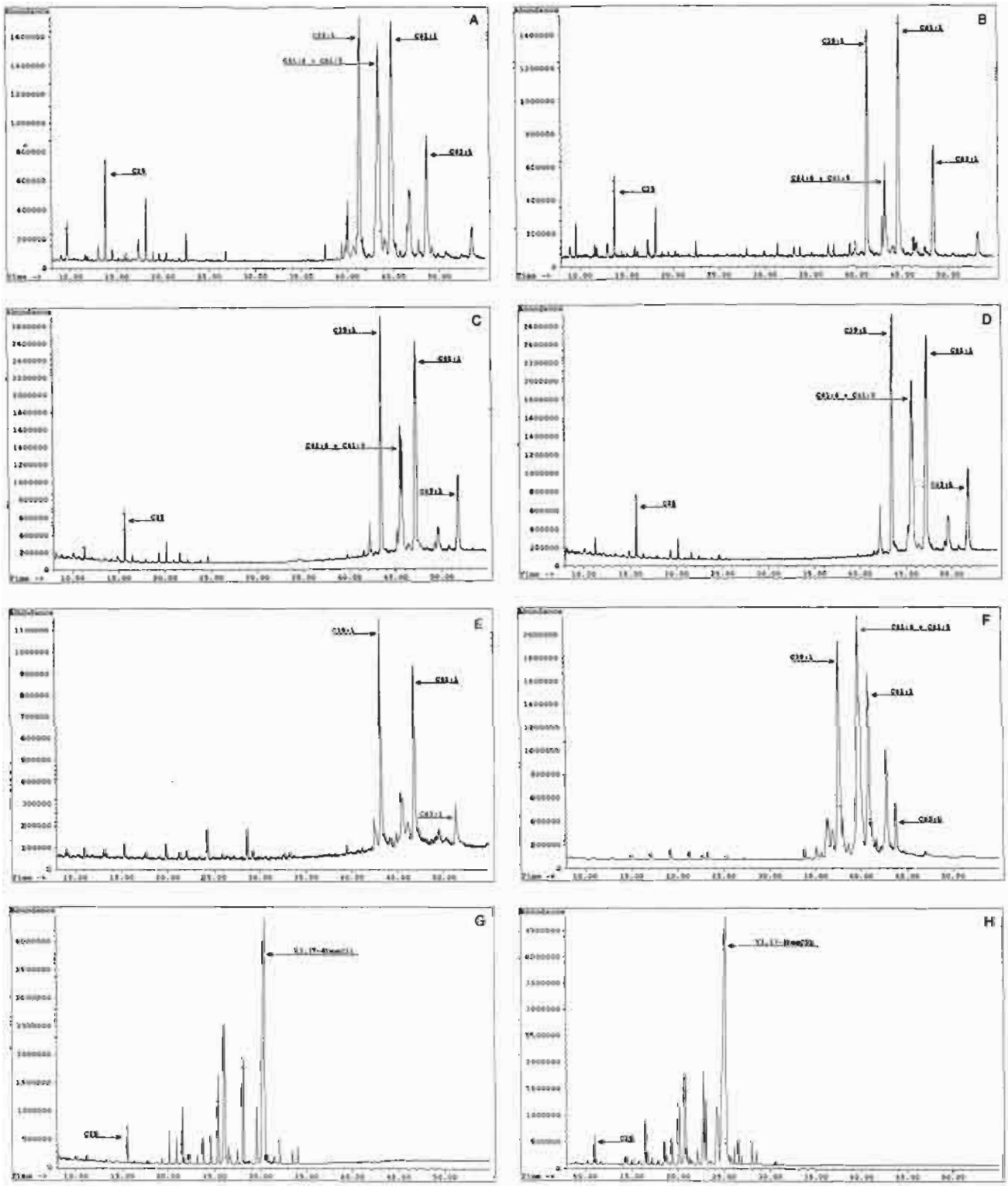

Fig. 3. Gas chromatograms of the surface hydrocarbons of three species of *Nasutitermes*. (A) *N. acajutlae* (colony 3) from Guana Island, British Virgin Islands; (B) *N. acajutlae* (colony 17) from Guana Island, British Virgin Islands; (C) *N. acajutlae* from Necker Island, British Virgin Islands; (D) *N. acajutlae* from Virgin Gorda, British Virgin Islands; (E) *N. nigriceps* from Grand Cayman, Cayman Islands; (F) *N. nigriceps* from Galeta, Panama; (G) *N. costalis* from Tortola, British Virgin Islands; (H) *N. costalis* from Trinidad.

mulating around the poles and slowly lowering sea levels until the glacial maximum 17,000 yr ago. At that time, land exposure was greatest and sea level fell to ≈120 m below modern level. During the interval of the Würm Glacial Maximum, the Nicaraguan area of Central America extended broadly to the east, forming a stepping stone land bridge to Jamaica.

