GUANA 2005

The Conservation Agency

Exploration, Education, and Research

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4 May 2006

Dr. Henry Jarecki
10 Timber Trail
Rye, NY 10580

Dear Henry,

It has been a busy year. Of course, my book, *Island...* is out and selling well, but I have not seen any reviews yet. I do know some heavyweights have prepared and submitted reviews for publication.

Science month 2005 was shifted forward to begin the last week of September. We are not too pleased. We like all October because, usually, rainfall picks up and the water table increases. This benefits all life forms on an arid island, especially insects. Collecting gets better and better. Also, North American bird migration increases later in October, so our bird catch also typically goes up.

The first reason, insect increase, includes "no-see-ums" and mosquitoes which we cannot help but believe will make things less pleasant for paying guests. We hope this change will be reconsidered.

Here is a glimpse of what is inside:

Cover Story - This is a color rendition of Bob Powell's article from the remodeled and now nifty journal *Iguana* 13(1): 22-23. John Binns, who has come to study our iguanas, produced this for us...p.1

Bats - Michelle and her sister Dr. Jen Theberge came, caught, and radio-tracked. They located the roost site of our fishing bats: the only known colony in the BVI, and one of only a few known anywhere...p.2

Birds - Clint Boal and his wife Tracy did manage to get some nifty neotropical migrants and began what may be the first ever demographic study of the elusive mangrove cuckoo. I also include page proofs of our peer-reviewed article on Guana's more remarkable birds, which will appear in the May issue of the *Wilson Journal*.........p.6

out no more. Here is a second mystery, although the genus *Mithrax* is marine, and so I would not probably have included it in the book. But that is not all: Note the dates on the question and answer; how'd I do that?..p.84

Insects - I begin with Dr. Daniel Perez's updated list of our roaches. Remember, number 6, *S. pararuficollis*, was described from Guana and seems not to be known from elsewhere. Our species are mostly innocuous woods-dwellers.............................p.85

Fruit flies are of great interest from an evolutionary point of view, but I am having trouble finding anyone who wants to study them on Guana. Herewith the only-recently discovered (by us!) paper that highlights Guana's fruit flies, followed by Dr. Jenny Gleason's answer to my letter to her. I'm still looking...p.86

"General Insects" includes the Valentines' reports on entomological progress and, finally, notes to and from Dr. Dan Hagan, a dipterologist (fly man). I need to get him in direct contact with Barry Vallentine to get his questions answered.........................p.104
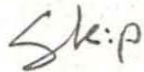
Archeology - Elizabeth (Holly) Righter's work continues and I provide an early draft report. Elizabeth Wing, retired from the University of Florida, is the prominent authority on Antillean animal remains in middens. She and Holly are conspiring to get our bones identified. I believe Dr. Samuel Turvey, Institute of Zoology, London, is the perfect person to identify our rodent bones. Dr. David Steadman, ornithologist, believes our rodent bones are not Hutia, but Agouti. Both are woodchuck-size, very delicious beasts (as are woodchucks). While Hutias are well-known from Puerto Rico Bank islands (and Taino middens), Agoutis are not. Turvey has identified several other large, edible rodent genera on Puerto Rico, any of which might have occurred on Guana. Correspondence about Holly's 2006 plans finishes up..p.110

A Nifty Cucurbit - Dr. Angela Davis, USDA geneticist and molecular biologist, provides a colorful portrait of a cucumber family plant, very little-known in the world, from Guana...p.119

The Still Virgin Island - I finish off with Larry Millman's article that appeared in *Islands Magazine*. Larry is an old friend. I think this is a very nice bit...............p.121

And so onwards to science month, 2006.

All the best

Skip

James Lazell, Ph.D.

3

Lizard Warfare

Robert Powell
Avila University, Kansas City, MO

M ale anoles (genus *Anolis*) are fiercely territorial. Although ritual behaviors involving posture, orientation, and head-bobs with dewlap extensions often dissuade an interloper from seriously challenging a resident male, battles sometimes ensue — and they can become violent, often leaving wounds that frequently become infected. Large abscesses on the jaws of males are a common sight.

In this instance, a male Crested Anole (*Anolis cristatellus*, the darker, slightly smaller individual with the notch in his tail crest) had established a domain dominated by a small palm tree on the grounds of the resort on Guana Island (British Virgin Islands) — only to find himself confronted one morning in October 2005 by a serious rival.

Each quickly assumed a threatening posture involving lateral presentation, erection of the nuchal (nape) crest, an inflated body, elevated tail, and gaping jaws (cover) in an effort to convince his counterpart that further efforts were futile. Each possessed an advantage, the resident was defending an established territory with which he was intimately familiar, but the interloper was slightly larger. Circling each other in an attempt to establish and hold the high ground (top left), all caution was thrown to the wind and jaws locked in an attempt to throw the other to the ground (top center and right). The interloper succeeded on three occasions, with the resident quickly reengaging in what had now become open warfare.

Eventually, larger size prevailed, and the former resident was banished to the outer reaches of the fronds from which he soon disappeared from view. Within minutes, the proud victor claimed the spoils of battle (right) and life went on in the sometimes violent world of lizards.

Guana Island Bat Research

October 13 – 24, 2005

*Michelle Theberge MSc,
Jen Theberge PhD*

Photo: J. Theberge

In the second year of a bat study on Guana Island, the objectives were: to continue building upon 2004's survey of bats and their ectoparasites; and to initiate research on fishing bat (*Noctilio leporinus*) behaviour.

Netting

Netting for bats occurred over 6 nights. Each night 2 to 6 nets were set giving a total of 25 netting nights. All nets were set on the resort section of Guana Island. In Table 1, the net locations were as follows: Patio (PL) refers to the patio above the water cistern; Resort refers to the turn around area (PB1 is in the saddle south of Barbados house, PG1 is the garden overlooking the flats); and Eden (E1, E2) refers to nets set in the Garden of Eden. Due to intense flooding on the low-lying areas, nets were not set in the Orchard or the Flats.

Summary of Bats Caught

Eight bats were caught on Guana Island between October 14 and 20, 2005. Three species were recorded: *Artibeus jamaicensis* (ARJA) (2M), *Noctilio leporinus* (NOLE) (4M), and *Molossus molossus* (MOMO) (2F) (Table 1). No bat samples were taken. All animals caught were marked with hair clippings, and none were re-caught.

Table 1: Measurements from live bats caught

Date	Location	Net #	Bat #	Species	Sex	FA	TL	HF	WT
Oct 14	Patio	PL	1	MOMO	F	39	85	9	10.5
Oct 14	Pool	Pool	2	ARJA	M	61.5	76.5	8.5	54
Oct 14	Resort	PB1	3	NOLE	M	89.5	106	30	75
Oct 16	Resort	PG1	4	ARJA	M	63	77.1	10	45
Oct 17	Resort	PB1	5	MOMO	F	38.7	92.8	7.5	11.8
Oct 17	Eden	E2	6	NOLE	M	88.3	105	29	69.5
Oct 17	Resort	PB4	7	NOLE	M	87.4	94	31	56.5
Oct 17	Resort	PG1	8	NOLE	M	88.3	102.8	30	69

**note: all measurements are from live animals

Parasites

Ectoparasites were found on 6 of the bats. Mite infestations, varying between 8 and 30 individuals, were on the lower abdomen and interfemoral membranes of all the *N. leporinus*. One batfly (Diptera: Streblidae) of the species *Trichobius intermedius* (Figure 1) (Peterson and Hurka, 1974) was found on *A. jamaicensis* (ARJA 4). Six specimens of an unknown species of parasite, likely a diptera, were found on *M. molossus* (MOMO1). This species is currently being identified (Figure 2).

Figure 1: Streblid batfly *Trichobius intermedius.*

Figure 2: Unidentified dipteran bat ectoparasite

Photos: L. Jarecki

Radio-tracking the Fishing Bat *Noctilio leporinus*

On October 16, radio transmitters were attached to 2 male *N. leporinus* (NOLE2, NOLE4) for the purpose of tracking them to their sea cave roost. The bats chosen for tagging were the largest ones caught weighing 67.5 g and 69 g each. The transmitters, weighing 3 g each, were attached to the backs of the bats with silicone glue (Figure 3).

After attachment, the bats were released facing west at the saddle where the Pyramid reaches the resort (beside Barbados house). The bats flew directly southwest around the Pyramid.

Figure 3: Radio tag attached to *N. leporinus*   *Photo: M. Theberge*

On October 17, we navigated around the Pyramid by boat with the radio receiver pointing the antenna towards the shore. Both bats' radio frequencies were located transmitting from a sea cave on the west side of the Pyramid (Figure 4) approximately 200 m north of the Guana Head. At dusk, NOLO4's signal left the sea cave with dozens of other fishing bats confirming the location of their roost. NOLO2's signal remained in the cave, and we presume that the radio tag had fallen off. One hour later, NOLO4's signal was detected east of the resort.

Figure 4: Observing the *N. leporinus* sea cave roost.
Photo: J. Theberge

Observations at Grenada House pool

The majority of the bats frequenting the Grenada House pool were likely the fishing bat, *N. leporinus,* due to their unmistakable musky odour (secreted by skin glands) and large size. A modified mist net was used to attempt to catch bats at the pool. However, catching them there is very difficult since the bats can easily detect the net. One *A. jamaicensis* was caught at the pool, and when flying it was significantly smaller than the more common fishing bats. While some bats swooped down to the pool to drink, others took large insects off the water surface.

Observations at the Bat Caves

The Bat Caves were visited on October 16. Twenty-six *A. jamaicensis* were observed in the lower cave (Figure 5), and none in the others.

Figure 5: *A. jamaicensis* at the Bat Caves   *Photo: M. Theberge*

Revisions to 2004 report

The ectoparasites from 2004 have been positively identified as 2 streblid species. The species reported to look like a nyterbiid was identified as Streblidae *Megistopoda aranea* (Wenzel and Tipton, 1966). This fly has very elongated legs and reduced wings. The second streblid, *Trichobius intermedius*, looks more like a fly with pronounced wings and short legs (Peterson and Hurka, 1974). Identification was done by M. Theberge and confirmed by Dr. C. Dick at the Chicago Field Museum of Natural History.

Acknowledgements

References

Peterson, B.V., and K. Hurka. 1974. Ten new species of bat flies of the genus Trichobius (Diptera: Streblidae). The Canadian Entomologist, 1049-1066.
Wenzel R.L., and V.J. Tipton. 1966. Ectoparasites of Panama. Field Museum of Natural History, Chicago Illinois.

Setting nets at dusk

Photo: L. Jarecki

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U.S. GEOLOGICAL SURVEY
BIOLOGICAL RESOURCES DIVISION

25 April 2006

Dr. James Lazell
6 Swinburne St.
Jamestown, RI 02835

Dear Skip:

Enclosed are two copies of my 2005 report on Ornithological Monitoring and Research on Guana Island, British Virgin Islands. The report is brief, but provides the relevant details. I have also included a copy of the Galley Proofs for our article in the Wilson Journal of Ornithology, which will be published in May or June.

2005 was an incredible year for avian work on Guana Island. As you know, we had a major fallout of blackpoll warblers. So many warblers came in that we had to shut down half of our nets completely, and the other half periodically just to keep up with the number we were catching. We captured 176 blackpolls, most of them in one day. This almost doubles the number of blackpolls captured on Guana since 1994. We could undoubtedly have kept capturing birds if we had not needed to close down our netting operation due to our departure date.

Of even more interest is the number of Swainson's thrushes we captured this year (26) and a gray-cheeked thrush. Swainsons' thrushes were first discovered for the Virgin Islands (indeed first for east of Cuba) on Guana Island in 2000, then again in 2003. Thus, the number we captured in 2005 suggests those were not anomalous events. I am currently drafting a manuscript discussing this, which I will be submitting to an ornithological journal.

Finally, my colleague Tracy Estabrook has done an incredible job surveying for, and locating, mangrove cuckoos on Guana Island. We anticipate her study to continue in 2006 to verify numbers and consistency of territorial occupancy, and to focus more on specific habitat selection. We are considering using telemetry on a few birds.

Thank you for all of your support with our research on Guana Island; I am looking forward to the 2006 seasons.

Sincerely,

Clint Boal
Assistant Leader – TXCFWRU
Associate Professor – TTU

ORNITHOLOGICAL MONITORING AND RESEARCH ON GUANA ISLAND, BRITISH VIRGIN ISLANDS

PROJECT REPORT 2005

Clint W. Boal, Ph.D.
USGS Texas Cooperative Fish and Wildlife Research Unit
Texas Tech University, Lubbock, TX 79409-2120

21 April 2006

Scarlet Tanager (female)

ORNITHOLOGICAL RESEARCH AND MONITORING ON GUANA ISLAND, BRITISH VIRGIN ISLANDS: PROJECT REPORT 2005

Dr. Clint W. Boal, *USGS Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409-2120*

INTRODUCTION

Ornithological research in the West Indies has focused on an array of topics including basic status surveys, ecology of individual species, effects of hurricanes on island bird populations, patterns of migration, and community dynamics (Wiley 2000). However, in terms of ornithological reports, the Virgin Islands, especially the British Virgin Islands (BVI), have received much less attention compared to the rest of the West Indies (Wiley 2000). Ornithological work on Guana continues to contribute toward developing a better understanding of the Virgin Islands as stop-over habitat for migrant birds, and basic ecology of Caribbean birds.

During October 2005 I continued directing research on avian ecology on Guana Island. The primary components of our research activities were 1) direction of mist-netting and banding of neotropical migrant birds, 2) continuing a population demography study of bananaquits, 3) general monitoring of resident bird species, and 4) supervise a newly initiated study of distribution and density of Mangrove Cuckoos.

RESULTS

Mist-Netting and Bird Monitoring

We operated mist-nets from 3 – 18 October 2005. Heavy rains limited mist-netting activities at times. Additionally, the heavy rain and subsequent flooding of the flat prevented mist-netting for shorebirds at the Salt Pond. Mist-netting efforts totaled 403 net hours during which we captured a total of 428 birds at a rate of 1.1 birds per net hour. This includes hummingbirds ($n = 13$) which were released at the point of capture and not banded. It also includes 85 recaptures of birds banded early in 2005 ($n = 18$) or originally banded in a previous year ($n = 67$) (Table 2). We captured and banded 330 individuals of 21 species in 2005 (Table 2).

Migrants

Guana Island experienced a huge neotropical migrant bird fallout in 2005. Hundreds of Blackpoll Warblers arrived in late October, and I banded 176, the majority on 18 October. Undoubtedly, we could have banded 200-300 more birds, but had to close nets down on the 19th in order to leave on 20 October. However, the number of Blackpoll Warblers banded in 2005 almost doubles the total number of Blackpoll Warblers banded on Guana to date.

Of greater interest is the number of Swainson's Thrushes captured and banded in 2005. Swainson's Thrushes were first recorded in the Virgin Islands when we capture a single individual on Guana Island in 2000. We subsequently captured another and saw a second bird in 2003. These records are so extraordinary that they are a key record in our

manuscript (Boal et al. 2006). In 2005, however, we captured and banded 26 Swainson's Thrushes and a single Gray-cheeked Thrush. Numerous other Swainson's Thrushes were also observed on Guana. This suggests that our earlier records were not anomalous events, and that the commonly held belief that Swainson's Thrushes do not migrate through the eastern Caribbean is either erroneous, or was correct but migration patterns are changing. We are preparing a manuscript to document the presence of Swainson's Thrushes in the east Caribbean.

Other uncommon migrants captured included 3 Connecticut Warblers, 2 Nashville Warblers, 3 Red-eyed Vireos, 2 Black-and-white Warblers, 1 Bay-breasted Warbler, and 1 Golden-winged Warbler, and a Scarlet Tanager (Table 2). To my knowledge, the Gray-cheeked Thrush, Connecticut Warbler, Bay-breasted Warbler, and Scarlet Tanager are the first records for the species on Guana Island. If this is correct, 4 new species were recorded on the island during our 2005 study period. All four are exceedingly rare in U.S. Virgin Islands, and may be new species records for the British Virgin Islands. We are going through historical records to confirm this. We also captured a Black-whiskered Vireo which may have been a migrant or resident, but has rarely been documented on Guana Island.

We visually documented White-winged Doves on Guana Island in October, which Dr. Lazell had observed earlier in the year. We also visually documented the welcome returns of 3 species that had been gone from Guana Island for several years. At least one pair of Northern Mockingbirds was active near the burro corral, as were several Smooth-billed Anis, and an adult Yellow Warbler was spotted in the mangroves near the salt pond.

Residents Bird Study - Bananaquit
The most commonly captured resident species was the Bananaquit, both in terms of new captures ($n = 63$) and recaptures of birds banded in previous years. As part of a population demography and dispersal study, we individually color banded 69 Bananaquits. This involves placing a silver metal band on one leg and a combination of two plastic colored bands on the other leg. This allows the identification of individuals without having to recapture them. This is important in that it lets us visually identify mated pairs of birds and breeding territories, pair and site fidelity over time, and dispersal across the island. We are currently examining patterns of annual survival based on mark-recapture analysis, and annual variation in age and sex composition of the Bananaquit population on Guana Island. These parameters will be included in a model examining the influences of weather patterns on the Bananaquit population. The Bananaquit can be viewed as a representative of other island resident bird species, and information we collect may be extrapolated to those other species. These are important data in context of assessing the probably impacts of global climate change.

Residents Bird Study – Mangrove Cuckoos
The lead field investigator for this project is Tracy S. Estabrook (M.S.). The Mangrove Cuckoo is one of the least-studied North American birds and baseline population estimates and habitat requirements have been identified as among the most important

research needs for the species. In 2005 we initiated a standardized call-playback survey along trails on Guana to try to establish baseline information on minimum number of individual cuckoos, pairs, and/or family groups present. A call-playback survey consists of broadcasting the call of the species over a loudspeaker. If an individual of the species is present, it may interpret the broadcast as the vocalization of a territorial intruder, and respond by approaching the surveyor while calling in response.

We established survey points ~100 m apart along established trails on Guana Island. At each survey point we played a pre-recorded Mangrove Cuckoo vocalization 3 times, spaced 1 minute apart, and recorded number, estimated distance, and estimated compass direction of visual and audio responses from Mangrove Cuckoos in the area. From 6 October to 17 October 2005 we surveyed 104 points. All surveys were conducted from 0800-1200 hours under appropriate weather conditions. Due to potential biases inherent in the call-playback technique, it is prudent to be conservative when estimating the actual number of birds present from response to vocalizations. Therefore, our estimates of cuckoo numbers should be considered as biased low.

We confirmed presence of ≥1 Mangrove Cuckoo at 28 of 104 (27%) survey points. Birds responded at 13 (13%) additional sites where we could not determine them to be 'new' individuals or previously detected individuals that had followed the surveyor. We observed 2 birds in close proximity at 13 of the 28 (46%) active sites, suggesting parents and juveniles or (more likely) mated pairs remain together on at least some territories through October.

In subsequent field seasons we hope to refine this technique to establish a more robust survey protocol and distribution data set, and repeat surveys to record potential changes in Mangrove Cuckoo numbers and distribution on Guana Island over time. We anticipate expanding these surveys to other islands in the BVI. The relevancy of this study, in addition to acquiring basic biological information for the species, is the utility of it as a tool for conservation of Mangrove Cuckoos. The species is suspected of being substantially impacted by habitat loss and degradation due to conversion of low lying vegetation areas on islands to urbanization, resorts, and agricultural production. Refinement of our survey protocol may enable detection, monitoring, and enhanced conservation of Mangrove Cuckoos not just in the Caribbean, but in coastal areas of North America.

Project Productivity
As reported in the 2004 report, I have co-authored a manuscript with Fred Sibley, Tracy Estabrook and Dr. James Lazell, Jr., documenting banding activities, examination of longevity of individual resident species, and reporting new island and regional records of species. This manuscript has now been accepted for publication by the Wilson Journal of Ornithology and will be printed in May 2006. The citation is:

Boal, C.W., F. Sibley, T.S. Estabrook, and J.D. Lazell. 2006. Insular migrant species, longevity records, and new species records on Guana Island, British Virgin Islands. *Wilson Journal of Ornithology* 118:218-224.

I am currently conducting analysis on different aspects of age and sex variation among Bananaquits. I anticipate producing a manuscript for publication prior to the 2006 field season on Guana Island.

Future Work

In addition to annual mist-netting, I will be initiating new directions of research on Guana Island in 2005.

1. Annual surveys to develop an understanding of bird species distribution patterns and relative abundances across Guana, and how these parameters change in relation to meteorological events (e.g., dry years, hurricane events, etc.).
2. Continuation of the new color-banding program with Bananaquits to examine population demography of the species.
3. Continuation of the Mangrove Cuckoo study.

ACKNOWLEDGEMENTS

Table 1. Captures and recaptures of birds on Guana Island, September-October 2005. All new captures banded except for hummingbirds

Species	New Captures in 2005	Recaptures of 2005 birds	Recaptures of pre-2005 birds	Total Birds
American Kestrel	2	0	0	2
Yellow-billed Cuckoo	1	0	0	1
Mangrove Cuckoo	1	1	0	2
Zenaida Dove	5	0	2	7
Common Ground-dove	1	0	0	1
Green-throated Carib*	11	0	0	11
Antillean Crested Hummingbird*	2	0	0	2
Gray Kingbird	1	0	1	2
Caribbean Elaenia	3	0	2	5
Pearly-eyed Thrasher	21	2	6	29
Swainson's Thrush	26	4	0	30
Gray-cheeked Thrush	1	0	0	1
Black-whiskered Vireo	1	1	0	2
Red-eyed Vireo	3	0	0	3
Bananaquit	63	8	46	117
Blackpoll Warbler	176	0	0	176
Black-and-white Warbler	2	0	0	2
Bay-breasted Warbler	1	0	0	1
Connecticut Warbler	3	0	0	3
Golden-winged Warbler	1	0	0	1
Nashville Warbler	2	0	0	2
Scarlet Tanager	1	0	0	1
Black-faced Grassquit	15	2	10	27
Totals	343	18	67	428

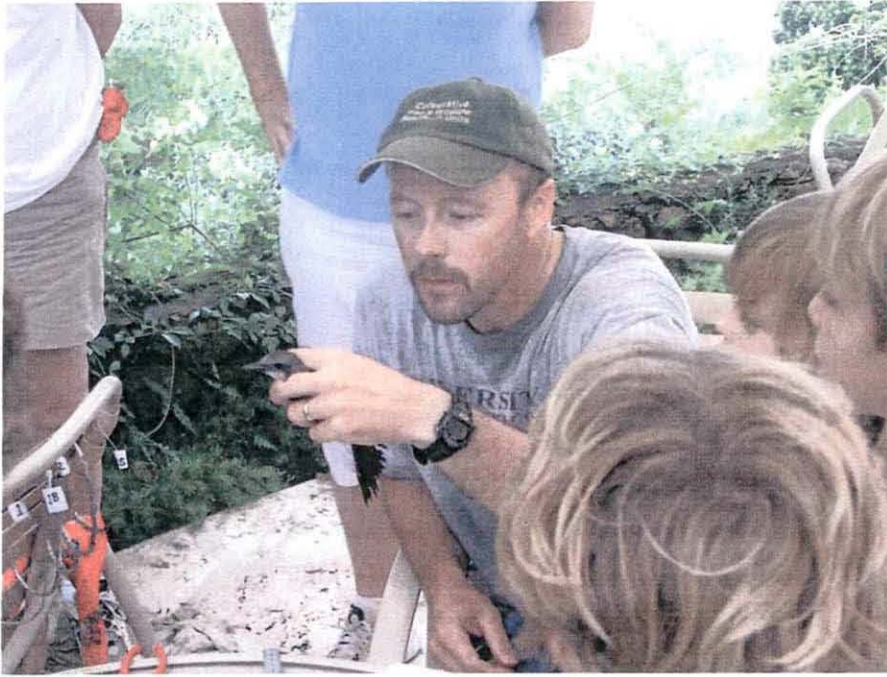Appendix I. Images of some activities and species captured and banded on Guana Island, British Virgin Islands, October 2004.

Golden-winged Warbler
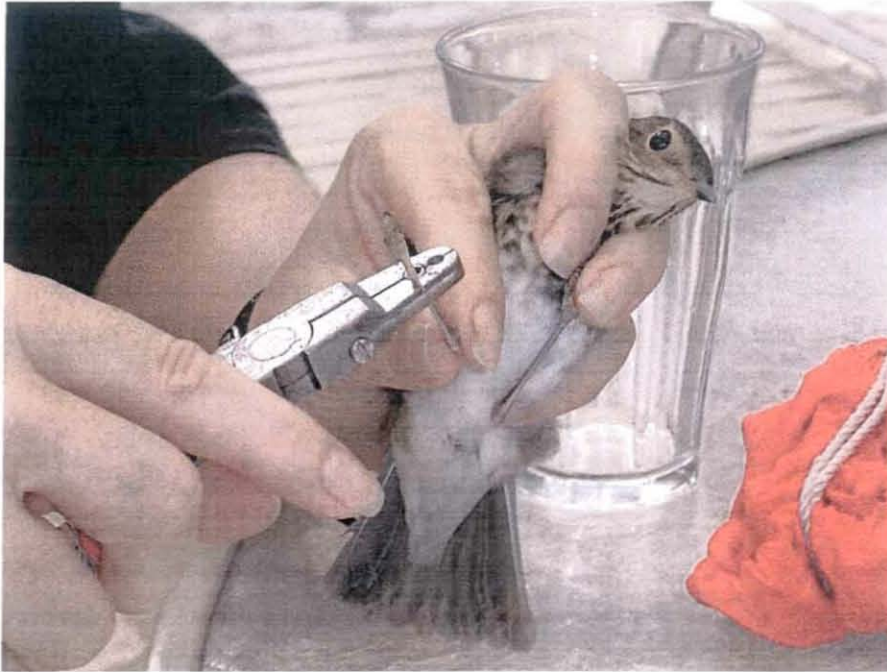

Gray-cheeked Thrush

Clint Boal shows a Gray Kingbird

Tracy Estabrook releasing a Swainson's Thrush

Banding a Swainson's Thrush

Clint Boal removes a Kestrel from a trap

Tracy Estabrook banding a Yellow-billed Cuckoo

Blackpoll Warbler

17

INSULAR AND MIGRANT SPECIES, LONGEVITY RECORDS, AND NEW SPECIES RECORDS ON GUANA ISLAND, BRITISH VIRGIN ISLANDS

CLINT W. BOAL,[1,4] FRED C. SIBLEY,[2] TRACY S. ESTABROOK,[3] AND JAMES LAZELL[2]

ABSTRACT.—We conducted mist netting each October from 1994 to 2004 on Guana Island, British Virgin Islands, and recorded bird sightings to develop a more complete inventory of the island's resident and migrant species. During our study, we recorded four new species for the British Virgin Islands: Magnolia Warbler (*Dendroica magnolia*; 1996), Golden-winged Warbler (*Vermivora chrysoptera*; 1997), Swainson's Thrush (*Catharus ustulatus*; 2000), and Red-necked Phalarope (*Phalaropus lobatus*; 2004). Blackpoll Warbler (*Dendroica striata*) was the most frequently captured Neotropical migrant landbird, despite only being first detected in the region in 1989. Captures and detections of other Neotropical migrant landbirds suggest that many species may be more common in the region than previously believed, or, as speculated by other researchers, that migrant routes may be shifting eastward due to habitat degradation on western Caribbean islands. We also used recapture data to establish longevity records of resident species, including Caribbean Elaenia (*Elaenia martinica*; ≥7 years), Bananaquit (*Coereba flaveola*; 7 years), Black-faced Grassquit (*Tiaris bicolor*; ≥9 years), and Zenaida Dove (*Zenaida aurita*; 5 years). Longevities of other resident species were similar to, or slightly less than, those reported elsewhere. *Received 22 February 2005, accepted 30 November 2005.*

Ornithological research conducted in the West Indies has covered an array of topics, including avian species occurrence and distribution, ecology of individual species, effects of hurricanes on island bird populations, migration patterns, and community dynamics (Wiley 2000). In the Virgin Islands region, researchers have addressed avifaunal occurrence and distribution (LaBastille and Richmond 1973, Mirecki et al. 1977, Norton et al. 1989), and species ecologies (Askins and Ewert 1991, Chipley 1991, Mayer and Chipley 1992, McNair et al. 2002); however, considerably less ornithological study has been conducted in the Virgin Islands—especially the British Virgin Islands (BVI)—than in other areas of the West Indies. In a bibliography consisting of 11,648 entries for ornithological work conducted in the West Indies from 1750 to 1994, only 7.5% of the entries included information for the Virgin Islands; only the extralimital islands of San Andres, Providencia, and the

Swans have received less attention (Wiley 2000).

We conducted mist netting on Guana Island, BVI, each October from 1994 to 2004. To our knowledge, the Guana Island station is the only current and consistently operated banding station in the British Virgin Islands and one of only three in the eastern Caribbean (St. Martin and Barbados being the others). Previously, information from the island has proven important in developing a better understanding of Neotropical migrant bird use of the region during the autumn migration (McNair et al. 2002). However, our data on species frequency of occurrence, which could be helpful in this effort, have not been made available until now. For example, Faaborg and Terborgh (1980) considered the Red-eyed Vireo (*Vireo olivaceus*) as a rare transient migrant encountered only in the Greater Antilles. In a status review of migrant landbirds in the Caribbean, Arendt (1992) did not list Red-eyed Vireos as even occurring in the British Virgin Islands. Indeed, Norton (1996) noted an account of a Red-eyed Vireo in Puerto Rico as one of only a few confirmed records for the species on the Puerto Rico Bank. The regular occurrences of Red-eyed Vireos at Guana Island (CWB and FCS unpubl. data), however, suggest that the species uses the Virgin Islands as a migration stopover more than previously believed.

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Here, we present an account of resident and migrant species banded during October each year for 11 years on Guana Island. For some species, we report longevity records based on recaptures of banded individuals. Additionally, we provide accounts of new or rarely reported species based on both banding and site records.

METHODS

The Virgin Islands, including both the U.S. Virgin Islands and the BVI, are a chain of approximately 76 islands and cays located 100–150 km east of Puerto Rico. Guana Island (18° 30′ N, 64° 30′ W) lies immediately north of Tortola, the largest of the BVI islands. Within the BVI, Guana Island is relatively small (3 km²) compared to other inhabited islands, such as Tortola (54 km²), Virgin Gorda (21 km²), and Jost Van Dyke (10 km²). The BVI has a subtropical climate tempered by northeasterly trade winds, with temperatures normally ranging from 28 to 33° C, and fairly constant humidity levels (~78%) throughout the year (Lazell 2005). Annual mean rainfall for Guana Island is estimated at 92 cm (Lazell 2005), but data are limited and the long-term average may be lower.

Guana Island is topographically rugged, with elevations ranging from sea level to 246 m. Approximately 90% of the island is covered by subtropical dry forest, with ghut forests (mesic forest; 5%) present in some drainages; miscellaneous covers include human-altered areas (3%), mangroves (1%), and beach (1%) (Lazell 1996; CWB unpubl. data). Lazell (1996) lists the primary native vegetation on Guana Island as tabebuia (*Tabebuia heterophylla*), gumbo-limbo (*Bursera simaruba*), loblolly (*Pisonia subcordata*), buttonwood (*Conocarpus erectus*), frangipani (*Plumeria alba*), acacia (*Acacia muricata*), and sea grape (*Coccoloba uvifera*). Tam-tam (*Leucaena leucocephela*) is common in disturbed areas. Introduced species include coconut (*Cocos nucifera*), tamarind (*Tamarindus indica*), and royal poinciana (*Delonix regia*).

We operated a mist-netting station each October from 1994 to 2004. Nets were located primarily along a northeast–southwest ridge and southeast-facing slope of a mountain on the island's west side. The majority of nets were in subtropical dry forest areas, but each year we placed 2–3 nets in human-altered areas along the ridge, all at approximately 100-m elevation. For one afternoon each year, we also netted along the shore of a salt pond to sample the shorebirds present. We attempted to use the same net locations each year, but during the earlier years of the project we conducted some "exploratory netting" in other areas. Duration of mist-netting operations and number of nets operated were subject to local weather conditions, the number of assistants available, and the amount of time we were allowed access to the island by its owners; thus, the number of nets used (mean = 8.1 ± 0.9 SE) and mist-netting days (mean = 8.8 ± 1.3 SE) varied annually. Weather permitting, nets were opened at 06:30 AST and closed between 10:00 and 11:00; occasionally mist-netting was also conducted in the afternoon.

We identified all birds captured to the species level, and, when possible, determined their sex and age (Raffaele 1989, Pyle 1997, Raffaele et al. 2003). We recorded weight (g), length of wing chord (mm), and banded each bird with a federal aluminum leg band. We did not conduct systematic avian surveys (e.g., point counts), but we did record species encountered while engaged in other studies and activities on the island. Combined, our observation records and mist-netting efforts allowed us to compile an annual species list for the island and document occurrences of species previously unrecorded on the island and/or the BVI. We compiled recapture records to determine longevity for both resident and migrant species. We considered all after-hatching-year birds (AHY) to be 1 year old at time of initial capture.

RESULTS

Banding.—We conducted mist netting for a mean of 252 ± 53 SE net-hr each October from 1994 through 2004. During the study period, we captured 1,410 birds, 188 (13%) of which were recaptures of birds banded in previous years (Table 1). These numbers do not include captures of birds that we did not band, such as the Green-throated Carib (*Eulampis holosericeus*) and the Antillean Crested Hummingbird (*Orthorhyncus cristatus*). We captured 44 species, the most common of which was the resident Bananaquit (*Coereba flaveola*; 676 captures). Other frequently captured

TABLE 1. Species and numbers of birds captured and banded at Guana Island, British Virgin Islands, 1994–2004.

Species	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	n	Percent recaptured
American Kestrel (*Falco sparverius*)	0	0	1	0	4	0	0	0	1	4	0	10	10
Wilson's Plover (*Charadrius wilsonia*)	0	0	8	0	3	10	2	2	1	0	2	28	29
Semipalmated Plover (*Charadrius semipalmatus*)	0	0	0	0	0	0	1	0	0	1	1	3	0
Black-necked Stilt (*Himantopus mexicanus*)	0	0	0	7	0	2	1	2	0	0	0	12	8
Lesser Yellowlegs (*Tringa flavipes*)	0	0	2	0	0	1	0	0	1	0	1	5	0
Solitary Sandpiper (*Tringa solitaria*)	0	0	0	0	0	0	1	0	0	0	0	1	0
Spotted Sandpiper (*Actitis macularius*)	0	0	6	2	2	2	0	0	0	0	3	15	7
Ruddy Turnstone (*Arenaria interpres*)	0	0	0	1	5	2	0	0	3	3	0	14	0
Semipalmated Sandpiper (*Calidris pusilla*)	0	0	2	0	2	3	2	1	1	0	1	12	0
White-rumped Sandpiper (*Calidris fuscicollis*)	0	0	0	0	0	0	0	0	0	2	0	2	0
Stilt Sandpiper (*Calidris himantopus*)	0	0	0	1	0	0	0	0	0	0	0	1	0
Scaly-naped Pigeon (*Patagioenas squamosa*)	0	0	0	0	1	0	0	0	0	0	0	1	0
Common Ground-Dove (*Columbina passerina*)	1	0	9	1	9	0	0	5	1	1	0	27	11
Zenaida Dove (*Zenaida aurita*)	0	1	3	4	8	5	0	26	4	1	5	57	14
Yellow-billed Cuckoo (*Coccyzus americanus*)	0	0	0	0	0	0	0	1	0	0	0	1	0
Mangrove Cuckoo (*Coccyzus minor*)	0	0	1	0	1	0	0	1	0	0	2	5	0
Caribbean Elaenia (*Elaenia martinica*)	0	4	5	4	11	0	0	7	1	6	5	43	11
Gray Kingbird (*Tyrannus dominicensis*)	0	0	0	0	3	0	0	7	1	0	0	11	0
Yellow-throated Vireo (*Vireo flavifrons*)	0	0	0	0	0	0	0	0	0	1	0	1	0
Red-eyed Vireo (*Vireo olivaceus*)	0	1	2	1	2	0	0	1	0	1	4	12	0
Swainson's Thrush (*Catharus ustulatus*)	0	0	0	0	0	0	1	0	0	1	0	2	0
Pearly-eyed Thrasher (*Margarops fuscatus*)	3	0	21	2	13	5	1	21	0	12	15	93	4
Golden-winged Warbler (*Vermivora chrysoptera*)	0	0	0	1	0	0	0	0	0	0	0	1	0
Northern Parula (*Parula Americana*)	0	0	2	0	0	0	0	0	0	0	2	4	0
Yellow Warbler (*Dendroica petechia*)	0	0	0	0	0	1	0	0	0	0	0	1	0
Magnolia Warbler (*Dendroica magnolia*)	0	0	1	0	0	0	0	0	0	1	1	3	0
Cape May Warbler (*Dendroica tigrina*)	0	1	0	0	0	0	0	0	0	0	0	1	0
Black-throated Blue Warbler (*Dendroica caerulescens*)	0	0	2	0	0	0	0	0	0	0	0	2	0
Blackpoll Warbler (*Dendroica striata*)	0	11	27	9	83	0	5	16	8	19	7	185	0
Black-and-white Warbler (*Mniotilta varia*)	0	0	4	0	1	0	0	0	0	2	0	7	0
American Redstart (*Setophaga ruticilla*)	0	0	0	0	1	0	0	0	1	0	0	2	0
Worm-eating Warbler (*Helmitheros vermivorum*)	0	0	0	0	0	0	0	0	0	1	0	1	0
Ovenbird (*Seiurus aurocapilla*)	0	0	0	0	3	0	0	0	0	2	1	6	0
Northern Waterthrush (*Seiurus noveboracensis*)	0	0	3	0	2	0	0	0	0	0	0	5	0
Kentucky Warbler (*Oporornis formosus*)	0	0	1	0	0	0	0	0	0	1	1	3	0
Hooded Warbler (*Wilsonia citrine*)	0	0	0	0	0	0	0	0	0	1	0	1	0
Bananaquit (*Coereba flaveola*)	12	68	50	85	145	9	16	131	45	72	43	676	20
Rose-breasted Grosbeak (*Pheucticus ludovicianus*)	0	1	0	0	0	0	0	0	0	1	0	2	0
Indigo Bunting (*Passerina cyanea*)	0	0	1	0	0	0	0	0	0	1	3	5	0
Black-faced Grassquit (*Tiaris bicolor*)	11	6	26	9	18	2	11	16	8	11	30	148	13
Lesser Antillean Bullfinch (*Loxigilla noctis*)	0	0	0	0	1	0	0	0	0	0	0	1	0
Totals	27	93	177	128	317	42	41	237	76	145	127	1,410	

TABLE 2.   Longevity records for species ≥4 years old on Guana Island, British Virgin Islands, 1994–2004.

Species	Age[a]	Sex	Year captured	Last recapture	No. of recaptures	Minimum age (years)
Wilson's Plover	AHY	F	1996	1999	2	4
	AHY	M	1996	1999	1	4
	AHY	U	1996	1999	1	4
	AHY	M	1996	1999	2	4
Black-necked Stilt	AHY	F	1997	2001	1	5
Spotted Sandpiper	HY	U	1998	2004	2	6
Common Ground-Dove	AHY	F	1998	2001	1	4
Zenaida Dove	AHY	M	1997	2001	2	5
	AHY	M	1998	2001	1	4
	AHY	M	2001	2004	1	4
Caribbean Elaenia	Unk	Unk	1996	2003	1	7
	Unk	Unk	1996	2001	1	5
Pearly-eyed Thrasher	AHY	Unk	1998	2001	1	4
Black-faced Grassquit	AHY	F	1996	2004	2	9
	AHY	F	1998	2004	1	7
	AHY	M	1998	2003	1	6
	HY	U	1998	2003	1	5
	AHY	F	2000	2004	2	5
	AHY	M	1996	2000	1	5
Bananaquit	AHY	M	1995	2001	3	7
	AHY	M	1997	2003	2	7
	HY	F	1998	2004	2	6
	AHY	M	1998	2002	2	5
	AHY	M	1997	2001	2	5
	AHY	F	1997	2001	1	5
	HY	M	1998	2003	2	5
	AHY	M	2001	2004	3	5
	AHY	M	1997	2000	1	4
	AHY	M	1995	1998	2	4
	AHY	M	1995	1998	3	4
	HY	F	1997	2001	2	4
	AHY	M	2000	2004	2	4
	AHY	M	2001	2004	2	4
	AHY	M	1994	1997	2	4
	HY	F	1998	2002	2	4
	HY	M	1998	2002	2	4
	AHY	M	1998	2001	1	4
	AHY	M	1998	2001	1	4

[a] AHY = after-hatching-year, HY = hatching-year, Unk = unknown age.

resident species were Black-faced Grassquit (*Tiaris bicolor*; 148 captures) and Pearly-eyed Thrasher (*Margarops fuscatus*; 93 captures). These three species are among the most abundant residents on Guana Island. We also captured 20 species of Neotropical migrant landbirds, the majority of which were warblers (Table 1). The Neotropical migrant captured most frequently was the Blackpoll Warbler (*Dendroica striata*; 185 captures), followed by the Red-eyed Vireo (12 captures, multiple additional sightings). Other Neotropical migrants encountered included many species

(e.g., Yellow-throated Vireo, *Vireo flavifrons*; Table 1) previously reported only from the western Greater Antilles or for which there were no records from the BVI or the Lesser Antilles (Faaborg and Terborgh 1980, Arendt 1992).

Longevity.—We determined longevity for all species recaptured on the island, and provide data for those older than 3 years (Table 2). Among shorebirds, the longevity records were 5 years for Black-necked Stilt (*Himantopus mexicanus*), 6 years for Spotted Sandpiper (*Actitis macularius*), and 4 years for

Wilson's Plover (*Charadrius wilsonia*); however, our recapture rate for these species was low and we suspect that our longevity estimates, especially for the resident Wilson's Plover, may be substantially lower than actual longevity. Among Columbiformes, our longevity records were 4 years for Common Ground-Dove (*Columbina passerina*) and 5 years for Zenaida Dove (*Zenaida aurita*). Among resident passerines, we recaptured Caribbean Elaenias (*Elaenia martinica*) that were ≥7 and ≥5 years old, and we recaptured a 4-year-old Pearly-eyed Thrasher. Among the 19 recaptured Bananaquits, two were 7-year olds, one was 6 years old, and the others were 5- and 4-year olds. The oldest bird recaptured was a ≥9-year-old female Black-faced Grassquit; we also recaptured one 6-year-old and three 5-year-old grassquits.

New species records.—During the course of our netting operations and surveys, we obtained species records for Guana Island and, in some cases, the British Virgin Islands. Our captures of a Magnolia Warbler (*Dendroica magnolia*) in 1996 and a Golden-winged Warbler (*Vermivora chrysoptera*) in 1997 were first records for the BVI. More significant, however, was our capture of a Swainson's Thrush (*Catharus ustulatus*) in 2000, the first record for the Virgin Islands and only the second from east of Cuba (McNair et al. 2002). In 2003, we captured another Swainson's Thrush and obtained a visual sighting of a second, unbanded individual. Finally, our observation of a hatching-year Red-necked Phalarope (*Phalaropus lobatus*) on the salt pond of Guana Island in October 2004 represented a first record for that species in the Virgin Islands.

DISCUSSION

Deriving longevity estimates from survivorship models is preferable to using simple longevity records (Krementz et al. 1989). The reliability of survival estimates, however, depends upon robust recapture data (e.g., Burnham et al. 1987), which often are not available for many species. Longevity records, therefore, are still valuable for providing some basic life-history information on little-studied species. This may be especially true for island settings, where longer-lived species are at lower risk of localized extinction (Newton

1998). Although longevity records have been reported for many North American bird species (e.g., Kennard 1975, Klimkiewicz et al. 1983), little information is available on the life spans of tropical birds (Snow and Lill 1974, Faaborg and Winters 1979, Johnston et al. 1997). The few Caribbean bird species for which there are longevity records are primarily Puerto Rican (Faaborg and Winters 1979, Woodworth et al. 1999), and there is virtually no published information on the longevity of birds in the eastern Caribbean. Thus, our data provide new age records for several Caribbean species. In Puerto Rico, Faaborg and Winters (1979) recaptured 36 of 219 Bananaquits, the oldest of which was 4 years and 7 months. Outside of the Caribbean, de Souza Lopes et al. (1980) reported a 4-year, 8-month-old Bananaquit from their study in Brazil. Our longevity record of 7 years for Bananaquits exceeds previous reports by a minimum of 2 years. Furthermore, our Bananaquit data suggest that ages of 4 and 5 years are not uncommon. Perhaps most unusual is our 9-year-old age record for a Black-faced Grassquit, with additional individuals aged 6 and 5 years. These far exceed the previous report of 2 years and 11 months (Faaborg and Winters 1979). The 4-year-old Common Ground-Dove in our study is similar to the longevity records of 4 years and 4 years and 1 month from Puerto Rico (Faaborg and Winters 1979). However, the 5-year and 5-month old Pearly-eyed Thrasher reported by Faaborg and Winters (1979) exceeds our oldest known thrasher by 1 to 2 years. We found no reports of longevity for Caribbean Elaenia with which to compare our records; however, our records of 7- and 5-year-old Caribbean Elaenia are similar to those reported for unspecified *Elaenia* spp. in Brazil (6 years and 3 months and 5 years; de Souza Lopes et al. 1980) and substantially exceed ages recorded for Yellow-bellied Elaenia (*E. flavogaster*; 2 years and 11 months) and Mountain Elaenia (*E. frantzii*; 3 years and 9 months) in Panama (Loftin 1975). We believe that the 5-year-old Zenaida Dove from our study also represents a longevity record for that species, as we could find no reports with which to compare our data.

Many of the Neotropical migrants captured or sighted during our study are known to occasionally occur in the BVI. Some of our

sightings and captures, such as Hooded Warblers (*Wilsonia citrina*) and Worm-eating Warblers (*Helmitheros vermivorum*), are unusual for the BVI. Still others, including Magnolia Warbler, Golden-winged Warbler, Swainson's Thrush, and Red-necked Phalarope, provide new records for the BVI. Detections of Swainson's Thrush and Red-necked Phalarope were particularly interesting. Within the Caribbean region, Raffaele et al. (2003) indicated that Swainson's Thrush was found only rarely in the western Greater Antilles and only during migration; thus, detections of Swainson's Thrush in 2 different years on Guana Island was notable. Raffaele et al. (2003) also indicated that Red-necked Phalarope is a very rare migrant in the Bahamas, Cuba, and Hispaniola (e.g., Greater Antilles); in Puerto Rico, the species has been recorded only twice (Raffaele 1989). In September 2003, however, a Red-necked Phalarope was reported on Guadaloupe Island (Norton et al. 2003), which lies 400 km southeast of Guana Island.

Our detections of Blackpoll Warbler and Red-eyed Vireo, and our consistent detections of other, less common species—such as Yellow-throated Vireo, Swainson's Thrush, Indigo Bunting (*Passerina cyanea*), and numerous warbler species—indicate that they may be more common in the eastern Caribbean during migration than previously believed due to a lack of searching or banding efforts in that region. For example, Blackpoll Warbler, the most common warbler encountered on Guana Island and the second-most frequently captured species overall, was not reported in the BVI until 1989 (Norton 1990); it had been considered a common Neotropical migrant through the Greater Antilles but uncommon to rare on other islands (Arendt 1992, Raffaele et al. 2003). Similarly, Red-eyed Vireo was thought to be very uncommon or vagrant in the Lesser Antilles (Faaborg and Terborgh 1980, Arendt 1992, Norton 1996, Raffaele et al. 2003); however, our regular sightings and captures of Red-eyed Vireos suggest that the species may be a more common migrant in the BVI than previously believed.

Overall, our detections of species previously believed to be uncommon or not present within the BVI may have been due to a lack of field surveys and banding efforts through-out most of the Virgin Islands and Lesser Antilles. Alternatively, our detections may be related to changes in habitat conditions in the western Caribbean islands. As habitat availability decreases in the western islands, some migrant species might be shifting their migration routes eastward (Arendt 1992). Regardless of possible shifts in migration routes, it appears that Guana Island—a functional ecosystem protected as a nature preserve (Lazell 1996)—provides important habitat for both resident and transient migrant species. A low-occupancy, private resort occupies less than 2% of the surface area of Guana Island; the remainder of the island is almost completely free of direct human impacts and exists in a near-natural state (Lazell 1996). Furthermore, exotic herbivores and carnivores, which are a severe problem throughout much of the Caribbean, occur at very low densities and are heavily controlled on the island.

As larger islands in the Virgin Islands (e.g., Tortola, St. John, Virgin Gorda) continue to undergo deforestation and development (e.g., Arendt 1992), smaller islands maintained in primarily natural states are likely to become increasingly important for conservation of both resident and migrant birds. However, small islands, such as Guana Island, may not provide a full range of landscape characteristics required for some migrant or wintering Neotropical songbirds. For example, Northern Parula (*Parula americana*) and American Redstart (*Setophaga ruticilla*), both common nonbreeding residents in the Virgin Islands (Raffaele et al. 2003), are seldom detected on Guana. Further examination of resource use and spatial needs of Neotropical songbirds migrating through or wintering in the BVI is needed to facilitate conservation efforts.

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Conservation of the Extremely Endangered Stout Iguana

Gad Perry and James Lazell

The stout iguana was originally found throughout the Greater Puerto-Rico Bank, but today survives only in the British Virgin Islands. With only about 200 individuals remaining, it is one of the most critically endangered species in the world. Until recently, the only surviving population existed on the island of Anegada, where increasing development and introduced herbivores and predators greatly degraded conditions and caused major population declines. To increase its chances of survival, the iguana has been reintroduced to a number of islands. The population on Guana Island appears to be flourishing, and the Necker Island population is also expanding. The severe threat currently facing the species makes detailed information about its biology essential to successful conservation and restoration efforts. We have been working to address this need by studying the natural history of the iguana and its reproduction.

Our findings are encouraging. The Guana Island population is consistently producing a large number of juveniles, with egg-laying occurring on sandy beaches in the summer and hatchlings dispersing into the interior of the island in the fall. Juvenile numbers are high enough that they are once again becoming part of the food cycle, and we have observed predation events by the native snakes and kestrels. During 2003 and 2004, we have captured, measured, and released nearly 50 juveniles, and have discovered that they begin growing almost immediately; weight gain is measurable even within the first few weeks of life. We hope to be able to track some of these individuals throughout their lives, documenting their ecology, growth patterns, and long-term survival.

A hatchling iguana who survived a predation attempt showing tail damage and regeneration.
Picture by Gad Perry

Longevity of Insular Tropical Birds in the Caribbean

Clint W. Boal, Fred Sibley, and Tracy S. Estabrook

Longevity records for North American birds have been reported for individual species and species groups, but little information is available on longevity of insular tropical species. The few longevity records available for species in the Caribbean are limited primarily to studies from Puerto Rico; virtually no information is available from the smaller islands of the eastern Caribbean. We have been monitoring resident and neotropical migrant bird species on Guana Island, British Virgin Islands each October since 1994. As part of our ongoing avian research program on Guana Island, we were able to assess relative abundances and longevity of some Caribbean land bird species, and longevity of some migrant shorebirds.

We operated mist-nets for an average of 253 net hours each October from 1994-2004. We captured 1410 birds, 188 (13%) of which were recaptures of birds banded in previous years. The most commonly captured resident tropical species were bananaquits (*Coereba flaveola*; 676 captures), black-

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faced grassquits (*Tiaris bicolor*; 148), and pearly-eyed thrashers (*Margarops fuscatus*; 93 captures). These three species are among the most abundant residents of the island. We obtained recaptures of several bananaquits, the oldest of which were two 7-year-olds, a 6-year-old, and multiple 5- and 4-year-olds. The oldest bird we obtained a recapture on was a minimum 9-year-old female black-faced grassquit; we also recaptured a 6-year-old and three 5-year-old grassquits. We also obtained recaptures of Caribbean elaenias (*Elaenia martinica*) that were minimums of 7 and 5 years old, and we recaptured a four year old pearly-eyed thrasher. Among resident dove species, our longevity records were four years old for common ground dove (*Columbina passerina*) and five years old for zenaida doves (*Zenaida aurita*). Among shorebirds, the longevity records obtained during our study were five years for black-necked Stilt (*Himantopus mexicanus*), six years for spotted sandpiper (*Actitis macularia*), and 4 years for Wilson's plover (*Charadrius wilsonia*).

Birds exhibit a general pattern of body size correlating to life-history features. Typically, the smaller the species is corresponds to a briefer life span. However, tropical species appear to be generally longer-lived than their temperate equivalents. Our longevity records for bananaquits (9-11g), black-faced grassquits (9-12g), and Caribbean elaenias (14-18g) support these patterns. We are currently continuing our data collection and applying mark-recapture analyses to model survival and population trends of these species.

The Invasive Cuban Tree Frog in the British Virgin Islands

Jennifer Owen and Gad Perry

Non-native species are now present in every nation's ecosystems and their economic impact is estimated at over $100 billion/year in the United States alone. In addition, invasives are the second leading cause of native species

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Over 200 Cuban tree frogs fill a residential cistern in the BVI. This cistern provides the only supply of fresh water for the attached house.
Pictures by Jennifer Owen

extinction worldwide. The Cuban Tree Frog (CTF) is one such successful invader, currently recorded as introduced in Florida, Puerto Rico, Hawaii, Anguilla and the US Virgin Islands. Our research focuses on the spread of the CTF in the British Virgin Islands (BVI) in order to determine what effects the CTF has in this new environment. We have three objectives: (1) document the CTF's ongoing invasion of the BVI; (2) determine the primary diet of the CTF in the BVI; and (3) examine the reproductive output of the CTF in the BVI.

We have been collecting data since 2002 and have so far examined over 450 specimens of CTFs collected for us by BVI residents. To date, CTFs have been collected on five islands within the BVI, and these include a number of new island records. Two of these new island collections were made within the past year, indicating ongoing spread, a source of concern. All preserved specimens have been dissected with stomach and intestinal tracts removed and contents identified to order. We found invertebrates such as spiders, scorpions, roaches, beetles, and ants, as well as native vertebrates such as frogs, geckos and snakes. Residents further report that native frogs disappear as the invader moves in. Examination of reproductive organs indicates BVI populations are capable of breeding year-around, helping explain their rapid spread.

Another source of concern with the CTF is zoonoses, diseases that can be transferred from animals to humans. The frogs invade, live, and breed in the cisterns that are a major source of drinking water in the BVI. From work conducted elsewhere, the CTF is known to be able to carry several diseases that can affect humans, but there is no information on the introduced population in the Virgin Islands. In cooperation with government agencies within the BVI and the US Virgin Islands, we recently sent CTFs to a laboratory in Madison, Wisconsin to test for diseases and bacteria that may be transmittable to humans. Tests for chytrid fungus, a possible factor in the worldwide decline of amphibian populations, are also being preformed.

Data analysis in this project is ongoing. So far, our work shows that the CTF is an aggressive invader that has broad diet and high reproductive output. Because of its association with human commerce (especially building materials and ornamental plants), it is rapidly being distributed throughout the Caribbean. There are clear indications of ecological impacts, and potential for human health hazards. We are therefore aiding several entities in the BVI in producing effective management tools for controlling current populations and preventing further spread. Gratifyingly, these efforts are already beginning to bear fruit, resulting in the disappearances of several small populations and better protections for currently uninfected islands.

OSTEOPILUS SEPTENTRIONALIS (Cuban Tree Frog).
COLONIZATION OF THE BRITISH VIRGIN ISLANDS.
Native to Cuba, the Cayman Islands, and the Bahamas, *Osteopilus septentrionalis* is widely introduced in the Caribbean and elsewhere (Lever 2003. Naturalized Reptiles and Amphibians of the World. Oxford University Press, New York. 318 pp.). It has only been reported in the British Virgin Islands (BVI) once, from Necker Island (Lever, *op. cit.*). Here we document the ongoing spread of *O. septentrionalis* across the BVI. GPS points are based on the WGS84 datum and all specimens were verified by Jose Rosado, Museum of Comparative Zoology (MCZ).

The first record of *O.* septentrionalis in the BVI (MCZ A-135386) was collected on Tortola at the Road Town dock (18°25.8'N, 64°36.8'W) during Fall 1990 by Everton Henry. The species is currently abundant throughout the island. Because the frog breeds in cisterns providing residential water, it is considered a pest and attempts are made by the local health authorities to remove it from specific locations. The second oldest specimen (MCZ A-119258) was captured on Necker Island (18°31.6'N, 64°21.6'W) on 19 Oct 1993 by A. Miller. No population was established (Meshaka 1996. Herpetol. Rev. 27:37–40; unpubl. obser.). The first collection on Beef Island (MCZ A-136611) was made at Trellis Bay (near the international airport, 18°26.44'N, 64°32.08'W) on 15 Oct 2002 by Gad Perry and Kate LeVering. Additional sites with breeding populations have since been identified on Beef island, which is connected to Tortola by a bridge and frequent traffic. One of these sites is a nursery, which supplies ornamental plants to many BVI establishments. The following year marked the first record of the species on Virgin Gorda (MCZ A-136432). The specimen was collected by Jim Egelhoff on 27 Oct 2003 in Spanish Town (18°26.6'N, 64°26.2'W). More extensive work in 2004 revealed the presence of a breeding population in the town. Most recently, a collection (field tag JO166) was made on Peter Island (18°21.231'N, 64°34.317'W) by Jennifer Owen and Gad Perry on 13 Oct 2004. Frogs are established at several locations on the island, suggesting a breeding population.

There appears to have been multiple introductions of the frog in the BVI, primarily through movement of cargo and ornamental plants. The Necker Island specimen was associated with ornamental plants arriving from Miami (Meshaka, *op. cit.*). BVI resident Elvit Meyers (interviewed October 2002) reported frogs arriving in Cane Garden Bay, Tortola during April 2000, long after the initial specimen was collected there. The frogs were located in concrete block pallets arriving from Florida and local populations then rapidly expanded. Other populations, such as Beef Island and Peter Island, may be the result of spread within the BVI, although human assistance may have been involved. As *O. septentrionalis* preys on native species (Lever, *op. cit.*), the ongoing range expansion of the species is a source of concern.

Submitted by **JENNIFER OWEN** and **GAD PERRY**, Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Box 42125, Lubbock, Texas 79409-2125, USA (e-mail: gad.perry@ttu.edu); **JAMES LAZELL**, The Conservation Agency, 6 Swinburne Street, Jamestown, Rhode Island 02835, USA; **CLIVE PETROVIC**, H. Lavity Stoutt Community College, PO Box 3097, Road Town, Tortola, British Virgin Islands; and **JIM EGELHOFF**, BVI Pest Control, PO Box 1109, Virgin Gorda, British Virgin Islands.

Sent: Mon 8/22/2005 11:53 AM
To: Owen, Jennifer L; Perry, Gad
Cc: CarSmith@gov.vg
Subject: The Cuban Tree Frog pamphlet

Dear Jennifer and Gad:

I have just returned to work from summer vacation to find a batch of pamphlets entitled "The Cuban Tree Frog." I am impressed by the fact that you chose to produce this very informative and impressively designed pamphlet rather than just sending us the empirical data you gathered during your research and the conclusions you arrived at.

I shall impress upon the Chief Environmental Health Officer by copy of this e-mail to convey a letter of acknowledgement to you on official letterhead for the assistance you have given to us.

Minchie

M. Minchington Israel
Vector Control Coordinator
Environmental Health Division
Community Health Services
Department of Health
Ministry of Health and Welfare
Government of the British Virgin Islands
Telephone (W): 1 (284) 468.3701 Ext. 4965/5110
Telephone (H): 1 (284) 494.8835
Facimile: 1(284) 494.7430
E-mail: enviro5@surfbvi.com

--
It was a pleasure speaking with you. Do come again, will you?

Perry, Gad

From:	Minchie [mec.archive@gmail.com]
Sent:	Monday, September 12, 2005 9:47 AM
To:	Perry, Gad
Subject:	Re: The Cuban Tree Frog pamphlet

Greetings Gad:

The leaflets are really coming in handy. I can assure you of that.

We will certainly take you up on your offer to send us some more of them. However, we have not yet exhausted the first batch so we will let you know when we need more. Many thanks again.

There is not much debate so far about the ecological disaster that has taken place next door to you in Mississippi and Louisiana, but I am sure that scientists like yourself are very much attuned to that. I would think that once the water settles (no pun intended), if not sooner, work will commence to assess the effects of the flooding on the wildlife, the future of the wetlands and their implications.

So far this hurricane season, we in the southern Caribbean have been spared. We will be keeping our fingers crossed because we are right in the middle of the peak period for storm activities. We are not out of the woods as yet, not for a long shot anyway. Wish us luck.

Well, you take care. So will I.

Minchie

On 22/08/05, **Perry, Gad** <gad.perry@ttu.edu> wrote:
> Minchie,
>
> Good to hear from you - it's been a while. Glad to hear the leaflets were of use. We have some more if
> you'd like them. The wildlife disease lab in Wisconsin is still examining the specimens, so there may
> be updates. Jennifer will e keeping in touch with them and will update as she learns more. She has
> finished he Master's, by the way, and currently works for Texas Parks and Wildlife.
>
> Happy to help,
>
> Gad

From: enviro5@surfbvi.com [mailto:enviro5@surfbvi.com]

9/16/2005

ALSOPHIS PORTORICENSIS ANEGADAE (Puerto Rican Racer). **SCAVENGING.**
Snakes occasionally scavenge food, but the use of carrion in snake diets has typically been undervalued. Evidence for scavenging by pit vipers and aquatic species continues to accumulate (DeVault and Krochmal 2002. Herpetologica 58:429-436), but cases of colubrids feeding on carrion are much less common. Here we report on a case we observed on 23 September 2005. At 1150 h we captured a racer on Guana Island, British Virgin Islands. The snake (male, SVL 56 cm, stumped tail length 24 cm, mass 82.5 g) was captured within the hotel complex on the island (18.4793^0 N, 64.5781^0 W). It had recently ingested a relatively large food item of unusually angular shape, which we obtained by forced regurgitation (Fig. 1). Further inspection revealed the ejected food item to be two chicken bones, a humerus weighing about 5 g and about half of an ulna, weighing about 1 g. Both are now in the Yale Peabody Museum (YPM F-4171-2). The snake was released at the site of capture. Chicken wings are provided to workers at a construction site located about 70 m from the capture site and leftovers are often discarded in the nearby brush. Based on the state of digestion, which had left few traces of meat on the bones, the snake had likely encountered the bones very recently. *Anolis cristatellus*, a major component of the diet of the racer, is abundant at the site, thus lack of food is unlikely to have been a factor. Although scavenging is not a large part of the diet of *A. portoricensis*, this observation emphasizes that many species have greater dietary flexibility than often believed.

College of Agricultural Sciences and Natural Resource Management, Texas Tech University.

Submitted by **Gad Perry** and **Kate LeVering**, Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Lubbock, Texas 79409, USA, **Arijana Barun**, Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37936, USA, and **James Lazell**, The Conservation Agency, 6 Swinburne St., Jamestown, Rhode Island 02835, USA.
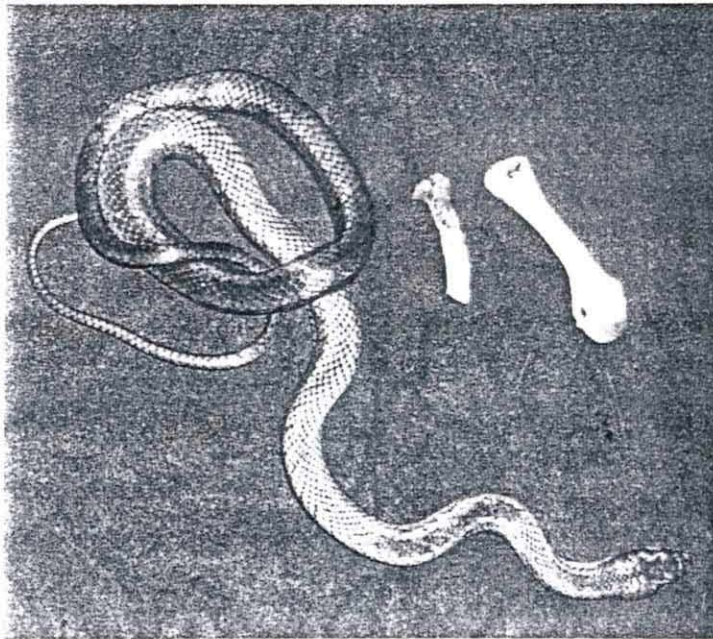

Fig. 1. *Alsophis portoricensis* and chicken bones it regurgitated.

James Lazell

Dear Skip and Numi,

I wanted to let you know that the ISG Steering Committee has voted not to re-invite you to join the group for the next quadrennium. This was a very difficult decision, and one that was not unanimous. While it is recognized that you are highly dedicated to iguana conservation and have performed much valuable work, we are under mandate to follow IUCN guidelines with regard to reintroduction and translocation efforts. The continued push to found new island populations of C. pinguis in the BVI without the approval of the National Parks Trust is in violation of the ISG's MOU with the Trust, which states:

"No floral and/or faunal species or part thereof, dead or alive may be transported from one Island/Cay to another or outside of the territory without the knowledge and express approval of the Deputy Director/Science Coordinator or his appointee."

The situation on Anegada is at a critical juncture. We are nearing completion of a comprehensive Species Recovery Plan and have finally successfully engaged the Trust in two long-standing key issues, feral mammal control and national park planning. To ensure that the ISG can maintain and grow our relationship with the Trust in the future, we cannot support further unapproved translocations. We recognize the potential conservation value of your work and hope that we can keep in touch concerning iguana conservation issues in the future.