In light of geological dynamics and recent glacial history, the most logical explanation to account for the current distribution of the closely related species *N. acajutlae* and *N. nigriceps* as-

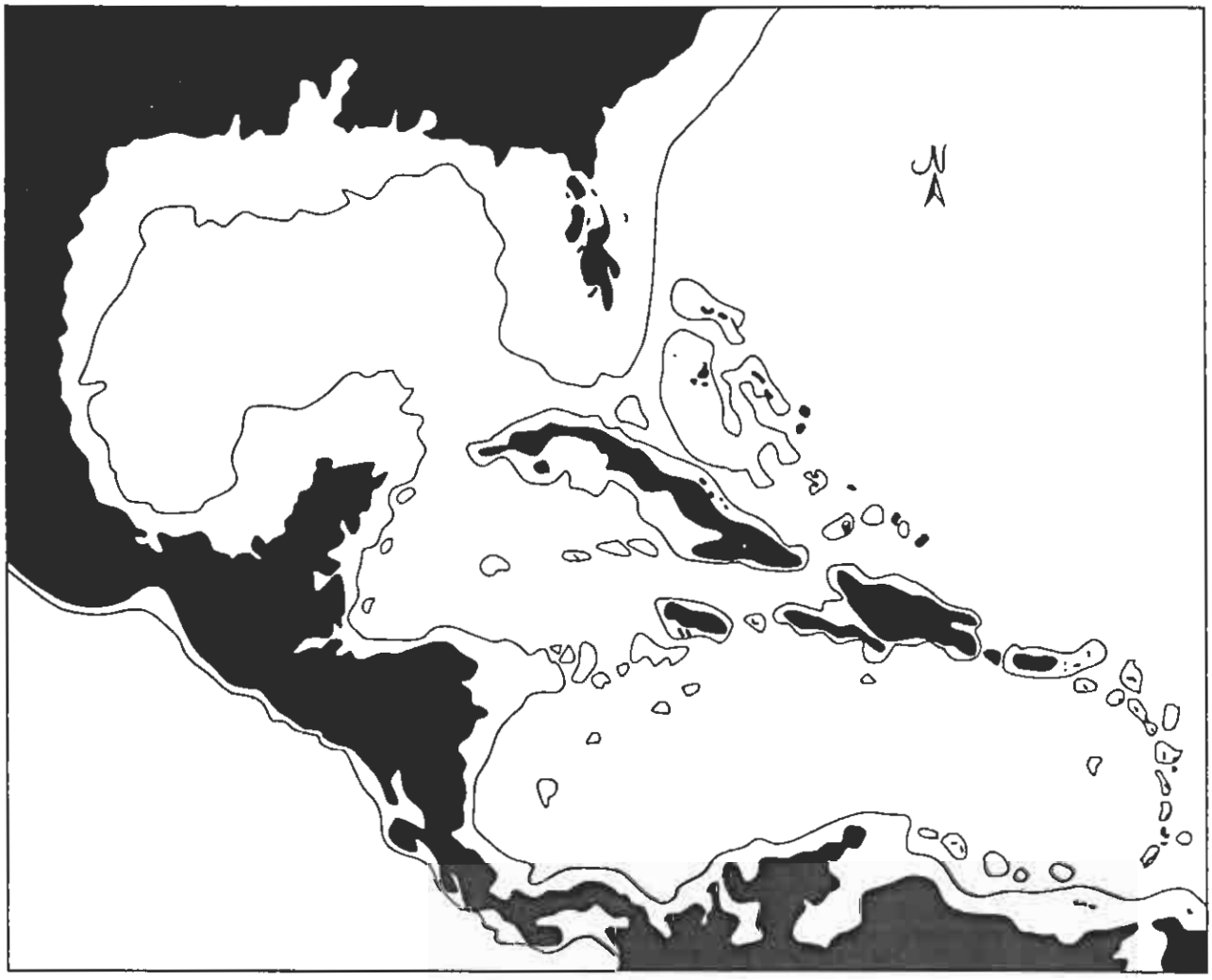

Fig. 4. Approximate land areas and distance relationships within the Caribbean Basin over the past 100,000 yr. Sea level during the Würm (or Wisconsinan) Glacial Maximum (17,000 yr ago; solid line) compared with land masses during the Sangamon Interglacial period (100,000 yr ago; stippled). Redrawn from Lazell (1989).

sumes an ancestral population of one or the other species inhabiting, most probably, a lowland region of northern South America, and perhaps ranging into either Central America or the Caribbean. Speciation occurred in a manner that is impossible to detail until more is known about discrete species boundaries or clinal variation of the fauna in northern South America. The modern range of *N. acajutlae* resulted from migration along the Caribbean arc from Trinidad to Puerto Rico. Once established in Central America, *N. nigriceps* probably dispersed over the Nicaraguan land bridge to the Cayman Islands and Jamaica during the Würm Glacial Maximum. Further sampling of termites from the British West Indies and from Colombia, Venezuela, and Guyana will help in understanding the historical biogeography and radiation of the group.

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