With many thanks for your service,
Allison

Allison C. Alberts, Ph.D.
Director of Conservation and Research
Zoological Society of San Diego
15600 San Pasqual Valley Road
Escondido, California 92027 USA

Tel: 760-291-5480
Fax: 760-291-5476
Email: aalberts@sandiegozoo.org
www.conservationandscience.org

GUANA IGUANA CENSUS

2005

BY

James Lazell

We caught, measured, weighed, pit-tagged, and paint numbered 15 iguanas between 22 and 30 September. One of these, a young female, had been first caught (and pit-tagged) in 2003. In two years she has grown from 38 to 42 cm snout-vent length and from 2 to 3.3 kg in mass.

We continued to sight marked and unmarked iguanas through 19 October, with a total of 47 seen of which 10 were marked. This gives a simple Lincoln Index point estimator of 71 in the small area we censused. Using more sophisticated estimates, we got:

Schnabel: 48, 95% confidence interval 27-100.
Schumacher: 49, 95% confidence interval 31-112.

To calculate the area censused we mapped extreme sightings, outlined the area they encompassed, and added a band equal to average travel distance, 57 m, of marked individuals to this on the landward edges (the sea defined most of edge): illustration follows. The area was about 70.4 ha, roughly centered on Dominica House (or more tightly, the Salt Pond).

Can we extrapolate to the entire Island?

There are two polar views of iguana density on Guana: They (like the observers) are concentrated around the buildings, or at least concentrated from the north end of North Beach southwest to the Pyramid, including the whole ridge with the buildings. Or, they are all over the Island with some concentrations just as dense as that around the buildings.

In support of the first view, iguanas do seem to like east and southeast facing slopes and ridge tops, presumably because these provide the best opportunities for morning sun basking, which the iguanas need to -- literally -- get up to speed for their daily activities. Further, the buildings area was the original sheep exclosure and remains sheep-free. Therefore it has the best, least-disturbed vegetation as a food supply. Lastly, if baby iguanas mostly hatch at North Beach, then the hatchlings might preferentially travel west and uphill in search of east-facing, sunny slopes to set up residence on. Indeed, one hatchling marked on the Peak Trail right above the North Beach hatching area traveled about 550m, in three days, to the south face of the first steep

hill past Crab Cove. This was by far the greatest travel distance recorded in 2005 and fits the first notion, above.

However, we do see iguanas all over the island and some of us believe there are dense concentrations on the southeast facing slopes of Guana's main massif, notably above Bigelow beach. Iguanas are regularly seen on the east-facing slope of Palm Ridge and in Shangri La (Grand Ghut). The few remaining sheep may present little competition to iguanas. Sizable areas within our 70.4 ha census area, like the Salt Pond and the open grass area of White Bay Flat, have few or no iguanas. One could, therefore, argue that our census area is representative of the whole Island. If this is true we can multiply our point estimator by about 4.2, giving a total population of about 200.

If the first scenario is true we could guess there might be about twice as many iguanas on the whole Island as in our 70.4 ha census area. Thus, about 100. In either case, the population does not seem to have changed much in the last several years, at least within our census area. There remains the possibility that this area was populated first and now iguanas are increasing on the rest of the Island. A sighting-per-km of trail hiked would be a way to test this hypothesis.

Question: There is one fact presented above that could invalidate both, or all, efforts to calculate population from the available data. Can you spot it?

Guana Island Iguana Census, 2005.

Dots indicate mark and resight points. Solid lines indicate outer limit of census. Dashed lines indicate travel distance edges.

305 m

N

-----Original Message-----
From: Alamillo, Hugo (UMR-Student) [hap4f@umr.edu] Sent: Thursday, June 10, 2004 4:31 PM
To: Perry, Gad
Subject: Amphisbaena fenestrata

Dear Dr. Perry,

I am a graduate student pursuing a Master's degree with Dr. Anne Maglia at the University of Missouri-Rolla. I saw your note on evaporative water loss in A. fenestrata as I was copy editing the latest issue of Herp. Review. Part of my thesis is describing the morphology of the tail autotomy of the Caribbean clade of Amphisbaena and I would like to include a phylogeny of the clade as well. Presently I have tissues for three species of Amphisbaena from the Caribbean and I am also contacting Dr. Kearney for others. At this time I am wondering if when you collected A. fenestrata you collected tissues for it? My plan originally was to go out there this summer and collect as many tissues as I could, but as a result of my grants not getting funded I am forced to ask other researchers who have collected Amphisbaena.

Thank you for considering my request. I look forward to your response.

Hugo Alamillo

Biological Sciences

The University of Missouri-Rolla

Rolla, MO

65401

Dear Hugo,

I'm afraid we have never collected any tissues, although there are formalin-fixed specimens at various museums. Finding these guys is not uncommon but not a foregone conclusion, but if collection is simple under primitive conditions I'll try to get you some this coming October. We can't do ultracold, but 95% EtOH should be fine, if that works for you.

Gad

****
Gad Perry, Assistant Professor
Dept. of Range, Wildlife and Fisheries Management
Box 42125, Texas Tech University
Lubbock, TX 79409-2125, USA

AMPHISBAENA FENESTRATA (Virgin Islands Worm Lizard). **EVAPORATIVE WATER LOSS.** *Amphisbaena fenestrata* is endemic to the Virgin Islands of the Puerto Rico Bank. Like most members of its fossorial genus, it is rarely seen and thus little-known (Gans and Alexander 1962. Bull. Mus. Comp. Zool. 128:67–158). In the course of evaporative water loss studies on Virgin Islands squamates (e.g., Perry et al. 2000. Biotropica 32:722–728, and citations therein), we had the opportunity to test 3 *A. fenestrata*.

The 3 individuals examined were collected on Guana Island (18°38'N, 65°25'W), British Virgin Islands. All were found surface-active during the day following heavy rains. Collection sites were up to 70 m above sea level and had thick plant cover, providing protection from direct sunlight. Substrates varied from sandy to rocky loam. Total water loss was measured in the laboratory using the methods of Dmi'el et al. (1997. Biotropica 29:111–116). Animals were tested within 24 h of capture, having been maintained with ad lib water but no food. Following the study, animals were euthanized and deposited at the Yale Peabody Museum (YPM 12061: TL 214 mm, 5.0 g; YPM 12986: TL 181 mm, 2.5 g) and the Sam Noble Oklahoma Museum of Natural History OMNH 39481 (TL 197 mm, 3.1 g). The sex of these animals was not determined. We report water loss calculated two ways. First, we used mass to calculate water loss in mg g^{-1} h^{-1}. Our results were as follows: OMNH 39481 had the lowest water loss rate: 5.64 mg g^{-1} h^{-1}. The largest individual (YPM 12061) lost 5.99 mg g^{-1} h^{-1}, and the smallest animal (YPM 12986) had the highest water loss rate: 6.56

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mg g^{-1} h^{-1}. We also calculated water loss per unit surface. To quantify surface area, we assumed an amphisbaenid is cylindrical in shape, and calculated surface area (A) from total length (TL, in mm) and average diameter (D, measured to the nearest 0.1 mm at three positions: just behind the head, at midbody, and just anterior to the cloaca), using the equation $A = p \cdot D \cdot TL$. We were unable to partition respiratory from cutaneous water loss, and thus used total water loss, which includes both. However, cutaneous gas exchange in amphisbaenians is especially large (Abe et al. 1987. J. Exp. Biol. 127:159–172), implying a relatively low breathing rate. We therefore expect water loss through the respiratory tract to be low as well, making our values only somewhat higher than actual cutaneous water loss rates. Our results were as follows (surface area and water loss rate, respectively, indicated for each): OMNH 39481: 32.2 cm^2, 0.55 mg cm^{-2} h^{-1}; YPM 12061: 44.3 cm^2, 0.66 mg cm^{-2} h^{-1}; YPM 12986: 26.2 cm^2, 0.62 mg cm^{-2} h^{-1}.

Few comparative data exist for amphisbaenians. Krakauer et al. (1968. Nature 218:659–660) graphed total water loss for 13 individuals. Of 7 species examined, 6 were comparable in size to *A. fenestrata*. These 6 species broke into two distinct groups based on habitat: humid or desert. However, the experimental design of Krakauer et al. (*op. cit.*) differed in that dried, flowing air was used rather than air at ambient humidity, preventing a precise comparison of results. Nonetheless, values for *A. fenestrata* seem most similar to the desert group, as might be expected from a species residing on a fairly dry island (Dmi'el et al. 1997. Biotropica 29:111–116).

Submitted by **GAD PERRY**, Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Box 42125, Lubbock, Texas 79409-2125, USA (e-mail: gad.perry@ttu.edu), **RAZI DMI'EL**, Department of Zoology, Tel Aviv University, 69978 Tel Aviv, Israel, and **JAMES LAZELL**, The Conservation Agency, 6 Swinburne St., Jamestown, Rhode Island 02835, USA.

ANOLIS PULCHELLUS (Grass Anole). **NECTIVORY.** Nectar feeding has previously been reported in anoles (e.g., Liner 1996. Herpetol. Rev. 27:78). Perry and Lazell (1997. Herpetol. Rev. 28:150-151) argued that, though episodic, it is more common that usually thought, and recent reports (Campbell 2000. Herpetol. Rev. 31:239; Echternacht et al. 2000. Herpetol. Rev. 31:173; Rios-López 2004. Herpetol. Rev. 35:386; Townsend 2004. Herpetol. Rev. 35:141-142) support that view. Here we report on another such case.

On the afternoon of 24 September 2005, we observed an adult (ca. 4.5 cm SVL) male *Anolis pulchellus* licking at a button-like structure near the base of the leaf of a small *Leucaena leucocephala* tree (Leguminosae). The lizard was about 1 m above the ground and the behavior continued for about a minute. Although nectar was not apparent to the naked eye, these structures are known to be extrafloral nectaries (Minu 1991. Acta Botanica Indica 19:49-54).

This is but the second report of lizards using extrafloral nectaries; the first (Rios-López 2004. Herpetol. Rev. 35:386) occurred in a closely related lizard species of similar size that also occurs on the Puerto Rico bank. Anoles also occasionally engage in outright herbivory (e.g., Lazell and Mitchell 1998. Herpetol. Rev. 29: 237), and episodic nectivory also occurs in other lizard clades (e.g., Perry and Ritter 1999. Herpetol. Rev. 30:166-167).

Submitted by **GAD PERRY,** Department of Range, Wildlife, and Fisheries Management, Texas Tech University, Lubbock, Texas 79409, USA, and **JAMES**

ANOLIS CRISTATELLUS WILEYAE (Virgin Islands Crested Anole). **SAUROPHAGY.** *Anolis cristatellus* primarily feeds on invertebrates, but has been recorded feeding on fruit (Lazell and Perry 1997. Herpetol. Rev. 28:150), *Sphaerodactylus* geckos (Schwartz and Henderson 1991. Amphibians and Reptiles of the West Indies. University of Florida Press, Gainesville, 720 pp.) and juvenile conspecifics (GP, unpubl. data). However, predation on lizards is uncommon, and feeding on *Hemidactylus* geckos is unreported. Hence, here we report *A. cristatellus* feeding on *H. mabouia* from the British Virgin Islands.

An adult male *A. cristatellus wileyae* (ca. 70 mm SVL) was observed capturing a juvenile *H. mabouia*, (ca. 25 mm SVL; Fig. 1) at 1000 h on 4 October 2004 on the side of a shed at the H Lavity Stout Community College, Paraquita Bay, Tortola (18°25'24.816N, 64°34'44.287W [datum: WGS84]; elev. < 1 m). When first discovered, the *Hemidactylus* was alive, writhing in the mouth of the *Anolis*. The anole proceeded to struggle with the gecko for the next 4 min, at which point the pair moved out of sight.

This observation expands the reported diet of *A. cristatellus*. *Hemidactylus mabouia* are generally nocturnal, whereas *A. cristatellus* are generally diurnal (but see Perry and Lazell 2000. Herpetol. Rev. 31:247). However, the shed pictured is a nocturnal foraging site for *H. mabouia*, which presumably also use it for shelter during the day, when *A. cristatellus* inhabit the same structure.

FIG. 1. *Anolis cristatellus wileyae* predation on *Hemidactylus mabouia*. British Virgin Islands. Photograph by Jennifer Owen.

Submitted by **JENNIFER OWEN** and **GAD PERRY**, Department of Range, Wildlife and Fisheries Management, Texas Tech University, Box 42125, Lubbock, Texas 79409-2125, USA; e-mail: gad.perry@ttu.edu.

The Influence of Temperature and Humidity on Activity Patterns of the Lizards *Anolis stratulus* and *Ameiva exsul* in the British Virgin Islands

KERRY L. NICHOLSON, SHANNON M. TORRENCE, DANA M. GHIOCA, JOYDEEP BHATTACHARJEE, ADRIAN E. ANDREI, JENNIFER OWEN, NIKKI JO A. RADKE, AND GAD PERRY. *Department of Range, Wildlife, and Fisheries Management, Box 42125, Texas Tech University, Lubbock, Texas 79409. Corresponding author: Gad Perry, email: gad.perry@ttu.edu*

ABSTRACT.—Many organisms modify their behavior to reduce exposure to unfavorable abiotic conditions, but detailed information is available for only a few species. We studied the diurnal activity patterns of *Anolis stratulus* and *Ameiva exsul* on Guana Island, British Virgin Islands, in order to determine how they are affected by temperature and humidity. We surveyed transects on foot between 0730 and

1700 h, scanned the ground and vegetation for visible lizards and recorded temperature and relative humidity. Lizard activity patterns were influenced by ambient conditions and body size. We found an inverse relationship between daily activity patterns and temperatures for juvenile *A. stratulus*; the cooler the temperature, the more juveniles were present. Adult *A. stratulus* did not show any significant correlations with temperature and time of day. Temperature and abundance were strongly positively correlated for *A. exsul*; the higher the temperature, the more abundant *A. exsul* became. Activity was strongly significantly correlated with humidity. Because temperature and relative humidity were significantly inversely correlated, we cannot identify which parameter most impacted lizard activity.

Thermoregulation is a primary activity of ectotherms. Many reptiles such as lizards act to reduce exposure to unfavorable abiotic conditions by simply moving out of direct sunlight or by increasing their water intake. Thus, understanding the relationship between abiotic conditions and activity patterns can be important in understanding the ecology of such animals. Here we report on the daily activity patterns of two lizard species in the British Virgin Islands (BVI), and the effects of temperature and humidity on those activity patterns. The two were chosen as representatives of the genera *Anolis* and *Ameiva* which constitute a large percentage of the diurnally active lizards in the West Indies.

Lizards have often served as model organisms in ecological studies (e.g., Milstead 1967; Vitt and Pianka 1994). Because of their abundance and visible nature, *Anolis* lizards have been especially extensively investigated (Roughgarden 1995; Reagan 1996). *Anolis stratulus* (adult mass approximately 1.7 g; Butler and Losos 2002) has received considerable attention, with studies ranging from habitat use (Dial et al. 1994) to molecular systematics (Jackman et al. 1999). *Ameiva exsul* (adult mass approximately 40 g; Lewis and Saliva 1987) is also a highly visible and well-studied species (Lewis and Saliva 1987). Both species are common on the islands of the Greater Puerto Rico Bank, including our study site,

Guana Island. Activity patterns and their correlates have not been previously reported for either species, although R. Powell and R.W. Henderson (unpublished data) observed that activity of *A. exsul* on Guana Island, BVI, primarily occurred between 0930 and 1400 hours.

Guana Island is a privately-owned wildlife sanctuary characterized by temperatures ranging from 27-33 °C, a relative humidity of 60-90% (Dmi'el et al. 1997), and an annual rainfall of about 900 mm (G. Perry, unpublished data). The island has an area of approximately 300 ha and a maximum elevation of 240 m (Lazell 1996, 2005). Additional details regarding the study island are in Lazell (1996, 2005).

To investigate the activity patterns of *A. stratulus* and *A. exsul*, we established twelve 50 m transects along existing trails during October 2003. A single observer repeatedly surveyed the entire length of each transect on foot between 0730 and 1700h. During each hourly pass, the observer would sample the same six stations, located 10 m apart. At each station, the observer examined the ground and vegetation for lizards, taking one minute to complete a 180° scan of the vegetation within a 2 m radius. The count was repeated five consecutive times at each station, for a total of 5 minutes at each location, and the number of lizards seen during the most productive one-minute count was recorded. Each transect required 30 min to complete, and a 30 min pause separated consecutive passes, resulting in 10 sampling cycles per day. Twelve daily samples were used in calculating the total numbers of animals observed.

Perry et al. (unpublished MS) reported that smaller body size makes individual lizards more prone to water loss in *Anolis cristatellus* and *A. exsul*. Based on this work, the authors predicted that water conservation requirements will force smaller lizards to be active during cooler and more humid times of day. They suggested that adults may be able to retain water more efficiently than juveniles, who have a larger surface-area-to-body-size ratio. Juveniles should therefore decrease activity during the hottest part of the day to avoid water loss, es-

pecially in water-restricted locations such as Guana. To test this prediction, we categorized *A. stratulus* into two size classes, juvenile and adult, based on lack of dewlap development in juveniles (G. Perry and K. LeVering, unpublished). Size classes of *A. exsul*, which is less common on Guana, were combined to obtain an adequate sample size. To avoid artifacts caused by aberrant climatic conditions such as heavy rainfall, counts were conducted only during days with climatic conditions conducive to lizard activity. We recorded ambient temperatures (°C) and relative humidities (%) at five minute intervals by placing a datalogger at the starting point of each transect. To avoid making assumptions about data distribution, we used Spearman's non-parametric test of correlation for all analyses of relationship between climactic conditions and lizard activity.

We recorded a total of 140 *A. stratulus* sightings during our surveys, of which 56 were adults and 84 juveniles. Both adult and juvenile *A. stratulus* showed variation in activity levels during the day. However, the activity levels of adults (Fig. 1A) were not significantly correlated with either temperature (Fig. 1D; rho = 0.534, N = 10, P = 0.112) or relative humidity (rho = −0.222, N = 10, P = 0.537). In contrast, juvenile ac-

FIG. 1. Numbers of adult (A) and juvenile (B) *Anolis stratulus* and all *Ameiva exsul* (C) recorded between 0730–1700 h on Guana Island, British Virgin Islands. Bars represent the combined totals from twelve transects. Ambient conditions (relative humidity and temperature) measured during a typical study day are presented in D. Adult *A. stratulus* showed similar activity levels throughout the day, whereas juveniles were less active during the hot and dry midday. In contrast, *A. exsul* are primarily active during midday.

tivity levels (Fig. 1B) were negatively related to temperature (rho = −0.684, N = 10, P = 0.029) and positively related to relative humidity (rho = 0.720, N = 10, P = 0.019). Temperature and relative humidity were also negatively correlated to one another (rho = 0.848, N = 10, P = 0.002), making it difficult to distinguish which factor lizard activity patterns were responding.

We encountered 43 *A. exsul* of all sizes, and their activity pattern was different than that observed in *Anolis*. Number of sightings was positively correlated with temperature for *A. exsul* (Fig. 1C; rho = 0.800; N = 10, P = 0.005), and negatively correlated with relative humidity (rho = −0.804; N = 10, P = 0.005). Few individual *Ameiva* were active during the cooler parts of the day and most observations occurred during the warmer, drier surveys. Activity peaked between 1430h and 1500h. R. Powell and R.W. Henderson (unpublished) report similar activity times in *A. exsul* from Guana. Similarly, activity in *Ameiva erythrocephala* begins at 0930, peaks midday, and ceases by 1600 h (Kerr et al. 2005). Thus, the activity pattern we describe, although not previously documented in detail, is not surprising.

Although both species are diurnal, *A. exsul* and *A. stratulus* are most active at different times of the day. The physiological differences between the two species may partially explain this patter: members of the genus *Anolis* are typically thermoconformers, whereas teiid genera such as *Ameiva* are normally heliothermic (Hertz 1992; Rivera-Vélez and Lewis 1994; Rogowitz 2003). However, this does not explain the intraspecific difference within *A. stratulus*. Juvenile *A. stratulus* are active during the coolest, most humid parts of the day, adult *A. stratulus* are active throughout the day, and the much larger *A. exsul* are preferentially active during the hottest part of the day. We believe that this difference is related to differences in body size and the effects they have on water loss rates (Perry et al. submitted). Our results thus support Perry's et al. (submitted) conclusion that smaller body size makes individual lizards more prone to water loss, and that this will affect their ecology, especially in water-
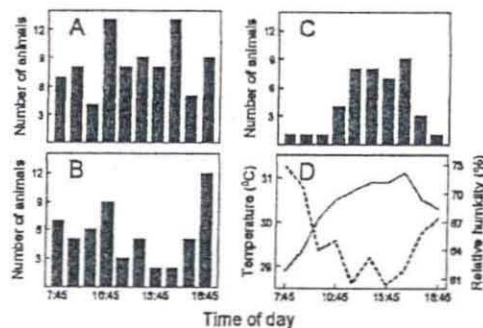
restricted locations such as Guana Island. Unfortunately, the need to lump data for *A. exsul* of different sizes may obscure size-related differences in activity patterns within that species as well. We predict that a larger sample size would show that adult *A. exsul* are active at warmer parts of the day than are juveniles. In fact, Kerr et al. (2005) cite studies in which bimodal activity patterns were seen in other *Ameiva*.

It has long been known that different species are active at different times of day, and that this has profound consequences for their ecology at both the autecological and community ecology level. The effects of body size at multiple levels are also well known (e.g., Perry and Garland 2002 for home range size). Our study demonstrates that the two factors interact in ecologically important ways. Further, it indicates that further research needs to consider this interaction when studying variability in activity patterns.

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The effects of body size on water loss in the lizards *Anolis cristatellus* and *Ameiva exsul*

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Running headline: Water loss in *Anolis* and *Ameiva*

ABSTRACT

As individuals grow their surface-to-volume ratios alter and whole-organism physiological traits such as water loss rates consequently change. However, the influence of allometry on reptilian water loss rates remains poorly studied. We used the lizards *Anolis cristatellus* and *Ameiva exsul* to test the hypothesis that water loss rates will also covary with body size in reptiles. Water is often a limiting factor on the Puerto Rico Bank, where these lizards live. We measured water loss rates and evaporative cooling and found a strong effect of body size. Larger animals lost more water per unit skin area, consequently showing a greater difference between skin and ambient temperature, and also lost less water per unit mass. In contrast, integumentary resistance to water loss did not covary with size.

Our findings demonstrate an advantage to large body size in lizards experiencing water shortage and high ambient temperatures. In hot climates, evaporative cooling may provide larger individuals and species with an additional advantage by allowing them to be active longer or use more exposed microhabitats. This suggests that ectotherms should display the opposite pattern to Bergman's Rule and evolve increased body size in warm climates, compared to colder habitats. Such a pattern has been previously reported but not explained.

Key Words: Allometry - British Virgin Islands - Reptiles

INTRODUCTION

As an organism ages, its morphology changes in ways that can have profound effects on whole-animal performance. Allometry describes the relationship between body mass and biological variables such as morphology or physiology (White and Seymour 2005). Although the importance of body size in large desert mammals has long been appreciated, its significance in other organisms and environments remains less well known. One well-documented area of effect is locomotor abilities, which vary greatly, at both the intra- and inter-specific levels, with body size and limb proportions (e.g., Garland 1985; Losos 1990; Irschick et al. 2000; Vanhooydonck et al. 2000). Another is mating success, which is linked to size both directly (i.e., larger individuals enjoy greater reproductive success: Fox 1983), and indirectly (i.e., size-determined locomotor abilities affect reproductive success: Garland et al. 1990; Robson and Miles 2000; Perry et al. 2004). The ratio of surface-to-volume tends to decrease as the animal grows, and individuals may consequently become less susceptible to physiological stresses imposed by the external environment, such as excessive heat, cold, and water loss (e.g., Schmidt-Nielsen 1964, Fig. 5; Mautz 1982a). Ecologically, juvenile lizards may use different microhabitats and are likely to eat smaller prey than do adults (reviewed in Vitt 2000), and small species often use especially protected habitats such as deep leaf-litter (e.g., Rodda et al. 2001; López-Ortiz and Lewis 2004). As these examples show, allometry can importantly affect the interactions of an organism with both its biotic and abiotic environment (Schmidt-Nielsen 1964; 1984).

The impetus for the present study was provided by previous work on lizard water loss conducted in the British Virgin Islands (BVI) and focusing on adults of *Anolis cristatellus* and the closely related *Anolis ernestwilliamsi* (Dmi'el et al. 1997; Perry et al. 1999; 2000). These studies showed that water loss rates vary geographically, in accordance with island aridity, and can further vary temporally in response to changes in rainfall patterns (Dmi'el et al. 1997; Perry et al.1999; 2000). They also suggested that body size is important in avoiding excessive water loss, something already well-established in mammals (e.g., Schmidt-Nielsen, 1964, Fig. 5) but poorly studied in poikilotherms (but see Dunson & Brahman 1981; Mautz 1982a; Dmi'el 1985). Dmi'el et al. (1997) pointed out that the relatively large body size of *A. ernestwilliamsi* allows it to survive on Carrot Rock, the most arid locality they studied. In a common garden study, Perry et al. (2000) documented that a rapid increase in ambient dryness stimulates a prompt increase in body size in *A. cristatellus*. If body size is important in mediating water relations in adult lizards, as these studies indicate, then ontogenetic changes in body size should be even more ecologically important.

The ecological importance and conspicuous behaviors of anoles make them excellent model systems for studying such questions (Trivers 1976; Perry 1996; Jenssen and Nunez 1998). Here we test the hypothesis that water loss rates will covary with body size in the lizard *A. cristatellus*. To examine the generality of this pattern we also test the same hypothesis in *Ameiva exsul*, a lizard that is phylogenetically distant from *A. cristatellus* and which inhabits the same islands but uses a very different microhabitat.

MATERIALS AND METHODS

Study site and species. Although located in the tropics, the British Virgin Islands show varying degrees of aridity (Dmi'el *et al.* 1997). All individuals included in this study were collected on Guana Island, BVI, between 1993 and 2002. Guana Island has an area of about 300 ha and reaches a maximum height of about 250 m. A private wildlife sanctuary, it has the richest fauna of any island of comparable size in the region (Lazell 1996, 2005).

Anolis cristatellus is a common trunk-ground anole found throughout the Greater Puerto Rico Bank, which includes the BVI (Lazell 1983; Schwartz and Henderson 1991). Adult males are highly aggressive and territorial (Perry et al. 2004) and reach a maximum mass of about 7 g (Fitch 1981). Females are much smaller, and only the males possess the large body and tail-crests that give the species its scientific name (Rivero 1978). Both sexes are highly sedentary foragers (Perry 1999). Considerable research has been conducted on this species, including several studies of water loss (Dmi'el et al. 1997; Perry et al. 1999; 2000). *Ameiva exsul* is an actively foraging (Perry 1999) ground-dwelling lizard reaching a maximum mass of over 100 g (Schwartz and Henderson 1991). Lewis (1986; 1989), Lewis and Saliva (1987), and Lewis et al. (2000) studied body size and some of its effects, but not water loss, in this species. However, Riveravelez and Lewis (1994) showed that these lizards actively elevate their body temperature to nearly 40 °C when thermoregulating, suggesting water conservation could be an issue in relatively xeric environments, especially for juveniles (typically 1-2 g).

Study animals were captured on Guana Island during normal activity times by noosing and brought into the laboratory. Following morphological (mass, snout-vent length [SVL]) and water loss measurements (see below), animals were released at the site of capture. Care was taken to ensure that our sample includes the entire range of sizes found in each species.

Water loss measurements. Our methods closely followed those of Dmi'el et al. (1997). Prior to testing, we housed lizards individually for 8-12 h. Food was not given, to reduce defecation during testing, but animals were provided with moistened paper during this period to allow them to regain water lost in transit. Experiments were conducted during normal activity times on the day following collection. Immediately prior to testing, we moved each lizard to a dry plastic box covered with small-mesh wire netting that permitted free exchange of air. Box dimensions were individually chosen to allow each individual some freedom of movement, but not so much that it continuously tried to escape during testing. Room air was continuously circulated by ceiling fans to prevent appearance of areas of stagnant air around test animals. We used a balance (Precisa 800M or Sartorius 1518) to monitor lizard mass change (measured ± 1 mg), assumed to mainly represent total evaporative water loss (EWL). Lizards were weighted at 60 min intervals over a period of 6-8 h. At the same time, we used a Psychro-Dyne psychrometer (Environmental Tectonics Corporation) to measure room relative humidity and a 36-gauge copper-constantan thermocouple connected to a Wescor TH-65 electronic thermometer to measure skin and room temperature. Animals that defecated during the experiment were immediately re-weighed and the study restarted. Lizard surface area was determined at the end of each session using the method of Dmi'el *et al.* (1997), which has proved superior to available alternatives.

To calculate integumentary resistance (R) we have previously used the equation CWL = $(eT_s - eT_a) / R$ (Lillywhite and Sanmartino 1993), where CWL is the cutaneous evaporative water loss per cm^2; eT_s and eT_a are the saturated water vapor density at skin temperature and the actual water vapor density in the air, respectively; and R is the combined resistances of the skin and the air boundary layer adjacent to it. However, separating CWL from the total evaporative water loss (EWL) is stressful to test animals. To minimize stress, we used the CWL values published for *A. cristatellus* by Dmi'el et al. (1997) and later shown to be consistent among populations and within populations between years (Perry et al. 1999). No such data exist for *Ameiva exsul*, and we limited ourselves to measuring total water loss for this species.

RESULTS

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Surface area was positively and significantly related to body size in both species (Figure 1a; *Anolis*: log(area) = -1.746 + 1.862 log(SVL), N = 60, r^2 = 0.964, p < 0.001; *Ameiva*: log(area) = -2.054 + 2.084 log(SVL), N = 13, r^2 = 0.989, p < 0.001; Pearson regressions). An ANCOVA revealed a small but statistically significant difference between the two species in the slope of the regression line (df = 1, F = 20.8, p < 0.001). As expected from a logarithmic relationship between a measure of area and a linear measurement [(SVL)], the slope was close to two in both species. However, although young anoles have similar surface areas to those of similar-sized *Ameivas*, increasing body size results in a lower increase in the surface area of *Anolis*. Mass was also positively and significantly related to body size in both species, and the relationship was not significantly different between species (Figure 1b; ANCOVA, SVL: df = 1, F = 2971, p < 0.001; species: df = 1, F = 0.564, p = 0.455; log(mass) = -4.520 + 2.996 log(SVL), N = 73, r^2 = 0.984, p < 0.001). Thus, despite belonging to distantly related families and having somewhat different body shapes, the masses of *Anolis* and *Ameiva* increase at a similar rate as body size increases. As expected from a logarithmic relationship between a measure of volume and a linear measurement, the slope of the regression line was close to three.

Although cutaneous water loss per unit skin area increased significantly with body size in *Anolis*, the predictive power of the relationship was low (Figure 2 ; N = 60, log(EWL) = -1.091 + 0.286 log(SVL), r^2 = 0.112, p = 0.009). We found no relationship between body size and surface-specific water loss rates in *Ameiva* (N = 13, r^2 = 0.140, p = 0.208). The difference between the two species was highly significant (ANCOVA; df =1, F = 2542, p < 0.001), with *Ameiva* losing much more water per unit surface area than did *Anolis*. The difference between skin and ambient temperature, a measure of water loss rates and the resulting cooling of the skin surface, was positively and significantly related to body size in both species (Figure 3; *Anolis*: log(ΔT) = -2.085 + 0.732 log(SVL), N = 58, r^2 = 0.147, p = 0.003; *Ameiva*: log(ΔT) = -1.758 + 0.479 log(SVL), N = 13, r^2 = 0.488, p = 0.008). The difference between the two species, although quantitatively small, was also statistically significant (ANCOVA; df =1, F = 31.457, p > 0.001), with cooling of *Anolis* skins increasing more rapidly with size than did that of *Ameiva*.

Mass-corrected water loss rates decreased significantly in both species as animals grew larger (Figure 4; *Anolis*: log(EWL) = 1.696 – 0.770 log(SVL), r^2=0.395, p < 0.001; *Ameiva*: log(EWL) = 1.823 – 0.758 log(SVL), r^2=0.649, p = 0.001). The difference between the two species was also significant (ANCOVA; df =1, F = 7.727, p = 0.007), with *Ameiva* losing more water at any given mass than did *Anolis*. Integumentary resistance to water loss (R) could only be computed for *A. cristatellus*, for which the relative importance of cutaneous and respiratory avenues of loss had been previously evaluated (see above). R was not significantly correlated with SVL in that species (Figure 5; N = 60, r = -0.18, p = 0.168), indicating that integumentary resistance to water loss does not change as anoles grow larger.

DISCUSSION

Not surprisingly, the relationship between body length [(SVL)] and mass was similar in *Anolis*, *Ameiva* (present study), and lizards in general (Pough 1973). Perry (1989) similarly noted that the relationship of SVL to mass in five lacertids was similar to the general equation for lizards provided by Pough (1973), and hypothesized that an overall similarity in body shape among lizards was responsible. However, because their body shapes are different (triangular cross-section in *Anolis*, compared to rectangular in *Ameiva*), it is also not surprising that *Anolis* and *Ameiva* slightly differ in how skin surface area increases as animals grow larger. In contrast, the

relationship between surface area and mass in five xantusiid lizards was similar to one another and to those for iguanid lizards published earlier (Mautz 1980).

That proportions change with ontogeny and body size, and that those changes have profound functional impacts, are well established generalities (e.g., Schmidt-Nielsen 1964;1984; Bookstein et al. 1985) that are also seen in reptiles (e.g., Dmi'el 1985). With the exception of mammals (mostly domestic species have been sampled to date), the effects of body size on water loss rate remain poorly studied, and body size is often treated as a "nuisance variable" in studies of water loss (e.g., Mautz 1982a; 1982b; Dmi'el et al 1997; Neilson 2002; for a review in insects see Chown et al. 2002; but see Dunson and Brahman1981; Dmi'el 1985). In this study we documented the importance of body size on water loss in two distantly-related lizard species inhabiting different microhabitats within a mesic tropical ecosystem. As expected from work on mammals (Schmidt-Nielsen 1964) and reptiles (Mautz 1982a; Dmi'el 1985), body size and water loss rates typically covaried in both species. In *Anolis*, but not in *Ameiva*, water loss per unit skin area increased significantly with body size. As expected on theoretical grounds (Mautz 1982a), per-mass loss significantly decreased with size in both species. At the same time, the difference in temperature between the lizard's skin and the surrounding air increased significantly as body size increased. Dmi'el (1985) reported a similar situation in *Spalerosophis diadema*, a desert snake weighing up to 1 kg. This suggests that the lack of a significant relationship between body size and area-specific water loss rate in *Ameiva* may be an artifact of small sample size, rather than a biological reality. Alternatively, the observed difference may reflect changes in the resistance of the boundary layer surrounding the animal (Ra), which we did not separately measure. Unlike skin resistance (Rs), Ra is significantly positively correlated with body size (Eynan and Dmi'el 1993; Lahav and Dmi'el 1996). However, the contribution of Ra to total resistance (R) has been shown to be very small, no more that 2-3% of the total (Eynan and Dmi'el 1993; Lahav and Dmi'el 1996), making this unlikely.

Our findings demonstrate the importance of body size in determining water loss rates in lizards. Larger animals lose more water per unit surface area than do small ones. The ecologically important measure, however, is water lost per unit mass, as this reflects how much water an animal can afford to lose before becoming dangerously dehydrated (e.g., Perry et al. 2000). The most obvious impact of body size in both species we studied is that larger individuals lose less water per unit mass, and are thus able to withstand conditions that would be prohibitively dehydrating for a smaller animal. This is true in both homeotherms (Schmidt-Nielsen 1964) and at least some poikilotherms (various reptiles: Mautz, 1982a; frogs: Navas et al. 2002; snakes: Dmi'el 1985; lizards: present study). However, it is difficult to compare our intraspecific results to the interspecific equation developed by Mautz (1982a), because the latter does not take into account phylogenetic relationships and thus suffers from phylogenetic pseudoreplication (see Garland 1997). Unlike the impact of size on area-specific water loss, the biological significance of the large difference in size-corrected water loss rates between the two species is hard to evaluate. As Gans (1989) and Garland and Adolph (1994) point out, comparisons based on two species offer severely limited inference about adaptation. Establishing whether the difference is a function of microhabitat, phylogeny, or some combination of these and/or additional parameters must await an explicitly phylogenetic analysis of multiple species that use a variety of habitats and microhabitats. These data can be used, however, to enhance our understanding of the importance of physiological and evolutionary adaptation and of phylogenetic conservatism in physiological traits (for comparison of species from different climactic regions see Dmi'el 2001)

Integumentary resistance to water loss (R), which combines the resistance of the skin and that of the boundary layer surrounding it, is less sensitive to changes in immediate environment than are EWL or CWL. R is therefore a reliable measure of actual impedance to water loss (Eynan and Dmi'el 1993). In contrast to Dmi'el et al. (1997) and Perry et al. (1999; 2000), who showed differences in R among island populations of *A. cristatellus*, we found no significant changes in intrapopulational integumentary resistance as a function of anole body size. Instead, lizards showed a wide range of R values at each body size. Dmi'el (1985) similarly found no effect of body size on R in the desert snake *Spalerosophis diadema*, and Perry et al. (1999; 2000) reported that lizards from wet islands always lost more water than did ones from dry localities. Combined, these observations suggest the existence of a genetically determined, population-specific integumentary resistance. This, in turn, shows individual variation that is determined by genetic, environmental, or combined factors. Long-term changes in R may primarily result from differential mortality and reproductive success, as suggested by Perry et al. (2000). Short-term changes, however, may primarily reflect behavioral changes, such as the increase in feeding and mass described by Perry et al. (2000) for wet-habitat lizards kept under considerably drier conditions. However, more detailed studies on the mechanistic processes underlying integumentary resistance are required, and further studies on intra-island variation may help determine the level of phenotypic plasticity involved.

As expected, changes in surface-to-volume ratio occur as body size increases, and the amount of water lost per unit surface area increases as animals grow, even though the loss per unit mass decreases. The greater degree of water loss in large individuals results in a previously unreported higher surface cooling and an increased temperature differential between the skin and environment in both species. We have been unable to locate previous work exploring this relationship, although Dawson and Templeton (1963, their Figure 5) found that water loss in the lizard *Crotaphytus collaris* increases rapidly once body temperature exceeds 40°C. Although they did not explicitly consider this a mechanism for cooling, they did note that "this acceleration starts a couple of degrees below the body temperature at which collared lizards begin panting." Dawson and Templeton (1963) concluded that water loss through panting "may lengthen slightly the duration of the period over which this lizard can remain abroad." We did not observe panting, and water loss in our study was primarily via the skin. Our observations suggest an unexpected ecological benefit of cutaneous water loss: larger animals should be able to maintain their body temperatures within preferred parameters under high-temperature regimes that smaller animals might not be able to withstand. Interestingly, DeNardo et al. (2004) recently showed that Gila monsters (*Heloderma suspectum*, approximately 400-700 g) can dramatically increase cloacal water loss rates at high ambient temperatures, that this increase helps reduce body temperature, and that dehydration causes this response to abate. We agree with DeNardo et al. (2004) that, in addition to sometimes being a source of water loss and potential stress, evaporation in desert reptiles may also serve as an important means of controlling body temperature and enhancing activity. The crucial element is availability of sufficient water reserves, or a high enough return to the additional activity time, to allow such additional water loss. Although the magnitude of evaporative cooling may not be large, the common perception of reptiles as mostly lacking physiological mechanisms for controlling body temperatures (e.g., Schmidt-Nielsen 1964) may need to be reexamined.

Varanid lizards and recent work on the genus *Liolaemus* (Espinoza et al. 2004) offer notable exceptions to the observation of Pough (1973) that large body size in lizards is primarily associated with herbivory. Our findings offer an alternative (but not necessarily mutually

exclusive) explanation for the evolution of large body size in lizards. This explanation produces a testable prediction: that large species, regardless of diet, will typically be found in warm climates. Indeed, large lizard species such as monitor lizards (genus *Varanus*) and iguanas (e.g., *Iguana*, *Cyclura*) often inhabit warm climates but are uncommon in cold environments, where additional cooling could be detrimental. Because most truly large lizard species belong to only two families (Iguanidae and Varanidae), this issue needs to be tested within a strictly phylogenetic context.

In homeotherms, Bergman's rule is used to explain, in physiological terms related to surface-to-volume ratio, why cold climates are inhabited by larger animals (Schmidt-Nielsen, 1964). This rule does not apply to poikilotherms, which in fact show an opposite trend for reasons which are less clear (reviewed in Ashton 2004). Our findings suggest the physiological cost of being active in exposed locations should become lower as individuals grow, and indicate that a cooling effect can help decrease the risk of overheating when large animals occupy an exposed perch. Our hypothesis can thus also be tested at the intraspecific level. We predict that in warm environments, smaller individuals will be less likely to be active during the heat of the day and during dry periods than would be larger animals. Much work remains to be done in this area, and we have only begun testing these hypotheses.

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12/13/2005

Review

Water relations of tetrapod integument

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Summary

The vertebrate integument represents an evolutionary compromise between the needs for mechanical protection and those of sensing the environment and regulating the exchange of materials and energy. Fibrous keratins evolved as a means of strengthening the integument while *simultaneously providing a structural support for lipids*, which comprise the principal barrier to cutaneous water efflux in terrestrial taxa. Whereas lipids are of fundamental importance to water barriers, the efficacy of these barriers depends in many cases on structural features that enhance or maintain the integrity of function. Amphibians are exceptional among tetrapods in having very little keratin and a thin stratum corneum. Thus, effective lipid barriers that are present in some *specialized anurans living in xeric habitats are external to* the epidermis, whereas lipid barriers of amniotes exist as a lipid–keratin complex within the stratum corneum. Amphibians prevent desiccation of the epidermis and underlying tissues either by evaporating water from a superficial aqueous film, which must be replenished, or by shielding the stratum corneum with superficial lipids.

Water barrier function in vertebrates generally appears to be relatively fixed, although various species have 'plasticity' to adjust the barrier effectiveness facultatively. While it is clear that both phenotypic plasticity and genetic adaptation can account for covariation between *environment and skin resistance to water efflux, studies of* the relative importance of these two phenomena are few. Fundamental mechanisms for adjusting the skin water barrier include changes in barrier thickness, composition and physicochemical properties of cutaneous lipids, and/or geometry of the barrier within the epidermis. While cutaneous lipids have been studied extensively in the contexts of disease and cosmetics, relatively little is known about the processes of permeability barrier ontogenesis *related to adaptation and environment. Advances in such* knowledge have didactic significance for understanding vertebrate evolution as well as practical application to clinical dermatology.

Key words: skin, vertebrate, permeability, lipids, evaporative water loss, phenotypic plasticity.

Introduction

One of the dilemmas in the evolution of multicellular organization was how increasingly larger organisms might continue to sense, interact and exchange with their environment while simultaneously achieving protection from the mechanical, radiative and chemical hazards with which they came into contact. Hence, the integument evolved as a heterogeneous, pluristratified, multimembrane system providing physical protection while simultaneously regulating the exchange of energy and materials between the organism and its surroundings (Chuong et al., 2002). An impressive array of body coverings evolved, with collectively diverse mechanical properties, appendages and multiple diffusion pathways, but with rate-limiting barriers for specific molecules. While integumentary coverings are highly variable in their morphology, they nonetheless exhibit common themes

of adaptation. An important aspect of vertebrate evolution was the orchestration of characters that rendered the skin increasingly less permeable to water as forms invaded and adapted to harsher and drier terrestrial environments.

Understanding water relations of vertebrate skin has been advanced by empirical knowledge and by perspectives based on several sources. These include descriptive studies of morphology, physiological studies of specific transport mechanisms and phenomena, physicochemical and physiological studies of integumentary lipids (thought to comprise the principal barrier to transepidermal water movement) and, generally, comparative investigations related to quantitative measures of water flux across the skin of animals adapted to specific environments. Holistic understanding of vertebrate adaptation to terrestrial environments requires considerable appreciation for the

integration of characters that determine the water relations of integument *per se*, as well as the water budget for the whole organism.

The integument is a complex structure comprised of epithelial cells derived from embryonic ectoderm and attached to an underlying fibrous and vascular dermis originating from mesoderm (Fig. 1). Epidermal–dermal interactions are critical for differentiation and specialization of cells. Mitotic activity takes place in the basal layer (stratum germinativum), which proliferates cells that are eventually lost from the animal's surface. All cell types that are present in the epidermis are represented by precursors in the basal layer. Localized cellular proliferation and differentiation produce appendages such as claws, glands, hair or feathers, which together with patterned folds or scales, contribute to integumentary form. Cellular renewal is a further characteristic of vertebrate skin and serves to maintain the integrity of structure and its various functions.

The earliest protovertebrate was likely a small, soft-bodied animal (Berrill, 1955), separated by millions of years from the radiation of early Paleozoic fishes. Subsequent trends to increasing body size required mechanical strengthening of the body surfaces, which, in turn, led to reduced mobility or to serial, patterned folding and the evolution of scales (Maderson, 1972). Patterns of ossified units suggest that Paleozoic amphibians possessed a scaled integument (Cobert, 1955; Kitching, 1957). Thus, it seems probable that the integuments of most modern vertebrates are derived secondarily from ancestral conditions of integument that were in some sense 'scaled' (Maderson, 1972, 2003; Maderson and Alibardi, 2000). Subsequent evolution of appendages such as hair or feathers is best understood by relating them to developmental fields, originally subdivided by embryonic epidermal–dermal cell populations reflecting a scaled integument of the adult (Maderson, 1972; Maderson and Alibardi, 2000). But scales *per se do not provide strength of structure*, nor, as commonly misunderstood, do they provide an effective waterproofing function (Licht and Bennett, 1972; Bennett and Licht, 1975; Roberts and Lillywhite, 1980). Total or near absence of scales is a derived character in many amphibians, mammals and birds (Maderson and Alibardi, 2000).

Keratin is a prevalent structural feature (Table 1) and is formed in epidermis of all living vertebrates by regulated processes involving synthesis of keratin filaments and cellular death. The synthesis of keratin polypeptides is known from epidermal cells of lampreys 450 million years ago (McLaughlin and Dayhoff, 1969). Subsequent evolution involved variable expression of genes to produce specific keratins and keratinized structures, as well as appropriate protective substances besides keratin. For example,
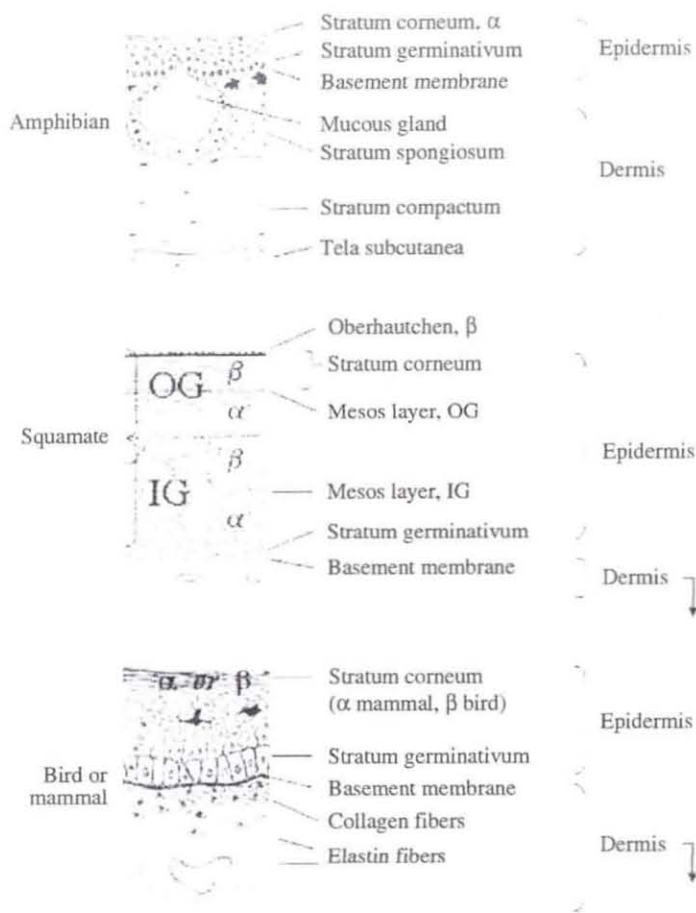

Fig. 1. Generalized features of integument in four major clades of vertebrates. α and β indicate alpha- and beta-type keratins, respectively. OG and IG indicate outer and inner epidermal generations that might be present in a squamate reptile prior to skin shedding. The mesos layer of squamates is a specialized cell type derived from α keratins and contains extracellular, laminated lipids, comprising the principal water barrier in these species.

cyclostomes, most osteichthyans, lissamphibians, and many larval or adult amphibians are protected principally by mucus (Maderson and Alibardi, 2000), and thus the body is covered by a largely non-keratinizing epidermis that suffices for aquatic life (e.g. Mittal and Banerjee, 1980). Additional mechanical protection is provided by a cytoskeleton and a terminal web of keratin filaments in superficial cells. During vertebrate evolution, elaboration of the keratinization process allowed adaptation to amphibious and eventually fully terrestrial life. Protection and movement on terrestrial substrates was facilitated by highly resistant cell envelopes encasing stabilized keratin frameworks in superficial cells. The formation of a corneous cell envelope is absent in fishes but occurs in epidermis of adult amphibians and forms in the periderm and first embryonic layers of amniotes, suggesting a primary role for terrestrial survival (Alibardi, 2003). Formation of a stratum corneum over the body surfaces

evolved in adult amphibians about 350 million years ago (Matoltsy and Bereiter-Hahn, 1986).

Mucus became associated with the filament matrix and was also discharged to fill the extracellular spaces of the stratum corneum. However, the requirement for an efficient permeability barrier to reduce significantly the exchange of water between the body and environment was met by production of neutral and polar lipids as well as mucus to become components of the cellular matrix and intercellular spaces in differentiating epidermal cells. The participation of lipids to form a competent water barrier likely began in reptiles about 300 million years ago (Matoltsy and Bereiter-Hahn, 1986) and has evolved independently in at least four principal lineages: reptiles, birds, mammals (Maderson, 2003; Maderson and Alibardi, 2000; Menon and Menon, 2000) and amphibians (Lillywhite and Mittal, 1999). The composition of Golgi-derived organelles related to barrier function changed during evolution from mucus and glycoproteins (fishes, amphibians) to glycolipids and lipids (amniotes; see below).

Most functions of the epidermis can be considered protective or defensive in all vertebrates. The majority of these functions reside in the stratum corneum, and of these, the protective function of the water permeability barrier is paramount (Chuong et al., 2002). If mammalian skin is damaged or diseased, cutaneous water loss can increase by several orders of magnitude. The rate-limiting process of water diffusion across the epidermis normally resides in the stratum corneum. Accordingly, many cutaneous phenomena, including immune responses, inflammation and hyperplasia, are recruited directly or secondarily to maintain a competent permeability barrier. Thus, mechanisms that normalize the stratum corneum and competent barrier function can abrogate susceptibility to diseases such as psoriasis, atopic and contact dermatitis, which are triggered, sustained or exacerbated by external perturbations (Chuong et al., 2002).

Transepidermal water permeation

Fundamentally, the integument of vertebrates consists of a large number of cell membranes organized essentially in stratified series with heterogeneous structure and variable resistances to movement of water molecules in both intracellular and extracellular pathways. While there is good understanding of permeation processes in membranes generally, the complexity and diversity of vertebrate skin greatly complicates a generalized analysis. Transepidermal water loss (TEWL) (and cutaneous water loss, CWL), is a passive diffusion process that is not dependent on cellular metabolic energy (Pinnagoda, 1994; Wilson and Maibach, 1994; Chuong et al., 2002). Therefore, limitations are expected to relate to tractable morphological features. Key questions are: (1) what are the pathways of molecular movement across the skin, and (2) which steps in the permeation process are rate-limiting?

Limitations for water transport through the skin can be related to movement through cell membranes and cytosol of cells, diffusion in the cutaneous matrix between the membranes, and passage of molecules across the membrane/environment interface, neglecting unstirred layers or secreted substances at the skin surface (see Feder and Pinder, 1988). Attributes of integument affecting water movement contribute to resistance of the skin to water passage (R_s), and this property combines with resistance of any external boundary layer (R_{bl}) to determine the actual rates of TEWL to ambient air in terrestrial animals. Measurements of R_s (in s cm^{-1}) range from negligible (near zero) in various aquatic or terrestrial amphibians to several hundred in xerophilic arboreal or cocooned frogs, to over a thousand in desert reptiles, with more moderate values (10–300 s cm^{-1}) being characteristic of birds and mammals (Table 2). Most measurements of boundary layer resistance are near zero to a few s cm^{-1} and thus constitute a negligible fraction of the whole-body R_s in most cases.

The ease of water movement through cell membranes can vary by orders of magnitude depending on the composition and physical state of the structure (Finkelstein, 1978; Carruthers and Melchior, 1983). Membranes that are in a liquid–crystalline state are far less permeable than are those that are fluid. In general, the relative diffusivity of molecules through cutaneous membranes will depend on their solubility

Table 1. *Properties of vertebrate integument that are relevant to integrity of the water permeability barrier. Features apply to the majority of species that have been examined in the listed taxa*

Taxon	General morphology	Keratin	Lamellar granules	Barrier location	Lipid–keratin complex	Facultative
Amphibians	Unscaled, glandular and pliant	α, few layers	Absent	Superficial	No; cocoons?	Yes
Reptiles						
Lepidosaurian	Scaled	α and β multiple layers	Present	Stratum corneum	Yes	Some spp.
Other	Scaled	α and β multiple layers	Present	?	?	?
Birds	Scaled or sculptured with plumage	α and β multiple layers	Present	Stratum corneum	Yes	Some spp.
Mammals	Scaled or sculptured with pelage	α, multiple layers	Present	Stratum corneum	Yes	Some spp.

Table 2. *Mean values of skin resistance to evaporative water loss (R_s) reported for various vertebrate species*

Taxon	Habitat	Resistance (s cm^{-1})	Source
Amphibia: Bufonidae			
Bufo cognatus	Xeric terrestrial–fossorial	1.5–5	1, 2
B. marinus	Mesic terrestrial	1.7	3
B. terrestris	Mesic terrestrial	~0	2
B. valliceps	Mesic terrestrial	~0	5
Amphibia: Hylidae			
Agalychnis annae	Mesic arboreal	10	2
A. callidryas	Mesic arboreal	2.8	1
Cyclorana australis (non-cocooned)	Fossorial–terrestrial	2.4	4
C. australis (cocooned, 3 weeks–6 months)	Fossorial–terrestrial	60–214	4
C. australis	Fossorial–terrestrial	5.4	3
C. longipes	Fossorial–terrestrial	3.5	3
C. maini	Fossorial–terrestrial	2.1	6
C. maini (cocooned, 1–5 months)	Fossorial–terrestrial	114	6
C. maini (cocoon only)	Fossorial–terrestrial	198	6
C. novaehollandiae	Fossorial–terrestrial	4.6	6
C. novaehollandiae (cocooned, 1–5 months)	Fossorial–terrestrial	37	6
C. platycephala	Fossorial	1.8	6
C. platycephala (cocooned, 1–5 months)	Fossorial	41	6
C. platycephala (cocoon only)	Fossorial	67	6
Hyla arenicolor	Terrestrial–riparian	4.2	1
H. cinerea (variable RH)	Mesic arboreal	1.7–3.6	2, 5
H. crucifer	Mesic terrestrial	1.4	2
H. gratiosa	Terrestrial–arboreal	2.2	2
H. gratiosa	Terrestrial–arboreal	3.3	1
H. triangulum	Mesic arboreal	2.2	2
Litoria adelaidensis	Mesic semi-arboreal	6.5	6
L. alboguttatus (= *Cyclorana alboguttatus*) (hydrated *vs* cocooned)	Terrestrial–fossorial	3.1–89.4	7
L. alboguttatus (=*Cyclorana alboguttatus*) (cocoon only)	Terrestrial–fossorial	89*	6
L. aurea (25–35°C)	Semi-aquatic	3.5–6.9	8
L. bicolor (wet season *vs* dry season)	Arboreal	47.6–79.7	3
L. caerulea	Arboreal	1.8	1
L. caerulea	Arboreal	~6	4
L. caerulea (25–50°C air temperatures)	Arboreal	2.9–8.5	9
L. caerulea (wet season *vs* dry season)	Arboreal	13.2–15.2	3
L. chloris (25–50°C air temperatures)	Mesic arboreal	2.5–29.6	9
L. coplandi	Terrestrial	9.6	3
L. dahlia (wet season *vs* dry season)	Aquatic	2.3–2.4	3
L. gilleni	Arboreal	11.5	3
L. gracilenta	Mesic arboreal	118	1
L. inermis	Terrestrial	1.4	3
L. meiriana	Semi-aquatic	0.6	3
L. microbelos	Terrestrial	~0	3
L. moorei	Mesic semi-aquatic	0.5	6
L. nasuta (wet season *vs* dry season)	Terrestrial	4.4–4.9	3
L. pallida (wet season *vs* dry season)	Terrestrial	5.4–6.6	3
L. peroni (25–40°C)	Arboreal–terrestrial	8.7–9.7	10
L. rothi (wet season *vs* dry season)	Arboreal	15.8–16.4	3
L. rubella (variable postures)	Arboreal	1.1–7.3	11
L. rubella (wet season *vs* dry season)	Arboreal	10.5–14.2	3
L. splendida	Arboreal	10.2	3
L. tornieri (wet season *vs* dry season)	Terrestrial	3.5–3.9	3
L. wotjulumiensis (wet season *vs* dry season)	Terrestrial	7.9–8.6	3
L. xanthomera (25–43°C air temperatures)	Mesic arboreal	32—8	12
Osteopilus dominicensis	Mesic arboreal	1.8	2

Table continued on next page.

Table 2. *Continued*

Taxon	Habitat	Resistance ($s\ cm^{-1}$)	Source
Amphibia: Hylidae			
O. septentrionalis	Mesic arboreal	1.4–1.9	1, 2
Pachymedusa dacnicolor	Semi-arid arboreal	14	2
Phyllomedusa azurae	Xeric arboreal	242	1
P. hypochondrialis	Xeric arboreal	364	2
P. iherengi	Xeric arboreal	277	2
P. pailoma	Xeric arboreal	336	2
P. sauvagei	Xeric arboreal	206	2
P. sauvagei	Xeric arboreal	300*	13
Pseudacris regilla (=*Hyla regilla*) (flowing *vs* still air)	semi-arboreal	2–15	13
Pternohyla fodiens (cocoon only)	Fossorial–terrestrial	414*	6
P. fodiens (cocooned)	Fossorial–terrestrial	457*	13
Amphibia: Hyperoliidae			
Afrixalus fornasinii	Arboreal	4.8	1
A. pygmaeus	Semi-arboreal	5.5	1
A. quadrivittatus	Semi-arboreal	3.2	1
Hyperolius argus	Semi-arboreal	24.5	1
H. kivuensis	Semi-arboreal	73.5	1
H. nasutus (still *vs* flowing air)	Semi-arboreal	96–257	13
H. pusillus	Semi-arboreal	34.2	1
H. tuberilinguis	Semi-arboreal	29.2	1
Leptopelis bocagei (cocooned)	Fossorial	40*	13
Amphibia: Leptodactylidae			
Lepidobatrachus llanensis (cocooned)	Terrestrial–fossorial	163*	13
L. llanensis (cocoon only)	Terrestrial–fossorial	116*	6
Amphibia: Microhylidae			
Austrochaperina adelphe	Terrestrial	0.1	3
Amphibia: Myobatrachidae			
Crinia bilingua	Terrestrial	0.1	3
C. georgiana	Mesic terrestrial	1.4	6
C. pseudinsignifera	Mesic terrestrial	2.7	6
Heleioporus albopunctatus	Mesic fossorial	1.6	6
H. eyrei	Mesic fossorial	2.5	6
H. inornatus	Semi-aquatic	2.2	6
Limnodynastes convexiusculus	Fossorial	2.7	3
L. fletcheri	Mesic terrestrial	1.5	10
L. ornatus	Fossorial	2.2	6
L. ornatus	Fossorial	3.1	3
L. spenceri	Fossorial	1.4–2.8	6
Neobatrachus aquilonius	Terrestrial–fossorial	0.8	6
Neobatrachus aquilonius (cocooned, 1–5 months)	Terrestrial–fossorial	32	6
N. centralis	Terrestrial–fossorial	2.2	6
N. centralis (cocooned, 1–5 months)	Terrestrial–fossorial	57	6
N. fulvus	Terrestrial–fossorial	2.7	6
N. fulvus (cocooned, 1–5 months)	Terrestrial–fossorial	59	6
N. kunapalari	Terrestrial–fossorial	2.2	6
N. kunapalari (cocooned, 1–5 months)	Terrestrial–fossorial	92	6
N. kunapalari (cocoon)	Terrestrial–fossorial	92	6
N. pelobatoides	Terrestrial–fossorial	1.1	6
N. pelobatoides (cocoon or cocooned, 1–5 months)	Fossorial	41	6
N. sutor	Fossorial	1.1	6
N. sutor (cocooned, 1–5 months)	Fossorial	93	6
N. sutor (cocoon only)	Fossorial	107	6

Table continued on next page.

Table 2. *Continued*

Taxon	Habitat	Resistance (s cm^{-1})	Source
Amphibia: Myobatrachidae			
N. wilsmorei	Fossorial	2.1	6
N. wilsmorei (cocooned, 1–5 months)	Fossorial	82	6
Notaden melanoscaphus	Fossorial	2.0	3
N. nichollsi	Xeric fossorial	2.1	6
Pseudophryne guentheri	Mesic terrestrial	1.7	6
Uperoleia lithomoda	Fossorial	~0	3
U. russelli	Terrestrial	1.7	6
U. sp. nov.	Fossorial	~0	3
Amphibia: Pelobatidae			
Scaphiopus couchii	Xeric fossorial	5	2
S. holbrooki	Mesic fossorial	~0	2
Amphibia: Plethodontidae			
Desmognathus ochrophaeus	Mesic terrestrial	0.09	14
Amphibia: Ranidae			
Pyxicephalus adspersus (cocoon only)	Terrestrial–fossorial	175*	6
P. adspersus (cocooned)	Terrestrial–fossorial	190*	13
Rana catesbeiana	Semi-aquatic	~0	2
R. pipiens	Mesic, semi-aquatic	0.05	14
R. pipiens	Mesic, semi-aquatic	1.6	1
R. temporaria	Mesic, semi-aquatic	3	2
Amphibia: Rhacophoridae			
Chiromantis petersi	Xeric arboreal	347	1
C. rufescens	Mesic arboreal	404	1
C. spp.	Xeric arboreal	400–900*	13
Polypedates maculatus	Semi-arboreal	1.9	15
Rhacophorus leucomystax	Mesic arboreal	14	2
Reptilia: Acrochordidae			
Acrochordus granulatus	Aquatic, marine	116	16
Reptilia: Agamidae			
Amphibolurus ornatus	Xeric terrestrial	521*	13
A. pallida (25–35°C air temperatures)	Xeric terrestrial	~175–~660	17
Agama sinaita (25–35°C air temperatures)	Xeric terrestrial	~180–738	17
A. savignii (25–35°C air temperatures)	Semi-arid terrestrial	~230–~410	17
A. stellio (5–35°C air temperatures)	Mesic and semi-arid terrestrial	~234–~270	17
Reptilia: Crocodylidae			
Alligator mississippiensis (20–30°C)	Mesic, amphibious	43.3–64	18
Reptilia: Colubridae			
Coluber rubriceps (25–34°C air temperatures)	Mesic terrestrial	~300–~380	19
Elaphe obsoleta (shed epidermis)	Terrestrial	700*	20
Lampropeltis getula (newborn)	Mesic–xeric terrestrial	866	21
Natrix tessellata (25–34°C air temperatures)	Semi-aquatic	117–~190	19
Nerodia rhombifera (shed epidermis)	Semi-aquatic	314*	20
N. sipedon (shed epidermis)	Semi-aquatic	308*	20
Psammophis schokari (25–34°C air temperatures)	Mesic semi-arboreal	~480–~540	19
Psammophis schokari (25–34°C air temperatures)	Xeric semi-arboreal	~700–1088	19
Spalerosophis diadema (25–34°C air temperatures)	Xeric terrestrial	~190–~260	19
Reptilia: Emydidae			
Terrapene carolina	Mesic terrestrial	78	14
Reptilia: Gekkonidae			
Gehyra variegata	Xeric terrestrial	198*	13

Table continued on next page.

Table 2. *Continued*

Taxon	Habitat	Resistance (s cm^{-1})	Source
Reptilia: Iguanidae			
Anolis carolinensis	Mesic arboreal	196	14
A. carolinensis (humid vs dry environment)	Mesic arboreal	~196–296	22
A. cristatellus (8 insular populations)	Mesic–xeric arboreal	29-199	23
A. ernestwilliamsi	Xeric arboreal	168	23
Iguana iguana	Mesic terrestrial	370*	13
I. iguana (shed epidermis)	Mesic terrestrial	108.3*	20
Sauromalus obesus	Xeric terrestrial	1360*	13
Uta stansburiana	Semi-arid, xeric	1080*	13
Reptilia: Scincidae			
Sphenomorphus labillardieri	Mesic terrestrial	248*	13
Reptilia: Trionychidae			
Trionyx spiniferus (15–35°C)	Aquatic	2.98–5.38	24
Reptilia: Viperidae			
Aspis cerastes (shed epidermis)	Xeric terrestrial	788*	20
Cerastes cerastes (25–35°C air temperatures)	Xeric terrestrial	~1200—1400	25
Crotalus atrox	Xeric terrestrial	1011	16
Echis colorata (25–35°C air temperatures)	Xeric terrestrial	~1300—1550	25
Pseudocerastes persicus (25–35°C air temperatures)	Xeric terrestrial	1463–1921	25
Vipera palaestinae (25–35°C air temperatures)	Mesic terrestrial	706–878	25
Reptilia: Xantusiidae			
Klauberina riversiana (15–35°C)	Semi-arid	1020–3080	26
Lepidophyma gaigeae (20–30°C)	Mesic	660–1430	26
Lepidophyma smithii (25–35°C)	Mesic	190–320	26
Xantusia vigilis (25–30°C)	Xeric terrestrial	2150–3310	26
X. henshawi (20–30°C)	Xeric terrestrial	1120–1510	26
Aves: Anatidae			
Anas platyrhinchos (30–35°C air temperatures)	Semi-aquatic	38.5–60.8	27
Aves: Caprimulgidae			
Phalaenoptilus nuttallii	Xeric	125*	27
Aves: Columbidae			
Columba livia (20–52°C air temperatures)	Widespread	9.4–99.8	28
C. livia (30–40°C air temperatures)	Widespread	45–130	27
Streptopelia decaocto (20–52°C air temperatures)	Xeric	16.2–77.1	28
S. risoria (29.4–34.8°C air temperatures)	Xeric	137–250*	27
S. senegalensis (20–52°C air temperatures)	Xeric	25.8–62	28
Zenaida asiatica (35–45°C air temperatures)	Xeric	38—200	29
Z. macroura (45–50°C air temperatures)	Widespread	24–41	30
Aves: Cuculidae			
Geococcyx californicus	Xeric	139*	27
Aves: Fringillidae			
Poephila castanotis	Xeric	112*	27
Aves: Passeridae			
Zonotrichia leucophyrys	Widespread	94*	27
Aves: Phasianidae			
Alectoris chukar (20–45°C air temperatures)	Xeric	80.9–309.1	28
Coturnix chinensis	Open, mesic	199*	27
C. japonica (20–45°C air temperatures)	Open, xeric	77.8–297.1	28
Gallus domesticus (=G. gallus) (30–40°C air temperatures)	Mesic, domesticated	88–101*	27

Table 2. *Continued*

Taxon	Habitat	Resistance (s cm⁻¹)	Source
Aves: Ploceidae			
Ploceus cucullatus	Savannah	123*	27
Aves: Psittacidae			
Melopsittacus undulatus	Xeric	87*	27
Aves: Pteroclidae			
Pterocles senegallus	Xeric	15–103	31
Aves: Remizidae			
Auriparus flaviceps	Xeric	197	32
Mammalia: Hominidae			
Homo sapiens (human stratum corneum)	Widespread	377*	6
Mammalia: Muridae			
Mus musculus (30–35°C air temperatures)	Widespread	116–201*	27

Values represent the reciprocal of water vapor conductance and express the water vapor density difference between saturated skin and external environment per unit of evaporative flux.

Note: Approximated values (preceded by ~) are either estimated from graphic data or from data involving assumptions regarding boundary layer resistance, or rounded if close to zero. Variation in reported values may also reflect conditions of temperature and rates of air flow employed during measurements. Data calculated from literature values of CWL are indicated with an asterisk.

[1]Withers et al., 1984; [2]Wygoda, 1984; [3]Young et al., 2005; [4]Christian and Parry, 1997; [5]Wygoda, 1988; [6]Withers, 1998; [7]Withers and Richards, 1995; [8]Buttemer et al., 1996; [9]Buttemer, 1990; [10]Amey and Grigg, 1995; [11]Withers, 1995b; [12]Buttemer and Thomas, 2003; [13]Withers et al., 1982; [14]Spotila and Berman, 1976; [15]Lillywhite et al., 1997a; [16]Lillywhite and Sanmartino, 1993; [17]Eynan and Dmi'el, 1993; [18]Davis et al., 1980; [19]Lahav and Dmi'el, 1996; [20]Lillywhite and Maderson, 1982; [21]Tu et al., 2002; [22]Kattan and Lillywhite, 1989; [23]Dmi'el et al., 1997; [24]Robertson and Smith, 1982; [25]Dmi'el, 1998; [26]Mautz, 1980; [27]Webster et al., 1985; [28]Marder and Ben-Asher, 1983; [29]McKechnie and Wolf, 2004; [30]Hoffman and Walsberg, 1999; [31]Marder et al., 1986; [32]Wolf and Walsberg, 1996.

and partition coefficients, as well as thermal mobility related inversely to molecular mass.

Cutaneous membranes are mosaic structures composed of lipid bilayers in addition to molecular channels or pores. Therefore, the penetration of water molecules into cutaneous cells will depend, in part, on the fractional membrane area that is occupied by channels and the characteristics of the channels (Finkelstein and Cass, 1968; Finkelstein, 1984). Various proteins that serve as membrane channels for specific ions may also facilitate the concomitant flux of water by means of processes that induce solvent drag and by forming a low resistance pathway between the protein and lipid. Solubility–diffusion mechanisms will depend on the degree of packing and thermal mobility of hydrocarbon chains and on the charge of phospholipid polar groups in the bilayer membrane. In addition to diffusion, water flow across skin may be related to concentration differences of impermeant solutes or a differential of hydrostatic pressure. In contrast to diffusion, bulk osmotic or hydrodynamic flow involves the vectorial movement of an assembly of molecules being driven by an imposed potential. Consequently, various permeability coefficients can differ quantitatively because of the physical nature of the water movement pathway (Schafer and Andreoli, 1972; Finkelstein, 1984).

Water appears to permeate lipid bilayers by interaction with lipid polar head groups rather than a mechanism involving solubility of water in hydrocarbons (Carruthers and Melchior, 1983). Driven by the transmembrane gradient of water concentration energy, water then diffuses (exchanges) between the various hydration shells of the polar group into the hydrocarbon core (Hauser and Phillips, 1979). Divalent cations potentially displace water molecules by competing for interaction with the negatively charged phospholipid groups (Hauser et al., 1976).

The number of ion channels in many cell membranes may be relatively too few to provide a significant pathway for water movement, and the bulk of transcellular water movement in cutaneous tissues appears to occur by a solubility–diffusion mechanism involving the lipid bilayer pathway (Finkelstein, 1984). However, aqueous pores do comprise a major route for water transport in certain epithelia including the skin of amphibians (Hevesy et al., 1935; Koefoed-Johnsen and Ussing, 1953). Moreover, if there is osmotic flow of water through a significant number of membrane-spanning channels, water transport is increased greatly because osmotic flux of water occurs by laminar or quasilaminar flow (Mauro, 1957). Water flow can also occur at the lipid/protein interface as well as *via* channels formed by protein, and thus water permeability may be governed significantly by the presence of membrane-spanning proteins and their interactions with the lipid bilayer. Diffusional movement of water through lipid bilayers is reduced by

orders of magnitude when proteins are absent (Carruthers and Melchior, 1983).

In addition to the cellular route of water movement across integument, water also can move between the lateral, intercellular spaces. Simple epithelia are often categorized according to the relative permeabilities of the intra- and intercellular pathways. So-called 'leaky epithelia' are ones in which most diffusion occurs between cells, whereas 'tight epithelia' have junctional contacts between cells that impose a high resistance such that most passive diffusion occurs through cell membranes and cytosol. These distinctions may become blurred in consideration of complex, multi-layered barriers such as skin. In the various layers, junctional contacts between cells have various organizations such that cells can adhere, interact and dissipate tensional stresses throughout the tissue.

Intercellular regions of membrane apposition may have junctional features that occlude the intercellular space. The term 'tight junction' has been applied loosely to a broad range of such intimate contacts between plasma membranes, although the term was originally introduced to designate the *zonula occludens* (Farquhar and Palade, 1963). Generally, tight junctions join cells at their apical edges by a continuous belt of fusion with the adjoining plasma membranes. So-called tight epithelia can support large osmotic and ionic gradients and maintain large transepithelial potentials by limiting the diffusion of electrolytes rather than of water (Staehelin, 1974; Madara, 1998).

The sealing elements of tight junctions consist, in part, of diverse fibrils within the membranes. In some tissues the junctional permeability appears related to the number of intramembrane fibers (Claude and Goodenough, 1973), but the packing geometry should be important as well. Sometimes the fibrils are not continuous, and leak pathways might be related to interruptions that occur at more or less regular intervals. Ionic selectivity of junctional zones also suggest the chemical composition of molecules within the junctions are important, as well as the pH of the junction environment. Low pH, for example, weakens intercellular junctions and increases permeation of ions through extracellular spaces (Ferreira and Hill, 1982; Marshall, 1985). The permeability properties of cellular junctions also may depend on the osmotic and hydration status of cells which, in skin, will be determined by the total water barrier properties, their location, and their interaction with blood capillaries and the environment at the skin surface. Changes in cellular volume can alter active transport as well as the structure of intercellular spaces (Erlij and Ussing, 1978). Moreover, the osmotic flow of water within epithelia can induce dilation or collapse of the lateral extracellular spaces, depending on the flow direction, and thereby affect diffusion in a manner that is independent of cellular shrinking or swelling. Such changes in the dimensions of lateral spaces can produce asymmetry of water flow, and

diffusion can become rate-limiting as such spaces collapse (Wright et al., 1972).

Tight junctions restrict the movement of both fluids and solutes between cells. While it is recognized that epithelia are selectively permeable, generally more than 90% of transepithelial ionic conductances appear to be localized in the paracellular pathways of leaky epithelia, whereas less than 10% of the total conductance is attributable to the paracellular route of tighter epithelia such as frog skin (Erlij and Martinez-Palomo, 1978). Some investigators believe that tight junctions also are effective in restricting water movement and estimate that hydraulic conductivity of the junctional pathway can account for only 10% of the entire epithelium (Wright et al., 1972). However, the junctional pathway can account for most of the water movement in leaky epithelia (Levitt, 1981).

The relative importance of paracellular *vs* transcellular pathways to diffusional water movement, and the significance of tight junctions in the overall resistance to water movement across multi-layered integuments, are unclear. There has been extensive use of amphibian skin and endothelial tissues as model systems for the study of membrane permeability, but little is known about the significance of tight junctions in skin from amniote species representing a broad range of requirements and morphologies. Tight junctions have been identified in skin of all classes of vertebrates, although they are considered to be sparse or absent in the skin of mammals (Matoltsy, 1984). Tight junctions joining cellular walls are present in the stratum corneum of adult anurans and possibly play a role in limiting transcutaneous water movement (Farquhar and Palade, 1965). However, these structures do not impede transcellular water flux and appear to be a general feature of anuran integument without variation among amphibian species from mesic and xeric habitats. Insofar as some anurans evaporate water from the skin at rates equivalent to a free water surface (Adolf, 1932; Spotila and Berman, 1976), it seems that tight junctions are not significant components of adaptation of R_s to desiccating environments. Moreover, skin permeability varies greatly among species that all possess these structures.

Thus, in a very broad sense, there is little evidence to suggest that the presence or nature of tight junctions is a significant determinant of the resistance of integument to transepidermal water flux. However, further research will be important to confirm or qualify this generalization. Recent studies implicate claudin-based cellular junctions to be important for barrier function in mammalian epidermis (Furuse et al., 2002). Claudins are integral membrane proteins that mediate cell-to-cell adhesion and are concentrated in the stratum granulosum of mouse skin. Genetic ablation of claudin-1 in mice induces rapid weight loss, increased TEWL, wrinkled skin and neonatal death, presumably attributable to dehydration. The deficient skin exhibits an impaired water barrier, although the organization of the stratum corneum appears to be normal. Genetic studies have demonstrated that absence of cross-linking molecules essential to organization of the cornified cell envelopes also results in a defective phenotype similar to that

of claudin-1 deficiency (Matsuki et al., 1998). These studies draw attention to the possible importance of correct organization of cellular junctions in the stratum granulosum and of the cornified cell envelopes in the stratum corneum for permeability barrier function in mammalian skin. However, further investigations are required to understand the proposed as well as alternative, complex, and possibly indirect roles for claudins that might relate to permeability barrier function (Bazzoni and Dejana, 2002).

Structural and secreted barriers
Structural barriers

During the evolutionary radiation of vertebrates, a variety of factors related to body size, locomotion, endothermy and especially terrestrial life, necessitated structural and protective reinforcement of the skin (Maderson and Alibardi, 2000; Maderson, 2003). On the one hand, terrestrial locomotion by tetrapods required loose connective tissues in the hypodermis and mechanical decoupling of the skin from underlying muscles. Exceptions are found among animals where skin is directly involved in transmission of locomotory forces, so in birds, for example, skin has extensive attachments to the underlying skeleton. On the other hand, fibrous keratins evolved as heterogeneous complexes of proteins containing sulphydryl (SH) and disulphide (SS) linkages of cystine. These provided variable protection from physical elements in the environment and formed the principal constituents of dead horny cells in the superficial layers of the epidermis and in derived hair or feathers (Figs 1, 2). Importantly, keratins provided mechanically resistant but relatively lightweight organic material in comparison with calcified dermal shields that were present in some earlier ancestral lineages (Alibardi, 2003).

Evolution of a protective epidermis initially involved α-keratin polypeptides, which are present in all living vertebrates and primitively represented in fish by 70 Å tonofilaments. Alpha keratinogenesis in amniotes became expressed as keratohyalin granules in mammals and keratohyalin-like granules in reptiles and birds (Maderson and Alibardi, 2000; Alibardi, 2001; Maderson, 2003). The capacity for epidermal synthesis of keratin appears to be a shared ancestral (plesiomorphic) feature of vertebrates, and the potential for α-protein synthesis is presumably ubiquitous (Maderson, 1972). In vertebrate embryos, α-keratinogenic cells form before β-keratinogenic cells, supporting the idea that α-keratin characterized the primitive condition in the first amniotes of the Carboniferous (Alibardi, 2003). Genes coding for β-keratin became expressed about 150 million years later than those coding for α-keratin and first appeared in the shells and claws of chelonians (Matoltsy and Bereiter-Hahn, 1986). The strengthening of integument with β-protein synthesis is a derived character shared among sauropsids (synapomorphy). The β-type keratin consists of polypeptide chains having a pleated sheet structure that assembles into an inflexible and inextensible conformation. These occur in the epidermis and scales of all reptiles, including scales, claws, beak and feathers

of birds. Within sauropsid lineages, the synthesis of β-keratin is variably expressed with horizontal or vertical alternation of α and β keratin domains (Baden and Maderson, 1970; Maderson and Alibardi, 2000). However, only α-keratin has been expressed in mammals, with physical strength being enhanced by innovations of orientation and addition of other proteins to the filament matrix.

Various conformations of β-keratin polypeptides played a principal role in the evolution of strong armament among reptiles and made possible the aerial locomotion of birds by means of stiff feathers. Wide variation in structure and mechanical properties are provided by association of keratins with fibrous globular proteins and by mineralization that involves calcium, usually bonded to phospholipid within the keratin complex. In both turtles and crocodilians, β-keratin forms thick, mechanically resistant corneous layers and may be mineralized with calcium. Scales in these animals are tough, dry and relatively non-pliable, except in the skin of the neck, limbs, and tail of chelonians where a softer corneous layer contains only α-keratin. Calcified structures are virtually impermeable to water, depending on thickness. At the other extreme, keratins of amphibians may form thin, transparent membranes, generally only 1–2 cell layers thick.

Desquamation of corneous cells results from wear and is a gradual and slow process. However, in the scales of lepidosaurs, deposition of β-keratin alternates with α-keratin to form a specialized, pluristratified 'shedding complex' that facilitates ecdysis (Maderson, 1984, 1985; Maderson et al., 1998). In both lepidosaurian reptiles and amphibians, periodic cellular proliferation (renewal phases) alternates with periods of inactivity (resting phases) and allows the skin to follow seasonal periods of rapid body growth or to restore skin functions such as gas exchange.

Issues of thickness

In very general terms, the permeability of a fibrous structure correlates with length of the diffusion pathway, or thickness, and where hard and soft tissues occur on the body surface, the important sites of exchange are at the softer sites. However, thickness is not an absolute predictor of permeability because the dimension is not independent of the tissue composition (Lillywhite and Maderson, 1982). The thickness of a diffusion barrier contributes to overall R_s across a structure, so overall thickness, and especially the number of keratinized cell layers in series, might have importance for water relations of skin. In this context, the location of blood capillaries determines the 'effective thickness' of skin, so vascularization of skin is an important related property.

Generally, in the majority of amniotes the outermost cutaneous capillaries occur within the dermal layers, sometimes just beneath the epidermis or at the dermis-epidermis boundary. However, capillaries penetrate the epidermis in certain fishes and amphibians where their presence is associated with cutaneous gas exchange (Mittal and Datta Munshi, 1971; Guimond and Hutchison, 1973; Hutchison et al., 1976; Toledo and Jared, 1993). The skin of

the pelvic region ('seat patch') of anuran amphibians is especially thin and richly vascularized, features regarded as specializations for water uptake from moist surfaces (Toledo and Jared, 1993). Such pelvic integuments also bear specialized structures, 'verrucae hydrophylicae', which are provided with vascular plexa and are sensitive to peptide hormones that influence permeability (Drewes et al., 1977; Hillyard, 1999). There is some evidence that Angiotensin II increases rates of blood flow through the seat patch of anurans (Slivkoff and Warburton, 2001), but observations also demonstrate that mere water exposure of the seat patch in dehydrated toads rapidly induces large increments of blood flow independent from hormonal influence (Viborg and Hillyard, 2005). These specialized features of integument are important in contexts of water gain ('cutaneous drinking'; Hillyard, 1999) involving ventral integument that is normally not exposed to atmospheric conditions. As in other vascular systems, cutaneous blood vessels are no doubt responsive to neuronal as well as endocrine control.

Studies of anurans suggest that overall skin thickness is related to habitat and is important for water balance in some species (for references, see Toledo and Jared, 1993). However, among amphibians as well as vertebrates generally, the thickness of skin *per se* does not constitute the principal barrier to the diffusion of water. Moreover, the keratinized surfaces of amphibian skin are typically no more than a few cell layers thick, and heavily keratinized integument does not appear to be an adaptive response of amphibian integument.

The periodic production of 'cocoons' in certain amphibian taxa is an exception to these statements. These structures impose considerable resistance to water passage (Table 2) and allow burrowing anurans to establish themselves at shallower depths during drought than do species without cocoons (Lee and Mercer, 1967; Ruibal and Hillman, 1981; McClanahan et al., 1983; Withers, 1998). Cocoons consist of multiple superimposed α-cell layers of stratum corneum resulting from multiple shedding of skin layers during periods of dormancy in drying soils. The phenomenon is present in the anuran families of Bufonidae, Hylidae, Leptodactylidae and Ranidae, as well as in a few urodeles. A cocoon may consist of some 40–60 layers of cornified cells with secreted lipids and proteinaceous materials sandwiched between them (McClanahan et al., 1976; Ruibal and Hillman, 1981; Withers, 1995a; Christian and Parry, 1997).

The presence of osteoderms or 'co-ossified' regions in skin where bone tissue occurs in the dermis is potentially significant for regional enhancement of R_s, and this subject has been given limited attention principally in amphibians (Seibert et al., 1974; Ruibal and Shoemaker, 1984; De Andrade and Abe, 1997; Navas et al., 2002; Jared et al., 2005). The integument of co-ossified regions of skin contains collagen fibres with reduced presence of blood vessels and mucous glands. Co-ossified head regions provide mechanical protection from predators and are used to seal off entries to refugia in plants where frogs seclude themselves (Duellmann and Trueb, 1986). Thickened or mineralized structures in the skin of various terrestrial amniotes no doubt increase regional R_s of the skin (Seibert et al., 1974), but few studies of regional differences in TEWL have quantified such contributions.

Water permeation in keratins

How do the structural features of keratin influence its resistance to water movement? Generally, structural features that alter the free volume (equivalent to pores or channels) should alter the permeation of water molecules accordingly. Resistance to diffusion is affected by the molecular mass of side chains and tends to increase with cross-linking beyond certain critical levels (Lieberman et al., 1972). However, proteins with bulky, globular side chains may inhibit close packing of molecules and thereby diminish the resistance of a complex membrane. Proteins with generally smaller and non-polar amino acids are able to pack more closely together, thereby permitting formation of many van der Waals forces. The stability of cross-linkages is dependent on a large number of intermolecular forces, including covalent, ionic, and hydrogen bonding in addition to van der Waals attractive forces between non-polar amino acid side chains. All of these act to influence the mobility and free volumes of the structure.

Water itself influences the molecular structure of fibrous protein polymers, and the hydrated status of the skin is an important property. Proteins, in general, bind water very strongly at low relative humidities, and the water content increases in proportion to humidity exposure. As water content increases, it enters between protein molecules and thereby increases the mobility of side chains and the distance between them, potentially reducing the degree of crystallinity. Thus, introduction of water molecules into the polymer structure increases the available free volume as well as the mobility of cross-linkage groups. Dehydration reduces permeability not only by reversing these effects but also by promoting non-covalent bondings between protein chains (Vincent and Hillerton, 1979). Consequently, the permeability of dry protein films can be extremely low (Lieberman et al., 1972), presumably enhancing water resistance in comparatively dry tissue.

Recently it was shown that the 'natural' hydration level of human stratum corneum *in vivo* is about 30–50% of dry mass (Caspers et al., 2001). When the stratum corneum is equilibrated over water, its mass increases to 300–400% of dry mass. At low hydration levels the stratum corneum is relatively brittle and fragile, whereas when water levels exceed a value of around 10–15% of dry mass, the tissue softens markedly. Hydration of the stratum corneum enhances the flux of hydrophilic as well as lipophilic substances (Bouwstra et al., 2003a).

Models of cutaneous water movement are subject to uncertainties related to the phase state of water moving through epidermal tissues and the potential effects of hydration and temperature (Scheuplein and Blank, 1971; Mautz, 1982; Marder and Ben-Asher, 1983). Webster et al. (1985) properly note that if liquid water moves into the superficial layers of the skin, water in the vapor phase might diffuse across a shorter

pathway and, hence, reduced resistance. Studies of human skin have indeed demonstrated that gradients of water exist in the stratum corneum (Warner et al., 1988; Bommannan et al., 1990; Caspers et al., 2001; Bouwstra et al., 2003a). Fourier transform infrared spectroscopy has demonstrated that free water content in stratum corneum is greater in central regions relative to superficial and deeper cell layers at moderate levels of hydration (57%–87%, w/w), whereas at higher levels of hydration (300% w/w) water swells corneocytes in a direction perpendicular to the skin surface except for the deepest cell layers adjacent to the viable epidermis (Bouwstra et al., 2003a). While the mechanism excluding free water from the deeper cell layers of stratum corneum is not understood, it is speculated to play a role in preventing dehydration of the viable epidermis. In relatively dry conditions (18%–26% w/w), only bound water is present in the stratum corneum (Bulgin and Vinson, 1967; Hansen and Yellin, 1972; Bouwstra et al., 2003a).

Extracellular matrices and organelles

Certain extracellular matrices and organelles have been hypothesized to reduce water exchange across the integument, notably in amphibians. Calcium salts and a hydrophilous, amorphous 'ground substance' are present in the stratum spongiosum of many amphibian species (Elkan, 1968; Moss, 1972). The so-called ground substance of the calcified dermal layer consists of a polysaccharide gel having glycosaminoglycans usually bonded covalently with proteins. The distribution of this layer among species exhibits little correlation with environment or susceptibility to dehydration, although the layer is generally more developed in dorsal than ventral skin. This layer was suggested to function in water conservation (Elkan, 1968), but the position of the layer and its mere hydrophilic property would appear to render it ineffective (Drewes et al., 1977). Other possible functions, such as a reservoir for mineral homeostasis (Moss, 1972), seem more plausible than a significant role related to water permeability.

Pigmented effector cells that impart color and capacity for physiological color change in vertebrates, have been suggested to influence R_s of amphibian skin. Chromatophore layers, mostly involving silvery iridophores, vary seasonally and appear to correlate with reduced rates of TEWL in some species of frogs exposed to dry conditions (Drewes et al., 1977; Withers et al., 1982; Kobelt and Linsenmair, 1986). However, these frogs remain exposed to sunlight during hot, dry conditions, and the iridophores likely function to increase reflectance of radiation (Kobelt and Linsenmair, 1986). Therefore, the role of iridophores as a causal mechanism for increasing resistance to TEWL is not conclusive. Moreover, the iridophore units usually have gaps between them, and they are overlain by living cells, so they cannot provide complete protection from dehydration of epidermis. Both pigments and structural colors in scales of reptiles, avian feathers and mammalian pelage potentially influence TEWL indirectly by modifying the reflectance and thermal properties of skin.

Secretions and secreted barriers

Fluid secretions can be important to the water relations of skin in a variety of vertebrates, particularly amphibians that lack a fibrous or mineralized covering of significant thickness. The skin of amphibians is glandular and produces three principal categories of secretions: mucus, various toxins, and lipids. A variety of toxic substances – largely alkaloids, peptides and amines – are usually secreted only during defensive interactions with other animals and are not considered further here (see Erspamer, 1994). Lipids are discussed in some detail in the next section. Mucous secretions are predominant and copious in many amphibian species, where they may form a virtually constant film overlying the outer skin layers. These secretions are largely water with varying amounts of proteoglycans and glycoproteins (glycoconjugates), various ions, and often lesser quantities of sugars, amino acids and lipids (Dapson, 1970).

Mucus plays multiple roles in the biology of integument and is especially effective in contexts related to lubrication and keeping the skin hydrated and moist (Lillywhite, 1971, 1975; Mittal and Bannerjee, 1980; Whitear, 1986; Lillywhite et al., 1998). While mucus has been conjectured to limit water movement where it is present in the extracellular spaces of the stratum corneum (Toledo and Jared, 1993), there is no demonstration that this is actually the case. Similarly, externally adhering mucus has been conjectured to reduce evaporative water loss from the skin surfaces (Wygoda, 1988) and to seal small gaps between the body and limbs of frogs (Geise and Linsenmair, 1986; Kobelt and Linsenmair, 1986). In these studies the composition of 'mucus' was not determined, so the lipid content is not known. Although mucus is hygroscopic, biophysical studies have shown it is not particularly impermeable, even when air-dried (Machin, 1966, 1972). Further, it was shown by direct measurements *in vivo* that intact mucus covering over the skin of *Litoria dahlia* provided no extra resistance to TEWL (Young et al., 2005). While quantitative comparisons are not available, it seems strikingly clear that any resistance to transepidermal water loss attributable to mucus is miniscule compared with that for lipid secretions (see below).

In terrestrial amphibians, secretion of mucus transfers water to evaporating surfaces that are sculptured to retain the fluid (Lillywhite, 1971). In bullfrogs and several other species of anurans, the rate of mucus secretion is regulated to keep up with increasing evaporative demands related to heliothermy, and thus water evaporates from a regularly replenished mucous film rather than the underlying epidermis (Lillywhite, 1971; Lillywhite and Licht, 1975; Kaul and Shoemaker, 1989; Lillywhite et al., 1998). If frogs are deprived of the external mucus film, the skin dries and is unable to maintain normal levels of evaporative water loss (Lillywhite, 1975). In contrast, anurans without a mucus film (e.g. bufonids) either avoid dehydrating conditions or, in the case of some heliothermic toads, rely on water that is transferred from moist substrates to wet the skin *via* capillary forces that 'wick' the liquid across a highly sculptured integument (Lillywhite and Licht, 1974).

Whether the external film overlying the epidermis is mucus or water, the aqueous evaporating film must be replenished by water that is either stored within the animal or is absorbed from a moist substrate. Thus, a mucus film cannot be utilized as a strategy for maintaining a hydrated skin during exposure without water in xeric habitats or during long-term drought.

The importance of mucous secretions in protecting exposed epidermal surfaces from dehydration suggests a fundamental dichotomy of skin organization and water balance (Lillywhite and Maderson, 1988). With few exceptions, either the epidermis is protected from excessive water loss by a lipid and/or fibrous diffusion barrier (some amphibians, reptiles, birds, mammals) (Fig. 2), or the epidermis is covered with a wet film so that evaporation occurs from secreted fluids rather than the epidermis itself (many amphibians). If the epidermis or whole skin dries excessively, its viability diminishes as the dehydrating cell layers compress and stiffen (Lillywhite, 1975). Superficial water films associated with sculptured skin surfaces (channels that act like 'blotting paper') appear to have an important role in protecting skin from dehydration in certain snakes (Lillywhite and SanMartino, 1993) and elephants (Lillywhite and Stein, 1987) as well as amphibians (Lillywhite and Licht, 1974).

Lipids and the evolution of cutaneous permeability barriers

The role of lipids in controlling transepidermal water loss is well established and has evolved numerous times during tetrapod evolution. Moreover, lipids are employed almost universally as a means of waterproofing the skin or body surfaces of terrestrial organisms, including plants, arthropods, some amphibians, reptiles, birds and mammals (Hadley, 1989, 1991). Thus, conferral of resistance to TEWL is fundamentally a matter of molecular rather than mechanical organization (biochemistry vs morphology), although these two attributes are intimately interconnected. Indeed, the efficacy of a lipid barrier depends in many cases on structural features that enhance or maintain its integrity and function (Table 1; Figs 1, 2). Understanding the significance and variation in permeability barriers requires first an appreciation for key features related to functional organization and structure of vertebrate integument.

The structural features of vertebrate integument are uniquely different from all other major phyla of animals. Clearly, mechanical strength is enhanced by a layered keratin structure, and the β keratins provide superior strength and rigidity. Importantly, the evolution in basal amniotes of a stratified squamous stratum corneum produced a tough yet flexible epidermis across the body surfaces (Maderson and Alibardi, 2000). Such a stratified corneum conferred an additional important advantage, which is a convenient means to preserve the structural integrity of laminated lipids that might function to impede the transcutaneous efflux of essential body water (Figs 2, 3). The corneous layers provide a framework and sufficiently rigid support for stability of lipid structure without undue movement or mechanical disruption (see below).
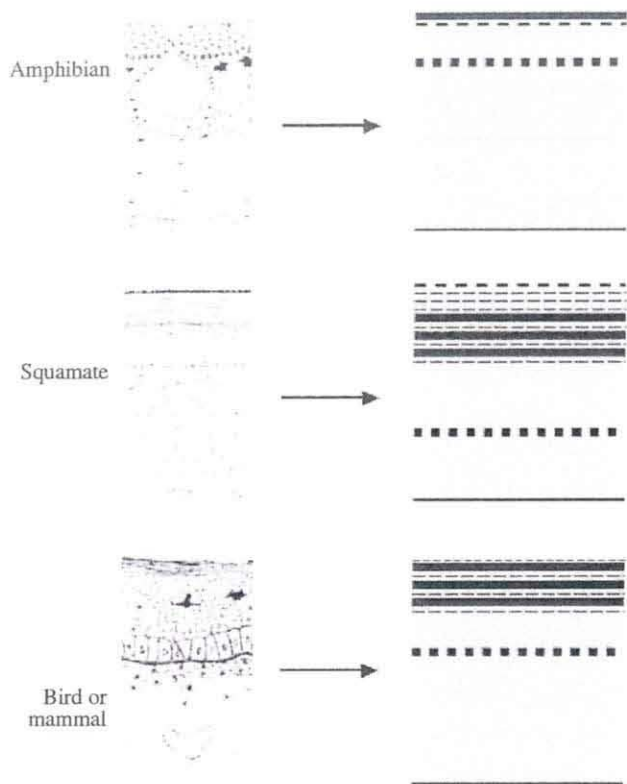

Fig. 2. Schematic illustrations of the water permeability barrier in relation to the stratum corneum of the epidermis in four principal lineages of vertebrates. The images on the left depict the morphology of generalized integument, and drawings to the right illustrate the stylized arrangement of barrier lipids (dark bars) in relation to horny layers of stratum corneum (thin broken lines). The stratum germinativum is represented by the thick broken lines. The features shown are not to scale.

Permeability barriers always contain a complex mixture of lipid molecules. Longer chain-length hydrocarbons tend to comprise a dominant category of lipids in most barriers examined. These tend to melt at higher temperatures and resist water permeation, whereas shorter chain-length molecules reduce the intensity of van der Waals interactions between hydrocarbon molecules and create a more fluid and permeable structure. Relative saturation of hydrocarbons also contributes to a tighter water barrier, whereas unsaturation and methyl branching tend to introduce kinks in molecules and disrupt packing. However, chain length alone is not necessarily a reliable indicator of water permeation because in some systems chain elongation and unsaturation offset each other (Gibbs et al., 1998). Hydrocarbons and wax esters are relatively non-polar, which assists in repelling water. Polar phospholipids and other classes of lipids having intermediate polarity, in addition to branching, might be important in structuring the geometry of a water barrier and providing a degree or specific orientation of fluidity important with respect to potential mechanical distortion or disruption of the barrier structure (Bouwstra et al.,

2000, 2003b). These and other properties of lipids might also play roles that are important in barrier assembly, prevention of degradation by enzymes or microorganisms, thermal properties relevant to thermoregulation, and interactions with non-lipid molecules.

Normal stratum corneum, as studied in mice and humans, demonstrates a markedly acidic pH, ranging from 4.5 to 5.5 at the surface to physiologic pH ($\approx$7.4) in hydrated, viable epidermis (Ohman and Vahlquist, 1994; Turner et al., 1998; Fluhr and Elias, 2002). The acidic pH is attributable to influence of key metabolites including urocanic acid, free fatty acids, and a non-energy dependent sodium–proton exchanger (Chuong et al., 2002). The acidic pH appears to regulate several key defensive functions of skin, including antimicrobial and permeability barrier properties, as well as integrity and cohesion of the stratum corneum. The stability of molecular structures is influenced importantly by pH, which also alters the water content of fibrous structures because bonding between chains are replaced by bonding between protein and water (Hackman, 1984). With respect to the permeability barrier, at least two key lipid processing enzymes affecting the barrier lipids within the interstices of stratum corneum are activated at low pH optima and are retarded by exposure to neutral buffers (Uchida et al., 2002; Schmuth et al., 2000). Moreover, the acidic pH also is important for direct influence of lipid–lipid interactions in the lamellar bilayers of the permeability barrier (Bouwstra et al., 1999). Hence, stratum corneum neutralization reduces competence of the permeability barrier lipids (Mauro et al., 1998; Hachem et al., 2003).

Lipid barriers of amphibians

The Amphibia present features, both in evolutionary history and specializations of extant forms, which are transitional between aquatic and terrestrial environments. Numerous species exhibit complex life histories with aquatic larval stages prior to acquisition of terrestrial adult characters, and the adults of extant taxa possess skin that is generally devoid of thick, rigid or heavily keratinized protective armor. Amphibians have retained integument that, in terrestrial as well as aquatic adults, is relatively thin and pliant – lacking extensive development of keratin and retaining permeability properties that allow significant gas exchange across the cutaneous surfaces. While only a small sample of some 4200 amphibian species have been examined in detail, it seems clear that many species of amphibians possess skin of characteristically high permeability and experience relatively high rates of TEWL.

Comparative investigations indicate that arboreal species of frogs tend to have generally higher R_s than do non-arboreal species (Table 2; see Withers et al., 1984; Wygoda, 1984; Young et al., 2005). Selection for increased R_s in arboreal habitats is no doubt related to exposure of animals to increased levels of radiation, temperature and convection relative to ground-dwelling, fossorial or amphibious relatives (e.g., McClanahan and Shoemaker, 1987). Various mechanisms have been suggested to play a role as determinants of R_s in amphibians, and these have been reviewed previously in attempt to evaluate the relative contributions of characters to the overall R_s (Toledo and Jared, 1993; Lillywhite and Mittal, 1999). As in other tetrapods, lipids appear to be of central importance to comparatively high R_s that has been reported for selected species (Table 2).

The role of cutaneous lipids in water economy is clear in some species, while problematic or elusive in others. Lipids confer exceptionally high resistance in certain tree frogs, which secrete lipids from cutaneous glands to form an external film that is subsequently wiped with complex and stereotyped movements involving all four limbs (Shoemaker et al., 1972). However, lipids that are located within the skin may or may not correlate with R_s (Withers et al., 1984; Toledo and Jared, 1993). Also, lipid mixtures that are produced by general extractions of whole skin undoubtedly include elements of membrane lipids not associated with a water barrier as well as precursor molecules that might be converted to other components if a barrier is present. Lipids that are structured within the dermis are purported to reduce TEWL (Amey and Grigg, 1995), but these cannot act to prevent dehydration of the overlying epidermis. Thus, the more effective lipid barriers among amphibians are those that create a barrier to TEWL at the skin surfaces (Table 1; Fig. 2).

Elaborate wiping of lipids on body surfaces was first described in phyllomedusine frogs, which exhibit very low rates of TEWL (Blaylock et al., 1976). The behavior is associated with a suite of behavioral and physiological characters, including selection of a permanent perch where frogs remain inactive during the day, secretion of lipids from cutaneous glands, wiping behavior, and the assumption of a condition resembling torpor. The secreted lipids are produced in specialized cutaneous glands and are a mixture comprising mostly wax esters, triglycerides, free fatty acids, hydrocarbons and cholesterol (McClanahan et al., 1978). Wax esters are dominant and average about 46 carbons in length. The externally wiped layer is estimated to be about 0.2 μm and 50–100 molecules thick, which is comparable to the epicuticular wax layers of various arthropods. This lipid barrier results in a R_s that is much greater than that demonstrated in typically amphibious anurans (Table 2) and enables frogs to remain exposed to sunlight in hot, dry weather where body temperatures approaching 40°C are tolerated (Shoemaker et al., 1987). The conservation of body water achieved by the high R_s of the waxy layer is complemented by excretion of uric acid, which also conserves water potentially involved in the excretion of nitrogenous wastes (Shoemaker et al., 1972; Shoemaker and Bickler, 1979).

Wiping behavior and secretion of lipids from cutaneous glands have been reported in several species of anurans having variable R_s (generally from about one to several 100 s cm^{-1}; Table 2). It seems likely that wiping behavior is more widespread among anurans than formerly supposed, and that components or precursors to the full behavior might have evolved in a number of different contexts (Blaylock et al., 1976; Lillywhite et al., 1997a; Barbeau and Lillywhite, 2005).

Wiping behaviors observed in a rhacophorid tree frog, *Polypedates maculatus*, stimulate secretion of lipids from cutaneous mucous glands, which are activated by a tactile neurogenic reflex (Lillywhite et al., 1997a). The lipids are secreted with mucus, and these frogs have a relatively modest R_s (Lillywhite et al., 1997a,b). Both wiping complexity and R_s are variable among six species of hylid tree frogs in Florida (Barbeau and Lillywhite, 2005). The lipids appear to be produced in granular rather than mucous glands and, like *Polypedates*, these frogs are not very waterproof. In all the examples of anurans investigated, elaborate wiping of secreted lipids is associated with arboreal species living in arid, semiarid, or ephemerally arid environments where there is potential for dehydration stress.

The water barrier in amphibian skin is very different from that of amniotes for two fundamental reasons. First, lamellar granules have not been definitively identified in any amphibian, although structures bearing resemblance to these organelles ('small dense granules' and 'tightly packed parallel lamellae') were reportedly seen in the stratum spinosum of *Hyla arborea* (Bani et al., 1985). These structures were possibly misinterpreted, as lamellar granules appear to be uniquely associated with keratinizing epithelia and are not generally recognized in the epidermis of amphibians or fish (Wertz, 1996, 2000; Alibardi, 2003). Secondly, the corneous layers of amphibian epidermis are too sparse to provide an effectively structured lipid–keratin complex, and lipid structures with complex lamellation have not been identified in association with interstices in the thin stratum corneum of any amphibian species. Hence, lipid layers comprising part of

the tela subcutanea have been described in several species of arboreal frogs, but these lipids underlie blood vessels and the frogs are not particularly resistant to cutaneous water loss (Wygoda et al., 1987). Similarly, a lipid band has been demonstrated histochemically in the uppermost aspect of the stratum spongiosum of the arboreal frog *Litoria fallax* (Amey and Grigg, 1995). These lipids appear to be derived from congealed lipid droplets, but there is no reported evidence for structured lamination or mechanical support associated with adjacent cell layers or other epidermal structures. This lipid layer possibly contributes to reduction of evaporative water losses in this species, but the reported rates of TEWL are not exceptionally low.

The limited keratinization of the epidermis of modern amphibians imposes a critical constraint, due possibly to developmental canalization related to aquatic life stages (Lillywhite and Mittal, 1999). Although keratin was presumably present in basal amphibians (Findlay, 1968; Maderson, 1972), neither extensive keratinization nor synthesis of β-type keratins characterizes the skin of modern amphibian lineages. The capacity for extensive synthesis of keratin is present in connection with a few specialized structures such as the cornified protrusions (spade) used in digging by fossorial anurans, so the generalized condition of the skin may be more related to a lack of genetic expression rather than gene deficiencies.

Amphibians thus differ from amniotes insofar as layered complexes of lipid and keratin are absent (Table 1; Figs 1,2). One possible exception might be the laminated structure characteristic of 'cocoons' that occur in various species during dormancy of estivation (Fig. 3A). In the Australian frog *Cyclorana auastralis*, the composition of both skin secretions and cocoon material was shown to consist of 5–10% neutral lipids and 78–85% proteinaceous material (Christian and Parry, 1997). The lipid fraction of these materials was chemically similar to the secreted lipids that provide an exernal water barrier in
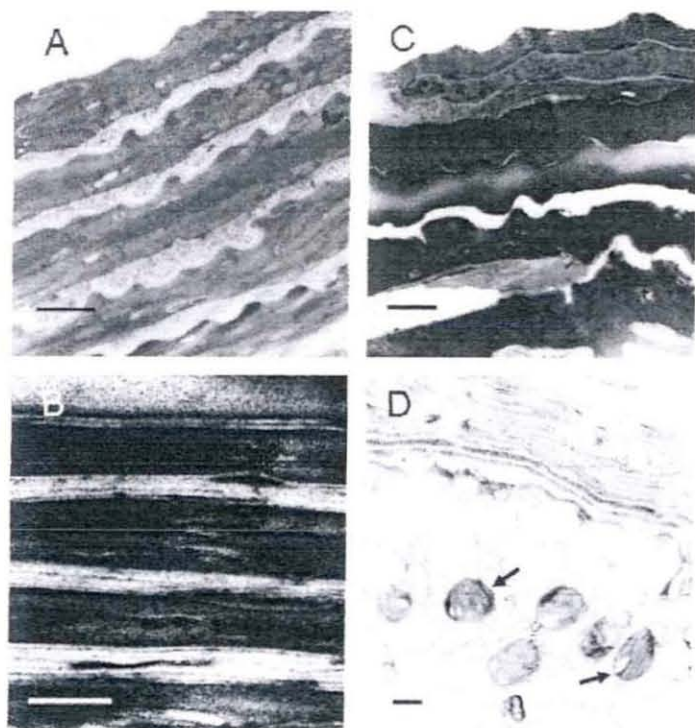
Fig. 3. Electron micrographs showing details of stratum corneum and permeability barrier of terrestrial vertebrates. (A) Section through a portion of cocoon of a burrowing hylid frog, *Pternohyla fodiens*. The layers of squamous epidermal cells are separated by granular extracellular materials in the subcorneal spaces. Scale bar, 500 nm. Reproduced with permission (Ruibal and Hillman, 1981). (B) Section through mesos layer of snake epidermis (*Natrix natrix*), which is the recognized permeability barrier of squamates. Laminated lipids occur between the darker bands of keratin layers. Scale bar, 100 nm. Courtesy of Lukas Landmann. (C) Section through stratum corneum of human skin. Lipids (unstained) occur between the distinct layers of keratin. Scale bar, 200 nm. Courtesy of Gopi Menon. (D) Section through epidermis of a canary, showing nucleated layers as well as stratum corneum (top). Lipids occur between the distinct layers of keratin toward top of figure. Note the multigranular bodies (source of lipids; arrows). Scale bar, 200 nm. Courtesy of Gopi Menon.

phyllomedusine frogs (McClanahan et al., 1978), but it is not clear whether these were extracted from the cell layers of the cocoon or were derived from the interspersed 'mucus.' Further investigations are required to examine the possibility that lipids are secreted in mucus (as in *Polypedates maculatus*: Lillywhite et al., 1997a,b) to provide a layered lipid–keratin complex in amphibian cocoons.

In the case of both secreted cocoons and the extraepidermal lipid layers that are secreted and wiped by arboreal frogs such as *Phyllomedusa* or *Polypedates*, subsequent function depends on immobility of the animal for otherwise the structural integrity of the lipid barrier is disturbed. Thus, amphibians produce cocoons when they are in soil and in a state of torpor, while wiping of lipids by tree frogs occurs characteristically prior to resting in a water-conserving posture while either in seclusion or on perches. In other systems, lipid water barriers occur either on external surfaces of rigid structures (as in plants, feathers and arthropod cuticle) or are contained in layered arrays within corneous tissues (as in reptiles, mammals and birds). With reference to the integument of amniotes, the corneous structure that contains barrier lipids is either comparatively rigid (scales) or somewhat supple (mammalian skin) while resilient to creasing. Importantly, the numerous layers of specialized corneocytes associated with the water barrier region of the stratum corneum of amniotes provide a tough and resilient framework for the intercellular lamellar lipids. On the other hand, the more delicate, pliant nature of amphibian integument and its limited keratinization appear to have constrained the possible evolutionary pathways for waterproofing mechanisms in amphibians (Lillywhite and Mittal, 1999). Due to the limitation of keratinization, in particular, external lipid barriers seem to provide the more effective and practical potential means of waterproofing in amphibians (Fig. 2).

Studies of 'waterproof' frogs have demonstrated that reduction of skin permeability by means of lipids to prevent dehydration also reduces cutaneous gas exchange, which normally contributes importantly to respiration in amphibians (Feder and Burggren, 1985). Thus, under these circumstances of periodic waterproofing, frogs rely increasingly on pulmonary ventilation to maintain arterial blood pH and levels of respiratory gases (Stinner and Shoemaker, 1987). Increased use of the lung also is characteristic in circumstances such as increased temperature or activity when the metabolic demand for oxygen exceeds the passive uptake across the skin (Hutchison et al., 1968). *A priori*, there seems no compelling reason why amphibians could not have evolved more effective lungs and used them more dominantly in gas exchange, similar to reptiles. However, this has not been a directional trend in amphibian evolution, and some have argued that reliance on cutaneous gas exchange is the explanation for retention of a generally permeable integument with minimal keratinization in the stratum corneum. Thus, a permeable and pliant integument might be characteristic of modern amphibians either because these attributes are important for aquatic larval stages, and the integument of metamorphosed adults is genetically

constrained, or the conditions are maintained by natural selection in the adult as well as larval stages of development. Either of these possibilities, of course, does not exclude the other (Lillywhite and Mittal, 1999).

Lipids and the evolution of cutaneous water barriers in terrestrial amniotes

The sources of lipids contributing to the epidermal water barrier of amniotes are lamellar bodies, which are discreet lipid-enriched secretory organelles first observed by Selby (1957) and later described in detail by Odland (1960) and Elias and Friend (1975). These and similar structures are present in amniote epidermis generally and are known synonymously as lamellar bodies (Elias and Friend, 1975), Odland bodies (Odland, 1960), keratinosomes (Wilgram, 1965), cementsomes (Hashimoto, 1971), membrane-coating granules (Matoltsy, 1966), lamellar granules (Breathnach et al., 1973), multigranular bodies (Matoltsy, 1969) or mesos granules (Roth and Jones, 1970; Landmann, 1979). These Golgi-derived organelles originate within the epidermal keratinocytes, and their synthesis is upregulated as the cells progressively differentiate. The lipid contents of these organelles appear to consist of stacks of flattened lipid vesicles (Landmann, 1986, 1988) comprising primarily glycosphingolipids, free sterols and phospholipids, which are precursors of the stratum corneum lipids (Fig. 4). Eventually, the lipid contents of the organelles are secreted into the extracellular domain, where they are further processed into compact lipid bilayers that occlude the extracellular spaces among adjacent and overlapping corneocytes (Fig. 4), a condition that has been likened to a 'bricks-and-mortar' organization (Elias, 1983; Elias and Menon, 1991). It has been proposed that acylglucosylceramides serve as molecular 'rivets' to promote flattening and stacking of lipid vesicles that subsequently fuse edge-to-edge to produce lamellae comprising paired bilayers that are stacked parallel to the skin surface (Engström et al., 2000; Wertz, 2000). These form multiple lamellar sheets with smooth surfaces shown in freeze-fracture studies. In this manner, the extracellular lipids form a continuous domain throughout the stratum corneum and function as the principal barrier to water diffusion (Elias and Friend, 1975; Fig. 2). The lamellar bodies also deliver a cocktail of lipolytic enzymes to the extracellular domain, and these convert the polar lipid precursors into nonpolar products that assemble into lamellar structures surrounding the corneocytes (Freinkel and Traczyk, 1985; Madison et al., 1998; Bouwstra et al., 2003b). Bound lipids that are chemically linked to the corneocyte envelopes are arranged in a crystalline packing and act as a template during formation of the extracellular lamellae (Bouwstra et al., 2003a).

In mammals and lepidosaurian reptiles, the lipid contents of lamellar granules are secreted and exocytosed into the extracellular spaces where they become visible as distinctive lamellae (Landmann, 1980; Elias, 1983; Elias and Menon, 1991; Hou et al., 1991; Menon et al., 1994). In most
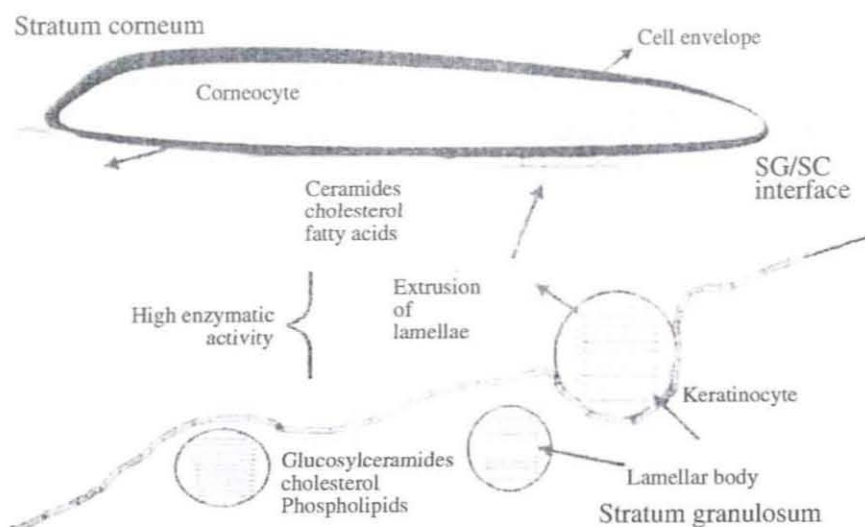
Fig. 4. Schematic illustration of the process involved in formation of intercellular stratum corneum lipids of a mammal following extrusion from lamellar bodies. The lipid content of lamellar bodies is altered in composition and rearranged into long lipid lamellae that fill the extracellular regions in the stratum corneum. Reproduced from Bouwstra et al. (2003b), with permission.

lepidosaurs lamellar granules are limited to the mesos layer of stratum corneum, but they are also present in the α-cells of *Sphenodon* (Maderson et al., 1998; Alibardi, 2003; Alibardi and Maderson, 2003a) and certain snakes (Tu et al., 2002). With reference to squamate integument, lamellar granules are secreted from differentiating α-cells and become organized into lamellar sheets filling the extracellular spaces of a specialized layer known as the mesos layer, which is subjacent to the β-keratin of the stratum corneum. The mesos layer consists of multiple layers of alternating keratin and lamellar lipids, forming a laminated structure sandwiched between the β- and α-keratin of the mature stratum corneum (Figs 1, 2, 3B) (Lillywhite and Maderson, 1982). The lipid sheets of the mesos layer prevent the outward flux of tracer molecules such as lanthanum and comprise the recognized water barrier in squamate reptiles (Landmann et al., 1981; Lillywhite and Maderson, 1982). The lipids concentrated in the reptilian mesos layer, as inferred from studies of shed epidermis, appear to be a complex mixture having a composition resembling that of the mammalian stratum corneum (consisting mostly of cholesterol, free fatty acids, phospholipids and ceramides: Roberts and Lillywhite, 1980, 1983; Burken et al., 1985; Itoh et al., 1990; Elias and Menon, 1991; Weldon, 1996; Ball, 2004). The biochemistry and modulation of these lipids are not well studied in reptiles, but the quantity of lipids in epidermis from a broad range of species correlates generally with the aridity of habitat (Roberts and Lillywhite, 1983).

In avian integument, the lipid contents of epidermis are derived from comparatively large lamellar granules (multigranular bodies; Fig. 3), indeed 3–5 times larger than those of mammals, in addition to large lipid droplets that resemble sebum or oil (Menon et al., 1986). The organelles break down to form large intracellular lipid droplets during terminal differentiation into corneocytes. These fuse with the nascent lipid droplets, and the resulting contents are passed through membrane pores into the extracellular domains of the stratum corneum (Menon et al., 1991, 1986). However, in the absence of water stress these secreted lipids fail to form bilayered structures, and some of the lamellar bodies are retained within the corneocytes where they remain visible as lamellar sheets when stained with ruthenium tetroxide (Menon and Menon, 2000). Thus, among amniotes there are pronounced differences as well as similarities in epidermal lipogenesis and the structural organization of water barrier lipids within the stratum corneum (Table 1; Figs 2, 3). Of course, the details of lipid composition and organization vary among species, environment and location on the body, albeit such variation has been explored in a very limited number of species.

Critical molar ratios among stratum corneum lipids are crucial for permeability barrier formation and function, most importantly cholesterol, ceramides and free fatty acids. A defective water barrier results if the synthesis of either of these lipids is inhibited, or if the enzymatic processing of the lamellar body derived discs is disturbed (reviewed in Menon and Ghadially, 1997). Ceramides account for as much as 50% of the total stratum corneum lipids in mammals (Raith and Neubert, 2000), and desert birds also have higher proportions of stratum corneum ceramides than free fatty acids (Haugen et al., 2003a,b). Adjustments of lipid ratios to favor ceramides over free fatty acids and sterols have also been shown to correlate with reductions of TEWL in desert larks (Haugen et al., 2003a,b). The comparatively higher ratios of ceramides in stratum corneum allow the lipid lamellae of the permeability barrier to exist in a more highly ordered crystalline phase, which creates a tighter barrier to water vapor diffusion (Velkova and Lafleur, 2002; Bouwstra et al., 2003b). Unlike terrestrial mammals, the stratum corneum of marine mammals retains appreciable amounts of glycolipids, and the bilayers are less tight (Elias et al., 1987). The water relations of skin in marine mammals are in need of further study.

Investigations of mammalian stratum corneum suggest that resistance to water permeation is related to tight, gel-like

packing of hydrocarbon chains and changes in lipid phase behavior related to component ratios and molecular arrangement of cholesterol (Potts and Francoeur, 1990; McIntosh, 2003). It also appears that fluid and crystalline phases of sublattices alternate vertically in repetition with stacked lamellae (Bouwstra et al., 2000). The presence and localization of fluid domains facilitates deformation of the lamellae, which evidently satisfies demands for elasticity as lamellae follow sharp edges of cell boundaries. Models further suggest that water diffusion is limited in directions both perpendicular and parallel to the plane of the lipid bilayers (McIntosh, 2003). Comparable molecular and biophysical details related to barrier function are not yet available for non-mammalian vertebrates. However, it seems clear that evolutionary or phenotypic adjustments in barrier efficacy will be founded in alterations of lipid composition, deposition and organization (Williams and Elias, 1987; Critchley, 1993; Menon and Ghadially, 1997).

The reptilian water barrier is fundamentally similar to that of mammals, but may show more taxonomic variation in addition to differences in morphology and renewal. The majority of studies of the water barrier in mammalian stratum corneum have employed skin from humans, neonatal rodents or hairless mice. Most reptilian studies have employed lepidosaurs, and little is known about the comparatively weaker water barrier of turtles and crocodilians. Overall, however, there is broader ecological and taxonomic coverage of reptiles than of mammals.

In addition to the stratum corneum barrier, glandular lipids are deposited exteriorly to the epidermis in both mammals and birds (Hadley, 1991). Sebaceous glands, unique to mammals, produce an oily secretion called sebum that moves outward along hair shafts to distribute generally non-polar lipids rich in triacylglycerols on epidermal or hair surfaces. In birds, 'preen waxes' from the uropygial gland are spread over feathers to prevent water penetration and ingress of bacteria and fungi. Uropygial secretions contain a complex mixture of lipids in which wax esters usually predominate. Glandular waterproofing lipids are also conjectured to pass onto scale surfaces in certain species of snakes (*Malpolon* spp., *Psammophis* spp.), and self-wiping movements by means of the head and chin perhaps function similarly to body-wiping behaviors described for various arboreal frogs (Branch, 1998; Dunson et al., 1978).

In birds and mammals, plumage and pelage appear to impede significantly the passage of water vapor from skin to atmosphere, although the skin remains the principal barrier to TEWL (Cena and Clark, 1979; Webster et al., 1985). In pigeons, for example, plumage contributes 5–20% of total resistance to water loss through the integument, and the plumage and boundary layer together account for 6–26% of total resistance to water vapor diffusion (Webster et al., 1985). Therefore, adjustments of plumage or pelage and seasonal shedding patterns are potential means of adjusting rates of TEWL.

Plasticity of the water barrier in vertebrate integument

Insofar as the more resistant barriers to TEWL among amphibians appear to be either cocoons or extraepidermal lipid sheets formed by wiping, as discussed above (see also Lillywhite and Mittal, 1999), effective waterproofing is clearly facultative among amphibians. Notwithstanding, there remains the enigma of certain frogs (*Chiromantis* and *Hyperolius*) that have been reported to have low skin resistance, and the mechanism has not yet been resolved (e.g. Drewes et al., 1977; Withers et al., 1982; Kobelt and Linsenmair, 1986). However, we have observed wiping behaviors in *Chiromantis petersi* akin to that of *Phyllomedusa* and *Polypedates* in my laboratory, and both extraepidermal lipids and wiping behaviors have been observed in six species of Florida tree frogs having moderate resistance to TEWL (Barbeau and Lillywhite, 2005). Wiping behaviors are context-dependent, and many persons familiar with various wiping frog species have not witnessed the behavior even though they might have observed the animals closely in other contexts (H.B.L., personal observation). Thus, I venture to predict that wiping, and by inference secretion of cutaneous lipids, will be found to occur in a number of anuran species inhabiting semi- or ephemerally arid environments. The stimulus for renewal of the lipid barrier is not known, whether osmotic and related to skin or body dehydration, or to sensory perception of low ambient humidity.

Aside from wiping, some studies have suggested that R_s in some amphibians varies seasonally (Table 2). While mean values of R_s in various anuran species are consistently higher during the dry season compared with wet season, these are not significantly different from wet season values (Young et al., 2005). Clearly, the possibility of R_s acclimatization beckons further investigation.

Water barrier function in amniotes appears to be relatively fixed and characteristic of species, and the heritability of TEWL has been demonstrated in mammals (Furuyama and Ohara, 1993). However, the capacity of animals to adjust R_s when environmental conditions change is also an important aspect of adaptation, including phenotypic plasticity, acclimatization and developmental plasticity. Although studies on the relative importance of 'plastic' responses and genetic variation of skin permeability properties are few, it seems clear that both plasticity and genetic adaptation can account for covariation between TEWL and environment (Eynan and Dmi'el, 1993; Dmi'el et al., 1997; Tieleman and Williams, 2002)

In reptiles, the barrier can be rapidly restored following trauma (Maderson et al., 1978), and some species have been shown to exhibit plasticity for enhancing R_s under conditions of water stress (Kobayashi et al., 1983; Maderson, 1984; Kattan and Lillywhite, 1989). Following injury to skin, hyperplasia of α-keratin ensues to form a wound epithelium, which, as in mammals (Odland and Ross, 1968), rapidly establishes a barrier to water loss in the disturbed tissue (Maderson, 1985; Alibardi and Maderson, 2003b). Experimental tape-stripping of squamate epidermis results in

α-layer hyperplasia, and in this type of barrier repair, no mesos layers are formed until the next cycle of ecdysis (Maderson et al., 1978). Further ultrastructural investigations of the α layers during the repair response might reveal whether newly formed α cells are secreting lamellar lipids to reseal the barrier-defective areas in advance of a pan-body renewal of epidermis and ecdysis that are required to form the mesos layer.

In the lizard *Anolis carolinensis*, barrier effectiveness is upregulated in response to dry environments, regardless of whether animals are allowed to dehydrate and regardless of whether animals undergo ecdysis (Kattan and Lillywhite, 1989). The changes in water permeability have been attributed to changes in skin lipids, likely in response to local stimuli acting at the integument. Recently it was shown that R_s increases considerably following the first postnatal ecdysis in California king snakes *Lampropeltis getula*, and this can be interpreted as an adaptive adjustment to the transition from the aqueous environment of the embryo to the aerial environment of the newborn (Tu et al., 2002). In the studies of neonatal king snakes, there was found a striking correlation relating a doubling of R_s with the first postnatal ecdysis to a doubling of the thickness and lipid histochemistry of the mesos barrier (Tu et al., 2002). While compositional features of these lipids are not yet known in detail, upregulation of the barrier during early postnatal ontogeny might be principally or entirely a quantitative phenomenon (Fig. 5). Others have also suggested that the principal determinant of barrier efficacy is the lamellar organization of exocytosed lipids in the extracellular domain, independent of the lipid molecular composition (Friberg et al., 1990; Menon et al., 1996).

A priori it would seem that permeability of mammalian and avian integument can be rapidly adjusted to the demands of the environment by virtue of more-or-less continuous exfoliation and renewal of corneal cells. In contrast, episodic synchronized sloughing of the entire epidermis in lepidosaurian reptiles involves periodic renewal of corneal cells interrupted by varying periods of stasis when the epidermis is in a resting stage. Insofar as the mesos barrier layer is completed prior to ecdysis and is underlain by keratinizing α-cells, shedding would seem to be a requirement for altering the efficacy of the

water barrier. Indeed, data for postnatal ecdysis of California king snakes in relation to upregulation of R_s suggest this is the case (Tu et al., 2002).

Few data are available for such responses to radically changing environments, and little is known about the processes underlying permeability barrier ontogenesis in vertebrates. The skin of full-term human and rodent newborns possesses a competent water barrier at birth, with rates of TEWL at least as low as in adults (Williams et al., 1998). Barrier formation begins during late gestation and involves a progressive increase in the thickness of skin layers, formation of a multilayered stratum corneum, secretion of lipid lamellar bodies in the interstices of stratum corneum, and transformation of short lamellar disks into compact, continuous, lamellar units (Aszterbaum et al., 1992). The keratinization and barrier formation in skin coincide with changes in the composition of amniotic fluid, and are thought to be essential for protection from amniotic fluid during late gestation (Hardman et al., 1999; Parmley and Seeds, 1970). Moreover, contact of rat fetal skin with air accelerates barrier formation (Williams et al., 1998). Similar changes occur in snake skin, but at a relatively later time in development. In the context of air exposure, it is of interest that newborn snakes undergo ecdysis within a short period (generally a few days) following birth or hatching (e.g. Greene et al., 2002; Tu et al., 2002).

In snakes, it appears the periderm and embryonic epidermis slough within the egg (Alibardi, 2002), and a permeability barrier (beta and mesos layers) of partial competence is formed within the epidermis prior to hatching (Maderson, 1985). In timing this crudely resembles the barrier formation in mammals (Williams et al., 1998). However, R_s increases at both the first and second postnatal ecdysis, indicating a continued capacity for improvement of barrier function, as shown in lizards. Unlike lizards, improvement of R_s appears to be incremented in association with ecdysis. Measurements indicate a threefold improvement of R_s over two postnatal shedding cycles (Tu et al., 2002).

Recent studies of skin from hatchling snakes include observations on lipid inclusions within the α-layer of epidermis (Tu et al., 2002). These inclusions show lamellar as well as electron-lucent morphologies within the mature α-layers, closely resembling what has been described for avian stratum corneum (Menon and Menon, 2000). In the
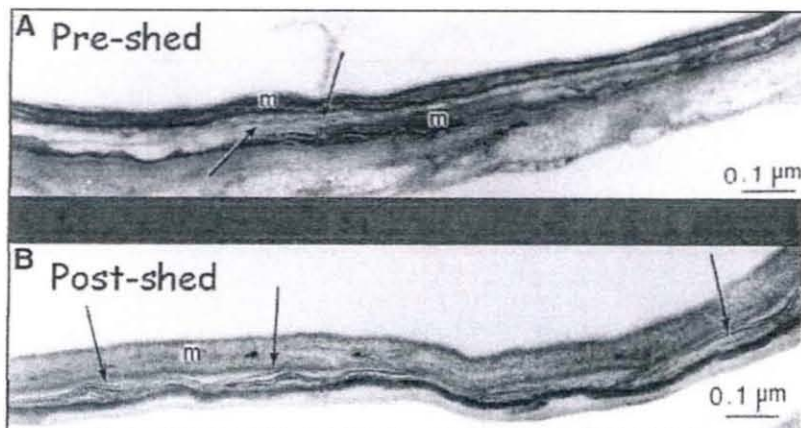

Fig. 5. Electron micrograph showing ultrastructure of epidermis in hatchling king snakes *Lampropeltis getula*, sampled on the day of hatching (A) and 2 days after the first ecdysis (B). The photos feature the mesos layer (m), which pre-shed has disorganized bilayer structures (arrows) but well-organized and continuous bilayers in the extracellular domains of post-shed skin (arrows). The number of bilayers is doubled in the post-shed skin relative to pre-shed. After Tu et al. (2002).

immature α-cells of pre-shed skin, multilamellar bodies and different stages of 'dissolution' of lamellar inclusions into electron-lucent lipids are dominant features, again very similar to what is seen in avian transitional cell layers. These observations point to the intriguing possibility that α-cells themselves might be involved in barrier homeostasis, which has not previously been suggested for ophidian epidermis. The facultative waterproofing ability of avian epidermis (Menon et al., 1996) resides in its capacity to modulate the type of lipids secreted, i.e. non-bilayer, electron-lucent lipids under basal conditions, but lamellar lipid structures under xeric stress, leading to significantly decreased TEWL. Whether snakes and other lepidosaurs can modulate R_s by secreting lamellar lipids from the α layer (previously named cholesterol clefts by Jackson and Sharawy, 1978) has not yet been evaluated, but clearly such a possibility might represent a reserve barrier mechanism. Such a mechanism could explain the upregulation of R_s in dry-acclimated *Anolis* lizards (Kattan and Lillywhite, 1989) and perhaps the variability of R_s observed among hatchling snakes (Tu et al., 2002). There is a trend toward increasing R_s for periods of weeks following hatching in king snakes, although again the largest and statistically significant increments are associated with skin shedding.

The ontogeny of barrier effectiveness demonstrated in snakes differs also from that of altricial species of birds endemic to arid environments. Nestlings of zebra finches *Taenyopygia guttata* have a remarkably tight water barrier that progressively decreases in efficacy as they fledge, evidently allowing evaporative cooling for thermoregulation (Menon and Menon, 2000). However, under conditions of water deficit, adult zebra finches appear to be capable of rapid facultative waterproofing. Within 16 h of water deprivation, TEWL measured in four adult zebra finches decreased by 50%, and the skin barrier efficacy continued to improve until mammal-like values were achieved (Menon et al., 1996). Similarly, TEWL decreased significantly in emus subjected to high ambient temperatures when they were first deprived of water (Maloney and Dawson, 1998). Studies of hoopoe larks, which are permanent residents in extreme arid regions of the Arabian Peninsula, demonstrated rates of TEWL about 30% lower than larks from mesic environments, and these rates decreased significantly when the birds were acclimated to high temperatures (Tieleman and Williams, 2002). However, acclimation of water loss rates was not observed in skylarks and woodlarks from mesic environments in Europe, nor in Dunn's larks from the Arabian Desert. Opposite changes in R_s occur in response to short-term thermal acclimation in other species (i.e. lower R_s in heat-acclimated birds; McKechnie and Wolf, 2004). Thus further investigations are needed to understand patterns of TEWL in response to environmental changes as well as confirm the nature of plasticity mechanisms.

Studies have convincingly demonstrated that TEWL in various vertebrates is closely related to temperature of the skin and generally increases with increasing ambient temperature (e.g. Grice et al., 1971; Mautz, 1982; Marder and Ben-Asher, 1983; Webster et al., 1985; Buttemer and Thomas, 2003). It is
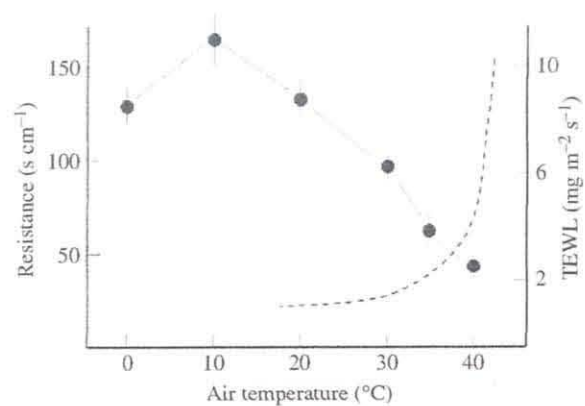

Fig. 6. Whole body resistance to water vapor diffusion (solid circles) and cutaneous evaporation rates (broken line) as a function of ambient temperature in pigeons, *Columba livia*. Data are re-plotted from Webster et al., 1985.

apparent from these and other studies that cutaneous evaporation increases with increased air temperature as a result of both higher skin temperature (Campbell, 1977) and decreased resistance (Fig. 6) to the passive diffusion of water vapor through cutaneous tissues (Webster et al., 1985). Patterns of blood flow changes are no doubt responsible, in part, for these changes in water vapor diffusion through skin. Furthermore, increased hydrostatic pressure in the cutaneous microvasculature of heat-acclimated rock doves *Columba livia* results from adrenergic control of arterial and venous blood flows and elevates water outflow from capillaries leading to elevated TEWL (Ophir et al., 2002). Blood-flow related adjustments in epidermal water supply presumably occur over shorter time scales than do structural changes in cutaneous lipids, and may be responsible for rapid adjustments in TEWL such as those reported for mourning doves by Hoffman and Walsberg (1999). In non-sweating endotherms such as birds, the temperature dependence of changes in R_s and TEWL suggests adaptation for increasing evaporative water loss *via* the skin during heat stress (McKechnie and Wolf, 2004). Studies of model mixtures of stratum corneum lipids demonstrate their stability as crystalline domains at temperatures below 40°C, whereas transitions to a liquid ordered phase occur at higher temperatures (Velkova and Lafleur, 2002). Therefore, adjustments of R_s over a non-stressful, physiological range of temperature are probably not due to phase changes of lipids (see also McClanahan et al., 1978).

Conclusion

There has been much detailed research concerning various physical and chemical attributes of vertebrate integument, including speculations regarding plausible effects on permeability. While virtually all features of integument can influence water passage to some degree, judgments about

effective barrier properties must consider the geometry and properties of blood capillaries as well as the multiple functions of skin in the species considered. The overall picture of water barriers in a broad range of vertebrate taxa shows a central role for lipids in determining R_s. Clearly, however, assessment of water relations of integument must include appreciation of the integrative features of multiple structures and how water relations of the skin relate to water relations of the whole organism. In this context, it is important to appreciate that effective barriers must protect the skin organ itself from desiccation, as well as serving a role in prevention of water loss from the whole body. Thus, barrier properties that appear most effective in terrestrial taxa involve features associated with epidermis, and usually its outermost aspect, where keratin provides a structural framework for laminated lipids.

Comparatively little is known regarding adaptive adjustments of permeability barriers in the contexts of evolution, phylogeny and environment (Lillywhite, 2004). In most cases we do not understand the environmental signals that activate genetic or phenotypically plastic mechanisms. For example, do facultative adjustments in barrier function involve direct sensory perception of ambient humidity, or do mechanisms depend instead on secondary effects such as internal signals related to dehydration and osmotic changes? *Such questions could drive new directions for research* following exploratory investigations such as those of Kattan and Lillywhite (1989). Future improvements in understanding the mechanisms and adaptive significance of permeability barriers will benefit from new questions as well as comparative and experimental approaches that include multidisciplinary contributions from genetics, morphology, physiology, ecology and evolutionary biology.

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The text below is what was submitted to and accepted by Herpetological Review. It includes much more information than was actually published, and may be of use.

PSEUDEMYS NELSONI (Red-Eared Slider). BRITISH VIRGIN ISLANDS: TORTOLA: Road Town: Pond in J.R. O'Neal Botanical Gardens (18° 25' 38" N, 64° 37' 22" W). 21 October 2003. Fred Sibley. MCZ R183688. Verified by Joe Martinez. Museum of Comparative Zoology, Harvard University.

These are the first records of this widely-distributed species from the British Virgin Islands. The pond constructed at the J. R. O'Neal Botanical Gardens in Road Town, Tortola, in the early 1980s (<100 m^2, <1 m depth) is one of the few permanent bodies of fresh water in the British Virgin Islands (BVI). Adult sliders were first noted there soon after construction was completed, and excavations and nests were first observed in the vegetation surrounding the pond in the mid-1990s. Hatchlings were first sighted and captured in 1998, and the population grew to 15-20 adults by the late 1990s (Arona DeWindt, pers. com.). Seining of the pond in 2003 produced 12 adults and approximately 20 juveniles. This indicates the presence of an established, reproducing population on Tortola. Additional capture efforts produced further adults and juveniles in July and October 2004 but did not deplete the population.

This species has been introduced around the world, including the Caribbean, as a result of its prevalence in the pet trade (Lever 2003. Naturalized reptiles and amphibians

of the world. Oxford University Press, New York. 318 pp; Emer 2004. Herp. Rev. 35:34-35). Staff at the O'Neal Gardens observe visitors releasing turtles into the pond several times per year (A. DeWindt, pers. com.), and the chief source of these animals is presumably the pet trade (the two pet stores on Tortola report importing baby sliders for at least the past 20 years). In addition to direct releases by owners, at least 10 turtles donated to the Humane Society on Tortola in recent years have been released at the Gardens (Tessa Gunter, pers. com.; it is also reported that sliders are occasionally "released" into the sea).

No additional populations are known from the BVI, and few suitable habitats exist. The Virgin Gorda specimen does not appear to be part of a population, but rather an escaped pet. It might have originated on Tortola, because O'Neal Garden staff report that visitors occasionally capture and remove juveniles, presumably for pets. At least one of these is known to have escaped elsewhere on Tortola (A. DeWindt, pers. com.). We expect further spread into agricultural ponds and other artificial water bodies to eventually take place, primarily on Tortola. However, scarcity of suitable habitat will likely restrict range expansion of this species in the BVI. Ecological impacts remain unstudied, but are likely to be small because of the localized nature of the invasion and the artificial nature of the habitat.

Submitted by **GAD PERRY** and **JENNIFER L. OWEN**, Department of Range,

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79409-2125, USA (e-mail: gad.perry @ttu.edu), **CLIVE PETROVIC**, H. Lavity Stoutt

Community College, Tortola, British Virgin Islands, **JAMES LAZELL**, The

Conservation Agency, 6 Swinburne St., Jamestown, Rhode Island 02835, USA, and **JIM**

EGELHOFF, BVI Pest Control, P.O. Box 1109, Virgin Gorda, BVI.

Folks,

Fred Kraus just pointed out to me that a paper with our names on it appeared in the last issue of Herp Review for 2005. As you'll see from the attached PDF, they did all kinds of terrible things to it, including taking just about everything we said out. Strangely, the red eared slider note, which was submitted and accepted about a year earlier, remains in limbo. Grrr!

Gad

****

Gad Perry, Assistant Professor
Dept. of Range, Wildlife and Fisheries Management
Box 42125, Texas Tech University
Lubbock, TX 79409-2125, USA

PSEUDEMYS NELSONI (Florida Red-bellied Turtle). BRITISH VIRGIN ISLANDS: Road Town, Tortola: Botanical Gardens (18°25'38.6"N, 64°37'22.1"W). 27 July 2004. Jennifer Owen and Gad Perry. MCZ R-184121. Verified by Jose Rosado. First record for this introduced species in the British Virgin Islands (Powell et al. 1996. *In* Powell and Henderson [eds.], Contributions to West Indian Herpetology: A Tribute to Albert Schwartz, pp. 51–93. SSAR Contrib. Herpetol. Vol. 12). The turtle was collected in a man-made pond that also contained introduced Red-eared Slider Turtles, *Trachemys scripta*.

The Conservation Agency, H. Lavity Stoutt Community College, and Texas Tech University supplied funding support for this project.

Submitted by **JENNIFER OWEN** and **GAD PERRY** (e-mail: gad.perry@ttu.edu), Department of Range, Wildlife, & Fisheries Management, Texas Tech University, Box 42125, Lubbock, Texas 79409-2125, USA; **JAMES LAZELL**, The Conservation Agency, 6 Swinburne Street, Jamestown, Rhode Island 02835, USA; and **CLIVE PETROVIC**, H. Lavity Stoutt Community College, P.O. Box 3097, Road Town, Tortola, BVI.

Another Mystery Crab

From: "Chad Walter" <Walter.Chad@NMNH.SI.EDU>
To: <hq@theconservationagency.org>
Sent: Monday, September 19, 2005 1:46 PM
Subject: Re: For James D. Lazell

> Hello,
>
> Back in 1989 you sent a spider crab from the British Virgin Island, Guana
> Island here for identification. the locality information is written on a
> Museum of Comparative Zoology label.
>
> I have recently found this specimen in the collections, with no
> identification on it, just the paperwork that indicates you sent it here.
>
> I am writing to find out what is to be done with this specimen.
>
> Is it to be returned to you or keep here at the USNM as a gift for
> deposit.
>
>
> Please relpy as to the status of this specimen.
>
> Thank you.

>>> wenhua@etal.uri.edu 09/15 7:14 AM >>>
Hi Chad, It's all yours! Back in those days Fenner Chace was still active
(I don't know if he is still alive!) and we were doing BVI crabs together.

I would like an idetification, though. My book, Island..., U Cal Berk Press,
is momentarily out and includes accounts of all the land and splash-zone
crabs. Nothing called a "spider" crab is in there, so I'm puzzled.

Thanks, Skip Lazell

Thank you for your reply.
The only identification on the specimen is Mithrax sp.

Regards,

T. Chad Walter
Smithsonian Institution
PO Box 37012 MRC-163
10th & Constitution Ave, NW
Washington, DC 20013-7012

ROACHES

wennuɐ

From: "Daniel Perez" <Perez.Daniek̟.............
To: <wenhua@eta:.un.ca:>
Sent: Friday. September 02. 2005 9:55 AM
Subject: Guana roaches

Hi Wennuɐ

...rrv about the long time without communicating. I have been sidetracked by
a miilion things.

Wanted to teil you that I decided to ieave the oniy two other roach species
to add to the list as sp. Both genera have a number of other neotropica
species. The Compsodes specimen is not in very good shape (sort of
flattened). I will return Barry his specimens asap. Most of the things he
gave numbers to were juveniles of species that we already seem to have.

So the "final" list of species is as follows:

1. Hemiblabera brunne:.
2. Eurycotis improcera
3. Peripianeta americana
4. Cariblatta antiguensis
5. Symploce ruficoiiis
6. Symploce pararuficoliis
7. Euthiastobiatta facies
8. Panchlora sagax
9. Plectoptera rhabdota
10. Pycnosceius surinamensis
11. Nyctibora sp.
12. Compsodes sp.

Regards.

- - - - - - -

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Rapid evolution of courtship song pattern in *Drosophila willistoni* sibling species

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Abstract

Courtship behaviours may provide a more reliable means of identifying reproductively isolated taxa than traits such as morphology or many genetic markers. Here we describe the courtship songs of the *Drosophila willistoni* sibling species group, which consists of several species and subspecies. We find that song pattern is species-specific, despite significant differences among strains within species. *D. paulistorum* has the most variable song pattern, which reflects this species' traditional subdivision into semispecies. All the other species could be unambiguously identified by song. The major differences among these species was in the interpulse interval, as has been found in other studies of fly song. However, the interpulse intervals of the species studied here were often multimodal. This was partly due to the presence of multiple song types within the courtship repertoire, but it also reflected changes in interpulse interval within a song type by some males. Unusually, some species had distinctively patterned variation in interpulse interval. Song must have evolved rapidly within the species complex, probably due to sexual selection.

Introduction

The evolution of premating isolation is an important step in speciation. In animals with sexual signalling behaviour, reproductive isolation may be caused by divergence in the structure of sexual signals and receiver biases or preferences (Butlin and Ritchie, 1994). However, whether signal-receiver coevolution or postmating hybrid dysfunction initiates reproductive isolation is unclear (Coyne and

Orr, 1989). If signal divergence is most important, studies of courtship signals in complexes of closely related species may most clearly resolve species boundaries. Following from the seminal work of Bennet-Clark and Ewing (e.g. 1969), the courtship songs of *Drosophila* have been shown to vary substantially among species of many different groups. These include the *melanogaster* group (Cowling and Burnet, 1981), the *virilis* group (Hoikkala and Lumme, 1987), the *repleta* group (Ewing and Miyan, 1986), the *auraria* group (Tomaru and Oguma, 1994), the *bipectinata* group (Crossley and Bennet-Clark, 1993), the *obscura* group (Ewing, 1969), and the Hawaiian planitibia group of *Drosophila* (Hoy et al., 1988). The species-specificity of fly song has often led to song being interpreted as a "species recognition" signal, although the differences could have evolved by sexual selection rather than through character displacement as implied by a species recognition function.

Fly song usually consists of a series of low frequency short pulses, though the *melanogaster* group also produces longer hum (or "sine") song, and the Hawaiian flies produce more complex high frequency songs. The most important parameter of pulse song is the interpulse interval (IPI). Usually it is the arithmetic mean IPI that is assumed to be important to female preferences, and this is typically unimodal (though it may have large variance). Flies of the *repleta* and *obscura* groups have bimodal IPIs, reflecting two different song types (Ewing, 1969; Ewing and Miyan, 1986). Species of the *melanogaster* group also have a complex periodic cycling of IPIs controlled by the *period* gene (Kyriacou et al., 1990; Wheeler et al., 1991) which is also important to preferences (Greenacre et al., 1993).

Here we describe the courtship songs of flies of the *Drosophila willistoni* sibling species group. This Neotropical group has been studied extensively since the 1940s and consists of six species, four of which are polytypic (Ehrman and Powell, 1982). The species have been defined primarily on the basis of hybrid dysfunction (Burla et al., 1949; Dobzhansky et al., 1957) and salivary gland chromosomes (Dobzhansky et al., 1950; Kastritsis and Dobzhansky, 1967). The group includes *D. paulistorum*, a complex "superspecies" of six genetically and behaviourally differentiated races. We examined the song of these species to determine if song resolves further the patterns of variability within this group. We found that most species can be unambiguously identified by song. We also found that song patterns were complex, frequently involving multimodal IPI distributions and some patterning of IPIs.

Methods

The species examined were *D. willistoni, D. equinoxialis, D. paulistorum, D. insularis, D. tropicalis, D. pavlovskiana* and the "Carmody" strain of uncertain taxonomic status (Carmody, 1965). *D. nebulosa*, the closest outgroup for the sibling species, was found not to sing. Interestingly, it had the most elaborate visual courtship display of the group, involving exhibiting patterned wings to the female. The strains of each species examined are listed in Appendix I. Many of these were old laboratory stocks. Perhaps because of this, courtship and song recordings were

often difficult to obtain, resulting in low numbers of individuals recorded for some strains.

Recordings were made onto reel to reel tape using an "insectavox" type of microphone (Gorczyca and Hall, 1987), filtered at around 150 Hz to 2 kHz and digitised at a rate of 4 kHz using a Cambridge Electronic Design 1401. Females were silenced by wing removal. Observations of unmanipulated females during courtship showed they occasionally make short buzzes, probably as rejection signals. Time of day was uncontrolled and male age was variable. Preliminary analyses suggested IPI was not correlated with male age. All recordings were made within the temperature range 20–30° C. IPI decreases at higher temperatures (e.g. Fig. 4), which is commonly found in studies of insect song (it is normally assumed that female preferences will show similar variation with temperature).

Analysis was carried out on a PC using custom written programs in the "Spike2" language (Copyright C.E.D.). These present histograms of all the IPIs detected in the recordings, an example of which is shown in Fig. 1. It is possible to edit the IPI information (to remove those due to background noise, for example) and to select subsets of histograms from which to extract statistical information. The programs allow the experimenter to examine both IPI and song pattern simultaneously (as in Fig. 2) to establish which song types are responsible for which peaks on the histogram. Further details of the methods used are given in Ritchie and Kyriacou (1994).

The major mean IPI was calculated from the largest peak of the histogram of pulse songs (e.g. the second peak in Fig. 1). Additional mean IPIs were calculated from the next largest peak, if present. These data were analysed using a generalised linear model (GLIM) (Horton, 1978) with strains or species as groups, temperature
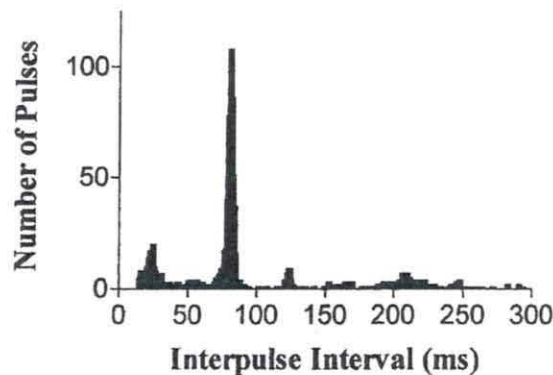

Fig. 1. An example of a histogram of interpulse intervals detected within a recording of fly song (in this case of *D. willistoni*). The multimodality is due to the presence of multiple song types. In this case the peaks (left to right) are due to rasps, a major primary pulse song peak, a smaller minor primary pulse song peak then secondary pulse song.
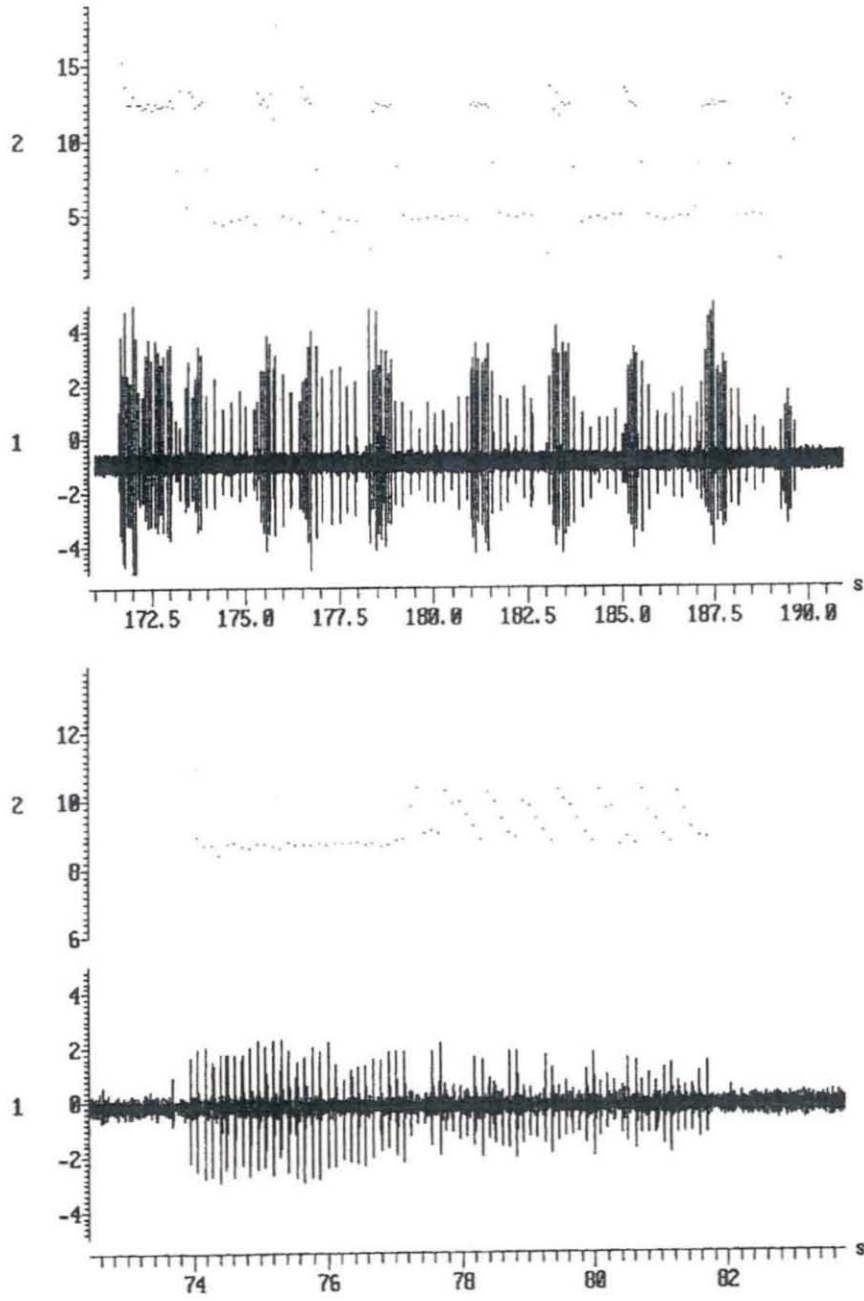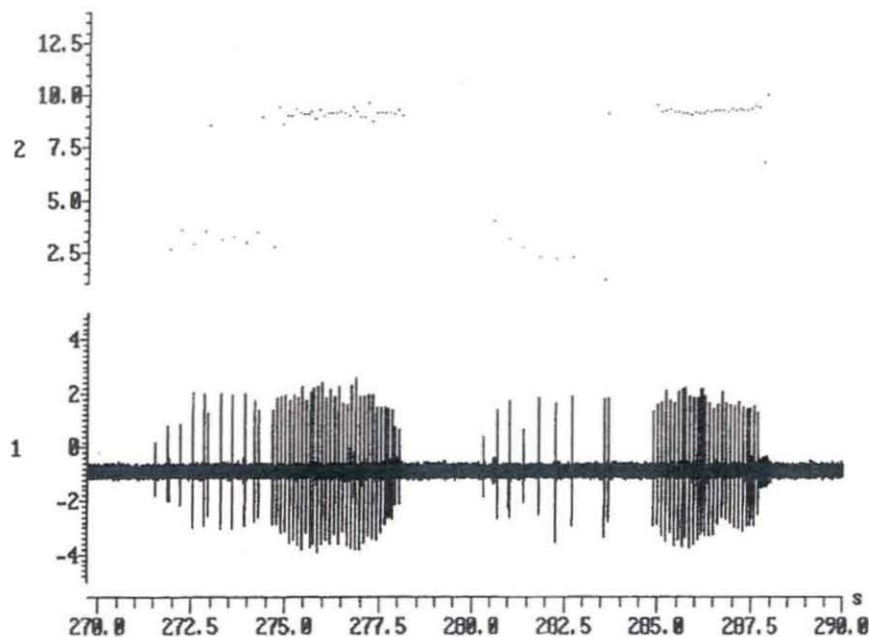
466

Fig. 2(a, b)

Fig. 2. A typical song burst of (a) *D. willistoni* (example shows a burst containing alternating primary and secondary song), (b) *D. equinoxialis* (example shows a clear transition from unimodal primary to patterned secondary song with rising and falling IPI) and (c) *D. paulistorum* (example shows bimodal primary song). Trace 1 is the song pattern (Y-axis arbitrary intensity units, X-axis time in seconds). Trace 2 shows each IPI expressed in Herz.

as a covariate and number of IPIs as a weighting factor (mean = 257). All quantitative data presented has been corrected to a temperature of 25° C using the regression coefficient from the GLIM.

Results

Song types

We have termed the major song types present in the *D. willistoni* sibling species: 1) pulse song, 2) rasps and 3) trembles. No equivalent of the hum song of the *melanogaster* group species is produced.

1) Pulse song is the most common song type and is similar to the pulse song of other species of *Drosophila*, i.e. rapid transients of low frequency noise. There are

two distinct types of pulse song produced by most of these species. "Primary" pulse song is produced by all the species, usually early in courtship. Both wings are involved and are held at an angle of about 40 degrees. Wing movements are small. The IPI of primary pulse song is generally unimodal, but bimodality was not uncommon. IPI sometimes increases very slightly within a burst. In later or more advanced stages of courtship, particularly after the male has contacted the female, a "secondary" pulse song is produced, in association with the primary song. This is particularly pronounced for *D. equinoxialis* and *D. willistoni*, and is produced by a single more fully extended wing moving through a greater angle. We distinguish these two song types as they occurred in differing contexts and could clearly be distinguished "by ear". In *D. equinoxialis* the mean IPI was similar in the two song types, although the pattern of IPI was more complex in secondary song (see later). In the other species, IPI differed between the two song types.

2) Rasps are short bursts (up to around 200 ms duration) of pulses with a much shorter IPI than pulse song, generally less than 30 ms. In some species (particularly *D. insularis*, *D. tropicalis* and some strains of *D. willistoni*), rasps are closely associated with pulse song and feature distinct pulses, so they are probably part of courtship signalling. In the other species it was not evident if rasps were part of courtship or served a rejection function.

3) Trembles were persistently produced during courtship by *D. tropicalis* only. They were produced by short (around 50 ms) rapid vibrations of the whole body and produced a low buzzing sound at around 350 Hz. Trembles sometimes have a pulsatile structure. It is possible that these were mainly vibrational, occurring once a male was in contact with a female. Note that here "tremble" refers to a whole body vibration that produces a sound. This is in contrast to Spieth's (1947) use of the term to signify the slight vertical wing movement performed by *D. willistoni* early in courtship before song is initiated.

Species descriptions

D. willistoni

D. willistoni produced all song types except trembles. Rasps had a mean IPI around 20 ms and could either be unassociated with pulse song, or occur at the start or end of a burst of primary pulse song. Primary pulse song was usually unimodal with a mean IPI around 90 ms. Secondary song involved louder pulses with a longer IPI, around 200 ms. Secondary song only occurred after a burst of primary song and alternated with primary song through the burst to produce a distinctly patterned train of pulses (Fig. 2).

There was significant variation in the IPI of both primary and secondary pulse song between the strains studied (Tab. 1). Secondary song was much more variable within a strain although there were fewer data points for secondary than primary song. Higher primary song IPI values do not correlate with higher secondary song IPI.

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Table 1. Interpulse intervals of *D. willistoni**.*

Strain	IPI (primary song)	SD	IPI (secondary song)	SD
Caño Mora	93.15	1.26	193.39	6.40
Habitatue	86.29	1.51	174.79	5.10
Belize VI	90.69	1.40	200.19	5.22
Lima	95.74	2.05	242.09	15.19
Atlixco	93.81	2.09	185.99	16.98
Belize II	92.69	1.03	190.99	5.63
Guana	85.82	0.89	214.19	3.62

* Strains with fewer than five individuals recorded were omitted from this analysis. Test for variation between strains; $F_{6.52} = 9.08$, $p < 0.001$ (primary song), $F_{6.31} = 8.65$, $p < 0.001$ (secondary song). IPI = interpulse interval, SD = standard deviation.

D. equinoxialis

D. equinoxialis produced all song types except rasps. The primary song was more likely to have a bimodal distribution (with one type per burst) than any other species, with about half of all recordings having a clearly bimodal distribution. The major peak was always that corresponding to a longer IPI. The secondary song of this species was highly distinctive. Mean IPI was unchanged from the major peak of primary song (and is not distinguished in IPI analysis), but showed greater variance. This reflected a clear patterning in secondary song such that IPI shortened then increased in a repeated manner (Fig. 2b). Secondary song occurred with individuals from several strains of *D. equinoxialis*, though it was often difficult to obtain clear recordings due to the lower amplitude of the patterned song. Burst length was longer.

Both the major and minor IPIs of *D. equinoxialis* showed similar variation between strains (Tab. 2). It should be emphasised that the secondary song of *D. equinoxialis* contributes only a little to the major peak of IPIs and that this is quite distinct from the secondary song of *D. willistoni*.

Table 2. Primary song interpulse interval of strains of *D. equinoxialis**.*

Strain	IPI (major peak)	SD	IPI (minor peak)	SD
0.741.0	129.92	1.10	78.27	3.15
Puerto Rico	136.62	1.11	91.80	2.96
Lee's A	131.42	1.90	79.98	6.29

* Strains with fewer than five individuals recorded were omitted from this analysis. Test for variation between strains: $F_{2.20} = 10.66$, $p = 0.001$ (major peak) and $F_{2.7} = 5.63$, $p = 0.04$ (minor peak). IPI = interpulse interval, SD = standard deviation.

D. paulistorum

D. paulistorum was particularly difficult to obtain song recordings from due to a lack of vigour and a low amplitude song. This species had the simplest song of the group, with secondary song rarely, if ever, heard. However, the low vigour possible makes it unwise to conclude that the species lacks this song. A few individuals (of the Andean-Brazilian and Transitional races) produced distinct rasps with a short IPI of less than 20 ms.

The pulse song was clear and very distinct. Two IPI peaks were found in about a third of recordings (from the Amazonian, Centroamerican and Transitional races). These reflected longer IPIs (Fig. 2c), often due to an individual doubling the IPI of pulses occurring between different bursts. Only the major peak was analysed statistically, and this showed substantial variation among strains (Tab. 3). This broadly supports the findings of Ewing (1979), although he concluded that only the Amazonian race differed in IPI.

Other species

The last four species were analysed together as few individuals were recorded of each (Tab. 4). D. tropicalis was characterised by a very high proportion of

Table 3. Primary song interpulse interval of strains of D. paulistorum*.

Strain	IPI (major peak)	SD
Amazonian	108.04	1.96
Cantroamerican	79.55	4.37
Interior	80.33	2.04
Andean-Brazilian	67.89	2.35
Transitional	84.43	2.56
Tame	71.44	1.55
S	54.37	3.04

* Many strains had very few individuals recorded. Even so, there was significant variation between strains; $F_{6,23} = 57.58$, $p < 0.001$ (major peak). IPI = interpulse interval, SD = standard deviation.

Table 4. Primary song interpulse interval of other species.

Species	IPI (major peak)	SD	IPI (minor peak)	SD
D. tropicalis	77.98	9.12	108.38	4.82
D. insularis	63.39	1.81	118.79	3.95
D. pavlovskiana	74.50	5.69	354.76	5.89
Carmody	84.98	2.26	127.49	1.76

There was significant variation between species for each song type ($F_{3,18} = 18.49$, $p < 0.001$ for major peak and $F_{3,19} = 514.67$, $p < 0.001$ for minor). IPI = interpulse interval, SD = standard deviation.

courtships involving trembles. A clear recording of secondary pulse song was obtained from only one individual and had a pattern like that of *D. willistoni*. *D. insularis* more regularly produced a secondary type of pulse song. The pattern was rarely clear, but seemed also to be of the *D. willistoni* type. *D. pavlovskiana* song closely resembled that of *D. paulistorum*, even having a small proportion of very long IPIs as was found for some races of *D. paulistorum*. Finally, the Carmody strain frequently produced pulsatile trembles like *D. tropicalis*. Secondary song was *D. willistoni*-like but unclear.

Interspecific comparisons

Pulse structure

Spectral properties of pulse song can be species specific (e.g. Bernstein et al., 1992), though there is little evidence to suggest that intrapulse frequency is likely to affect female preferences. We compared the spectral properties of the songs of these species using FFT techniques, by comparing averaged 1024 point FFTs taken over individual bursts, and carrier frequency was estimated as the largest peak from the resulting averaged spectrum. Figure 5 shows examples of individual pulses and FFTs for each species. There was no significant variation among species over the samples analysed (Tab. 5). However, all four individuals of the Cuba strain produced pulses with a low carrier frequency and a distinctive pulse shape (see Fig. 3), though otherwise this strain was typical for *D. equinoxialis*.

Pulse widths were all around 4 ms, which only allows a maximum of two cycles to occur within a pulse. This will be a major cause of the "messy" (broad spectrum) FFTs and implies that intrapulse frequency cannot be a reliable component of the information content of song. Surprisingly, pulse and spectral strcture did not vary consistently between song types within individuals. Given the lack of resolution and probable unimportance of intrapulse frequency, we did not concentrate on this

Table 5. Spectral analysis.

Species	N	CF(Hz)	SD
D. willistoni	9	514	52
D. equinoxialis	7	471	149
D. paulistorum	8	562	115
D. insularis	6	579	72
D. tropicalis	1	430	--
D. pavlovskiana	3	529	117
Carmody	5	674	197
Combined	39	546	127

Strains are ignored in this classification, though more than one strain was included wherever possible. There was no significant variation between species ($F_{6,32} = 1.8$, NS). N = number of flies examined, CF = carrier frequency, SD = standard deviation.
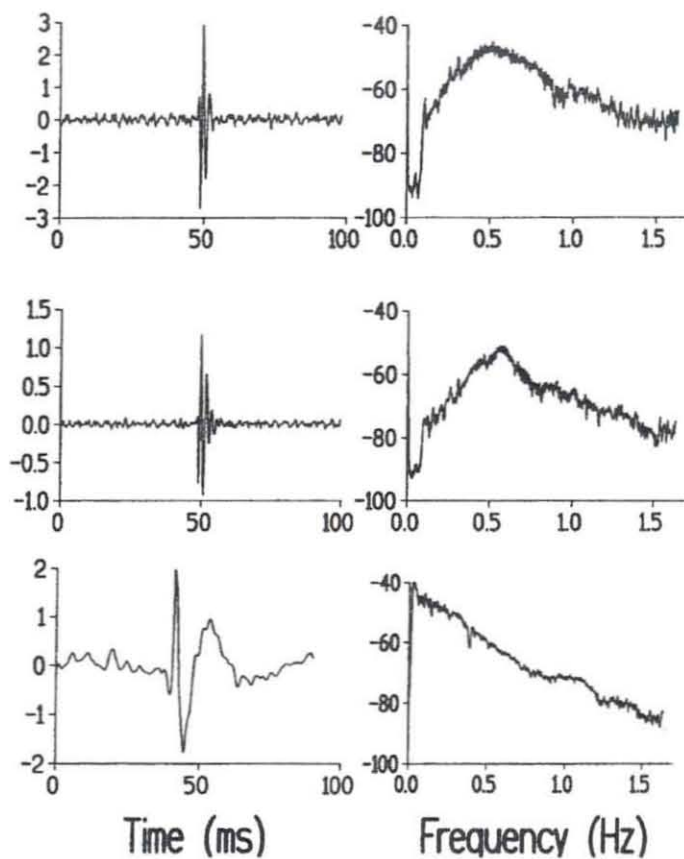
Fig. 3. Examples of (left) pulses and (right) FFT frequency spectra of the primary pulse song of (top to bottom). *D. willistoni*, typical *D. equinoxialis*, Cuban *D. equinoxialis*, *D. paulistorum*, *D. insularis* and *D. tropicalis*.

aspect of analysis. It is possible that there are differences that a more thorough analysis could detect.

Pulse song comparison

Figure 4 shows the major peak of IPI versus temperature for the main species examined; there is species-specific divergence for this single character despite the differences between strains within species. *D. paulistorum* is more variable than the other species with the A28 and S strains being most distinct.
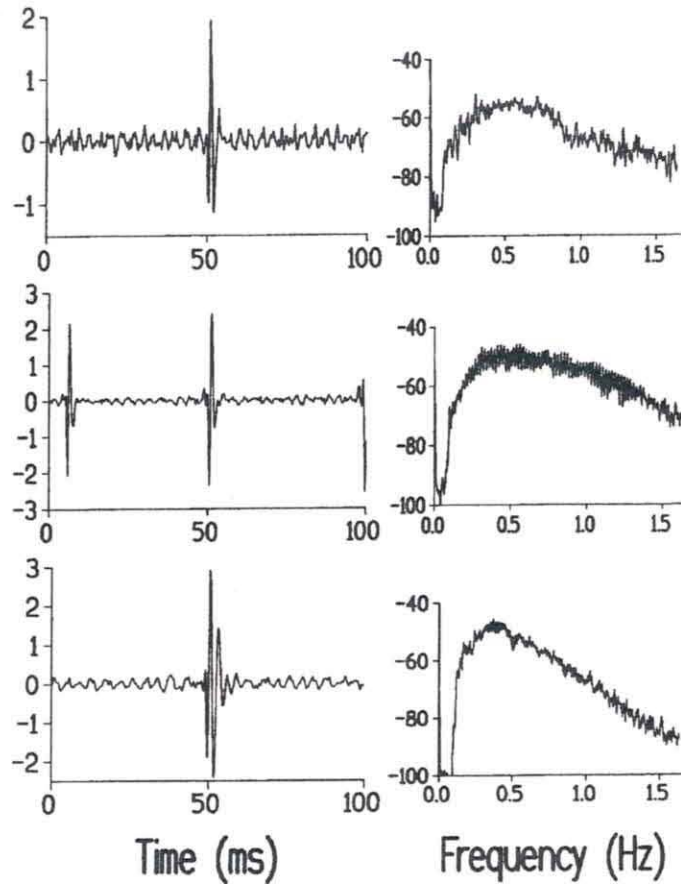
Fig. 3. (continued)

To examine species differences further, a canonical variate analysis was carried out (using the "Discrim" function of the MINITAB statistical package). Strains within species were ignored so that species were used as groups and the first and second peaks of IPIs were used as the characters. First, all data were temperature corrected to 25° C using the regression coefficients from Table 6 (except for the heterogeneous second peak in *D. paulistorum*; note that the effect of temperature on IPI did not vary between species). A reallocation routine for this canonical analysis, where all individuals are reallocated to groupings based on the composite variate, successfully reallocated every individual to the correct species except for the heterogeneous *D. paulistorum* and the Carmody strain (which can form hybrids with some *D. paulistorum* and some *D. equinoxialis* strains), see Table 7. Mean IPI

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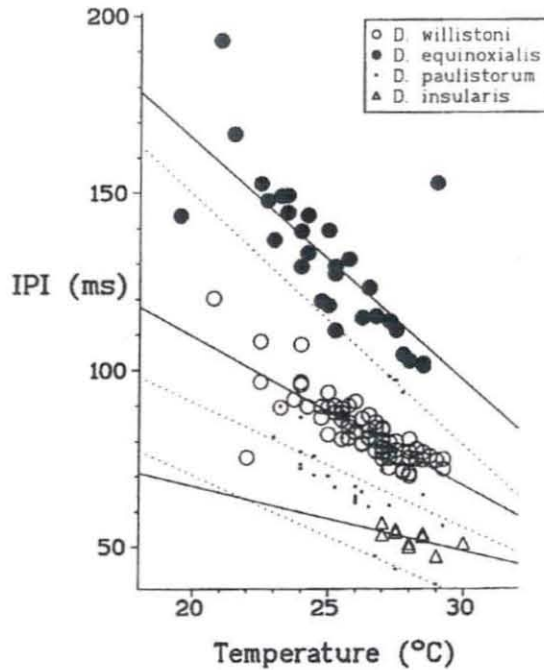
Fig. 4. The interpulse interval of the major pulse song versus temperature for *D. willistoni*, *D. equinoxialis*, *D. paulistorum* (separate lines are fitted for the A28 and S strains) and *D. insularis*. Each point is the mean IPI of the major peak in the IPI histogram of one male, and the figure clearly shows how distinct each species is.

Table 6. Regression coefficients for temperature*.

Species		Regression coefficient	SD	p
D. willistoni	peak 1	−5.42	0.39	<0.001
	peak 2	−10.06	1.95	<0.001
D. equinoxialis	peak 1	−8.20	0.55	<0.001
	peak 2	−3.15	1.46	0.07
D. paulistorum	peak 1	−3.96	0.60	<0.001
Other species	peak 1	−3.98	1.04	<0.001
	peak 2	−5.47	1.31	<0.001

* Results are from a separate GLIM analysis for each species (the interaction term between population and temperature was ommitted from these analyses as many strains were poorly sampled). The data combined showed no heterogeneity in the effect of temperature between species for the major IPI (peak 1 of primary song, coefficient = −5.64, interaction between species and temperature NS).

Table 7. Results of canonical variate analysis.

Species	W	T	E	Pa	I	Pv	C
N	41	3	10	10	9	2	6
NA	41	3	10	0	9	2	1

Squared "Song Distance" Between Species

	T	E	Pa	I	Pv	C
W	3.97	38.64	2.47	11.25	16.48	3.21
T		49.99	0.74	3.47	23.69	0.08
E			54.19	79.79	99.33	50.84
Pa				3.19	16.06	0.32
I					19.79	3.31
Pv						20.97

Groups are *D. willistoni* (W), *D. tropicalis* (T), *D. equinoxialis* (E), *D. paulistorum* (Pa), *D. insularis* (I), *D. pavlovskiana* (Pv) and the Carmody strain (C). N = number in group, NA = number correctly allocated to group by Minitab reallocation routine.

alone therefore contains enough information to allow identification of most of the species.

Discussion

The *Drosophila willistoni* sibling species consists of a "cluster" of closely related species and subspecies, most of which were defined by the presence or absence of fertile hybrids in crosses between laboratory strains. The species are difficult to diagnose by morphological analysis. Here we show that a courtship signal, male song, varies greatly in both qualitative and quantitative aspects among species.

The basic unit of *D. willistoni* sibling species song is typical of *Drosophila*, a low-frequency rapidly transient pulse. This was structured into apparently distinct song types; a primary song typically occurring early in courtship, which often led into a different secondary song later in the courtship sequence. Most species also produced short rasps during courtship. IPI was therefore multimodal, sometimes within a song type. At a gross level, there were three major forms of song corresponding to the three "main" species studied. *D. willistoni* had a primary song IPI around 100 ms and a relatively simple secondary song with a longer IPI. *D. paulistorum* had a major IPI peak around 70 ms, with occasional longer minor peaks. This species rarely if ever produced secondary song. *D. equinoxialis* often produced bimodal primary song with a major peak around 120 ms and a smaller peak around 80 ms. Secondary song had a similar IPI to the major primary song peak, but was sometimes strikingly patterned. Such patterned temporal variation in IPI has rarely been described for fly song, though a notable exception is the approximately one minute long rhythm or cycle in flies of the *melanogaster* group

(Kyriacou et al., 1990). While the pattern we describe is not analagous to the song cycle of *D. melanogaster*, it is interesting to find another example of such "higher level" patterning of IPIs.

Fewer individuals of the other species studied were recorded. *D. pavlovskiana* produces a song that is the most paulistorum-like, as might be expected because it was originally designated as a *D. paulistorum* race. *D. tropicalis* and *D. insularis* are very similar, though the former is distinguishable by a high incidence of trembles during courtship. In song form these two species were more like *D. willistoni* than *D. equinoxialis* (because they lacked a secondary song with a patterned IPI), though IPI information clearly separated them from *D. willistoni*. The Carmody strain had elements of both paulistorum-like and willistoni-like song. These results are generally similar to the proposed taxonomic grouping of Spassky et al. (1971) and somewhat like the proposed phylogenetic relationships based on allozymes (Ayala et al., 1974). The much greater differences among strains of *D. paulistorum* than the other species also corresponds to the accepted phylogenetic interpretation, with this species being subdivided into many subforms. Although it is possible to construct a phylogeny based upon our 'song distance' data, we have not done so because of a lack of confidence that similar IPI values will reliably represent homology, and the lack of a well defined alternative phylogeny with which to compare it.

The extent of the song differences, as observed Fig. 4, the success of the discriminant function in reallocating individuals, and the existence of substantial differences among strains within species suggests that song is evolving more rapidly than other traits (such as morphology) within this group. This is consistent with general comparisons that imply that courtship behaviour may vary more than morphology or other traits in closely related species groups (Butlin and Ritchie, 1994). There are many selective forces which may determine the evolutionary rate of such behaviours; direct environmental sources of selection, reproductive character displacement or reinforcement, sexual selection and genetic drift. It is rarely possible to identify which particular source is most important in any particular system. Rapid evolution, as seems to be occurring to song pattern in the species studied here, may be particularly likely to occur in species in which signal-receiver sexual communication is dominated by one or a few signals. Coevolution of such systems could result in rapid divergence between geographically isolated races (Lande, 1981), perhaps even in the absence of postmating isolation or direct selection on the mating signal. However, comparisons of post- and premating isolation between sympatric and allopatric populations of *D. paulistorum* (Ehrman, 1965) and *Drosophila* in general (Coyne and Orr, 1994) suggest that the relationship between these distinct causes of reproductive isolation is complex. Premating isolation is greater between sympatric forms, as might be expected, but the extent to which premating isolation may evolve as an evolutionary response to hybrid unfitness is not clear. There are numerous theoretical problems with the reinforcement or reproductive character displacement models of speciation, yet the most rapid evolution of premating isolation observed between sympatric forms of *Drosophila* provides indirect evidence in favour of these models.

Unfortunately, the strains of *D. paulistorum* examined here do not allow a comparison of song divergence between pairs of semispecies showing different levels of premating isolation (Ehrman, 1965). Such a comparison might indicate if these songs were directly correlated with premating isolation. It seems likely that the courtship song will influence premating isolation, though it is difficult to directly analyse the influence of song variation on female preference in flies as they do not lend themselves well to playback experiments. Some such experiments have shown that song influences female mating receptivity in other species groups (e.g. Greenacre et al., 1993; Crossley and Bennet-Clark, 1993), but we have not had any success in playback experiments with *D. willistoni* and *D. equinoxialis*, primarily due to short mating times and the lethargy of *D. equinoxialis*. It is highly likely that other behaviours also contribute to reproductive isolation.

There are both potential advantages and disadvantages in using traits such as courtship song for taxonomic investigations. Many behaviours are not easily quantified, as they require qualitative differences to be evaluated alongside quantitive ones. The relative importance of such differences must be evaluated subjectively. The "song distances" resulting from the discriminant analysis broadly follow the subjective categorisation of song differences presented above.

Our results suggest that, although sexual signals evolve rapidly during evolution, they may retain some phylogenetically useful information as distinct clusters are maintained. A great advantage of behavioural studies is their ability to produce diagnostic traits among clusters of closely related species. If sexual selection is a more common initiator of the speciation process than hybrid dysfunction, these traits may be the most relevant for the identification of reproductively isolated taxa.

Acknowledgements

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Appendix 1

Species and strains used in the study

Species	Strain	Location	Source	# Recordings
D. willistoni				
	Guana	Guana Island	P. Chabora	18
	Atlixco	Atlixco, Mexico	F. Ayala	9
	Belize II	Belize	F. Ayala	9
	Caño Mora	Costa Rica	F. Ayala	8
	Belize VI	Belize	F. Ayala	7
	Guadeloupe	Guadeloupe	P. Chabora	7
	Lima B	Lima, Peru	F. Ayala	7
	L' Habitatué	L' Habitatué	P. Chabora	6
	Willi7		L. Strausbaugh	6
	0811.4	Cuernavaca, Mexico	Bowling Green	5
D. equinoxialis				
	0741.0	La Hina, Honduras	Bowling Green	10
	Puerto Rico	Puerto Rico	P. Chabora	6
	Lee's A	Aguas de los Rios, Brazil	L. Ehrman	6
	La Maya	La Maya, Cuba	P. Chabora	4
	0741.1	Tefe, Brazil	Bowling Green	3
D. paulistorum*				
Amazonian	A28	Belem, Brazil	L. Ehrman	5
Andean-Brazilian	AB	Mesitas, Brazil	L. Ehrman	3
Centroamerican	C2	Lancetilla, Honduras	L. Ehrman	3
Interior	I-Colo	New Llanos, Colombia	L. Ehrman	3
Transitional	TI	Santa Marta, Colombia	L. Ehrman	3
Unknown	Tame	Tame, Colombia	F. Ayala	12
Unknown	S		J. Powell	4
D. insularis				
	FA	Lesser Antilles	L. Strausbaugh	6
	WE2		L. Strausbaugh	5
D. tropicalis				
	0801.0	San Salvador, El Salvador	Bowling Green	7
D. pavlovskiana				
	P – 1	Georgetown, Guyana	L. Ehrman	4
"Carmody"				
	K2	Brazil	L. Ehrman	8

* *D. paulistorum* is a cluster of 6 semispecies. Semispecies designations are given, when known.

Dear Dr. Gleason, Did you get my snail mail? Our server went down and all our email 20-24 Sept went to Blue Heaven with Buddha's elephant. Onwards! Skip Lazell

The Conservation Agency
6 Swinburne Street
Jamestown, RI 02835, USA

FRUIT FLIES
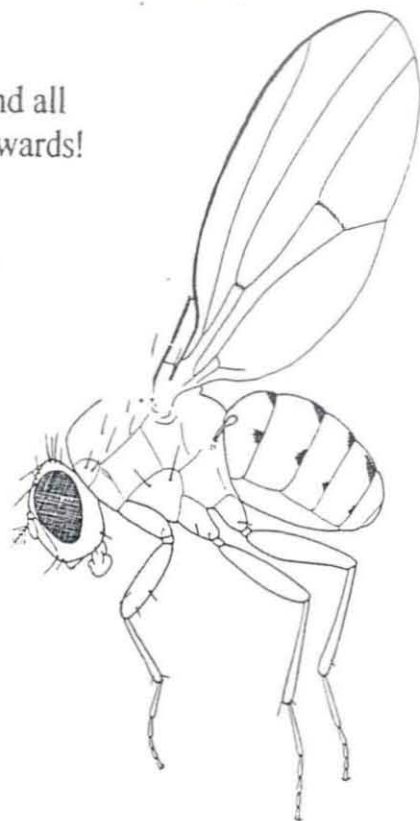
A pomace fly, *Drosóphila* sp.·

Dear Skip,

I'm terribly sorry, I haven't been good at keeping up with email lately. I got your emails and your regular US mail. School started in August and it always takes me a couple months to catch up again.

I haven't really thought about working on Guana island Drosophila anymore, which is not to say that I might not be interested sometime in the future. At the moment I've switched direction and I have been working in the Drosophila melanogaster group (boring flies, found everywhere, but genetically tractable). However, I've always wanted to get back to the willistoni group. Besides willistoni, I think that D. insularis should be found there. If anyone were to collect flies, I'd be happy to take them.

Hope Hollocher, at Notre Dame, has worked quite a bit on Lesser Antilles Drosophila, although in a completely different species group, the cardini group. She might also be interested in Drosophila from Guana (though, she like me, has turned to D. melanogaster lately because of the genetic tools available).

Cheers,
Jenny

GENERAL INSECTS

From:	"Dr. Barry & Buena Valentine" <buenavalentine@comcast.net>
To:	"Wenhua Lu" <wenhua@etal.uri.edu>
Sent:	2005年12月23日 7:23
Subject:	Guana update

Skip: when you phoned the other day, we were at the Entomological Society of America meetings in Fort Lauderdale; reception was poor and ended abruptly. So here is an update. You mentioned mordellids...all I know is that Wenhua had a female of a new species, and I sent her a male - I have no others, and was not aware of other specimens until you mentioned them.

We are finally starting to get input from some of the specialists to whom we have sent specimens, and we have also added a bunch of new species records plus new voucher records for the synoptic collection. We have 31 beetle species to add, raising the total to 442, plus 36 more voucher records, and there is still a backlog of specimens to be mounted and studied.

Now that we are interested in climate change, yearly fluctuations in abundance are being watched more closely. Based on our notes, October, 1999 and 2000 were dryer, 2001-2003 were intermediate, and 2004-2005 were wetter. Examples: Carabidae: during 1999-2001 we took a total of four species and 12 specimens. In 2002-2003 we got 11 species and 61 specimens, and in 2004-2005 we took at least 16 species and 272 specimens. Note: all visits had similar collecting techniques. In contrast, Tenebrionidae: which we expected to peak in the dry years were most diverse and abundant in the "average" years 2001-2003, and did less well in both dry and wet years. In fact, checking of about 20 families reveals no family with a dry year peak. It appears (from preliminary data) that most Guana beetles are adapted to average or wet years...this on an island usually characterized as "semi-arid".

Hemiptera: We have been checking our corixids and were surprised to find three distinct species on Anegada, and what tentatively looks like two species (both Trichocorixa?) on Guana. Since we have been invited to visit and work in the Smithsonian this Spring, we will take specimens for checking. Our four families of Pentatomoidea (stink bugs and relatives) have been identified by Dr. Joe Egar, Tampa, FL. Of 21 V.I. species, we have 19 on Guana, while seven other Virgin Islands total 13 species with a maximum of eight (on Tortola). The two species not yet found on Guana are known from one specimen each from half-way up Sage Mt. I haven't seen the Necker species. In your book on p.261, you list a Guana pentatomid genus: Chinavia, which is unknown to me. Can you tell me where the specimen is, or who identified it?

Homoptera: Charles Bartlett has agreed to identify our Fulgoroids so I am ready to send him a mess of them. We have over 20 obvious species but I don't know the final total.

Orthoptera: The long-horned grasshoppers and crickets have been checked by Dr. Thomas Walker, a recognized authority, at the University of Florida. It turns out they are in chaotic condition. Of 19 species on Guana, 13 are uncertain, and for some, even the genus is uncertain. However, there is some good news...Dr. Dan Otte at the Academy of Natural Sciences of Philadelphia, is revising the Caribbean crickets, and wrote asking to borrow them. I'm packing them now and should have them back this Spring. The Guana fauna as I know it at present has: Gryllidae - 7 genera and 10 species; Tettigoniidae: 5 genera and 7 species; and Gryllacrididae: 1 or 2 genera, and 2 species. A number of other orthops are conspicuous by their absence - mole crickets, and tetrigid grouse locusts. Of these 19 species, only six have firm identifications! The only additional grasshoppers are three species of short-horns (or locusts) family Acrididae, bringing the total Orthoptera to 22. One interesting note: among the many crickets we collected, two which we killed and pinned turned out to harbor tachinid fly parasites. One large maggot emerged from each, apparently unharmed, pupated, and transformed in Florida to adult. These must be pretty tough critters...both crickets became moldy after mounting, and were submerged in 75% (Cruzan) alcohol for several hours. One of the maggots emerged subsequent to this treatment, and the other emerged while IN the alcohol. I dried it with a paper towel and in a few days it pupated and about three weeks later an adult fly emerged. I still find this hard to believe, but it is true.

Coleoptera: Guana now has approximately 440 species. Of these I have 370, and Ivie has 345. Mike has 70 which I lack, and I have 94 which he lacks. I don't know how many uniques and doubles Mike has, so I ran a Chao estimate on my stuff. Chao predicts 467. I haven't computed the variance, I don't have Chao, 1987. It is my gut feeling that Chao is too low...we'll see.

There is lots of other minor stuff: a second species of Dermaptera; a new tribe of ants: Dacetini, species unknown; one less termite name because of new synonymy - my notes are in Ohio, but if memory serves, it involves Nasutitermes, I'll get you the data and citation when I head back north. The Neuroptera count is now doubled to at least 12 sp., but the specialist has just retired and I have temporarily lost contact. Etc. etc.

2005 was a particularly good but also exasperating session. We have masses of unmounted material which is being processed now. The delay was due to two factors. We used up 1200 pins, and ran out during the session; and it was so humid, I couldn't point mount the small fry because our water-soluable glue would not hold. Then, even though Buena rigged up heaters using light bulbs, we still had problems with mold and rust...so much that we have now switched to more resistant (and more expensive) stainless steel pins. As you know, collecting in the tropics has some unique problems.

Dermaptera: earwig

We will keep you posted on progress. And we send our very best wishes.
Barry, Buena, and Susan
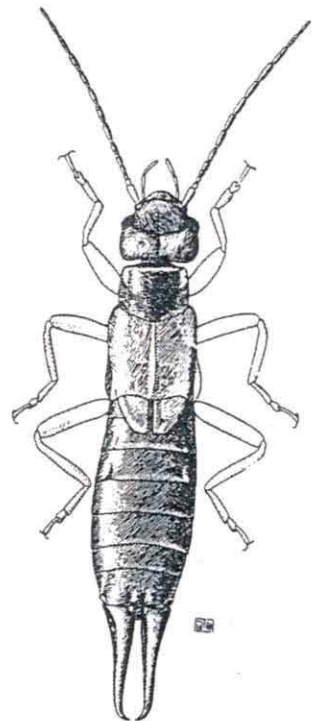
>===== Original Message From "Dr. Barry and Buena Valentine"
<buenavalentine@comcast.net> =====
>13 April, 2006
>Skip and Wenhua:
>
> I sent a sample (of several hundred Guana fulgoroids) of every species
>we have to Charles Bartlett, and still have about 600 more duplicates of the
>common species in Sarasota. Here is what I know so far. He writes that
>since publishing his paper, one additional species has turned up, making his
>count 28. We sent him 26 species, including 5 new records from Guana,
>plus a new V.I. record from Little Thatch. So Guana now has 33 species,
>and I suspect there will be more...a surprising number of ours are uniques.
>I'm still going through the older Liao samples, especially looking for males
>which are necessary for some identifications. I haven't made an analysis
>yet, but I'm getting the impression that adults are peaking during the two
>wet seasons.
>
> We have also sent a small batch of cicadellids to Dr. Paul Freitag, and
>I'm watching for new faces as I go through the samples.
>Still in the Homoptera, we have added a species of the whitefly family
>Aleyrodidae to the Guana list. Also, in seven years we have found only one
>specimen of membracid - but it has horns. Not exactly inermis. I sent it
>to Bartlett; he does not know it and will try to get a det. Incidentally,
>he writes that the descriptions of the new species of Neopunana have been
>delayed by need to see types, males, and the generic type species in Europe.

>We are planning to get together in early May, so I'll keep you up to date.
>
> Tachinid flies, corixid, and mirid bugs, etc. are in the plans for our
>upcoming Smithsonian visit. I wrote Lianna that we have two species of
>corixids (one very rare) on Guana, and three on Anegada. I assume she
>knows this and I don't want to invade her turf, but she has mentioned only
>one species to me. Oh, speaking of tachinids, the two maggots we reared
>from Guana crickets are not tachinids! When I checked them, they turned
>out to be sarcophagids. They look like tachinids, and fooled me
>completely.
>
> Is the above e-mail address the best? Hope China has been
>fascinating. We look forward to getting together again. Best wishes.
>
>Barry and Buena
>bv@nwcs.com
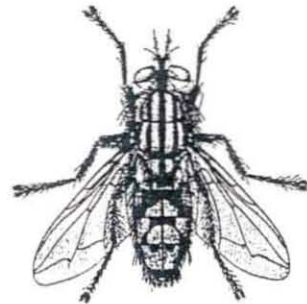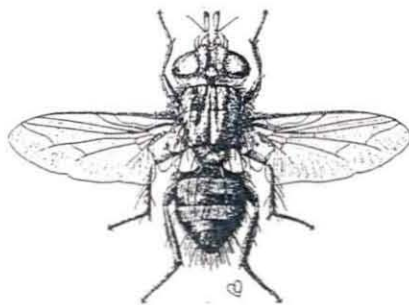
flesh fly, *Sarcóphaga*
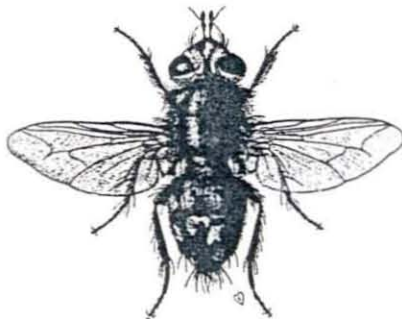
Tachinid flies.

Skip,

I forgot about the annual report! Here is some more info. Another
family of Homoptera has turned up: Psyllidae, the jumping plantlice, one
species so far. The Neuroptera count has changed. Four families, not
five (the fifth is tiny, like a coniopterygid, but in too poor shape to be
certain), its better not to count it. However, the species count has
risen from 6 to 24, with an astonishing 14 Chrysopids, 5 Myrmeleontids, 4
Ascalaphids, and 1 Mantispid. The absence of Hemerobiids, etc. is weird,
and unexplained.

Walking sticks have doubled...2 to 4...unless thay are extremely sexually
dimorphic, which I doubt. Orthoptera have gone from 14 to 19, including
10 species of crickets; there is another roach - now 16; Diptera appear to
be over 90 species and some dets are in progress; Coleoptera is growing,
now about 440, but some data are in Ohio...I still expect to hit 500.

We will be at the Smithsonian later this month, and are taking a
variety of Guana insects for further checking. They don't have specialists
for all groups, but we'll do the best we can. Again we send BEST WISHES as
does Susan.

Barry and Buena
bv@nwcs.com

-- No virus found in this outgoing message.
Checked by AVG Free Edition.
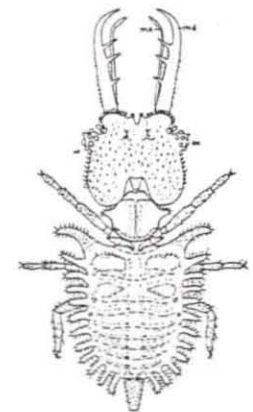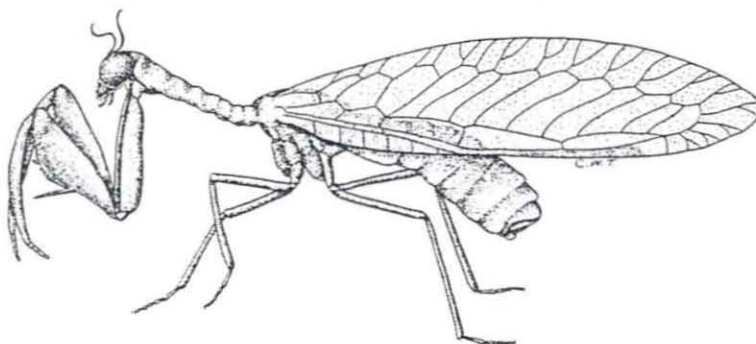Version: 7.1.385 / Virus Database: 268.4.0/305 - Release Date: 04/08/20
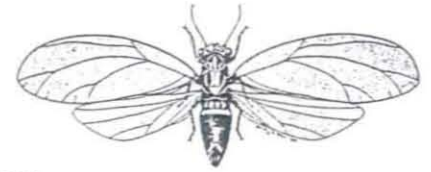
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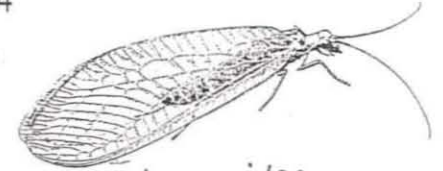
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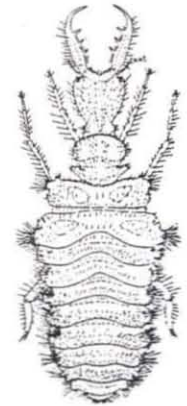
Myrmeleonidae

Ascalaphidae

A mantispid

NO-SEE-'UMS or PUNKIES

Dear Dr. Hagan, Season's Greetings!

I run The Conservation Agency (TCA), a small non-profit that, in turn, manages Guana Island in the BVI as a wildlife sanctuary and sometime research site. We are attempting an island inventory and Dips are a very neglected group. Would you be interested in looking at them? What we know appears in:

Lazell, J. 2005. Island: Fact and Theory in Nature. U. Cal. Press, Berkeley.

We know of 35 families, but few species (pp. 310-312). Ceratopogonids there, of course, but none identified yet.

2006-1-2

Sent: 2006年1月1日 19:16
Subject: Re: Diptera: Virgin Islands

Hi Skip,
1. Best wishes for the New Year.
2. I am interested in your e-mail message regarding the inventory of Diptera of Guana Island in BVI. As you know I have been interested in the Ceratopogonids for a number of years; my students, collaborators and I have gotten a chance to study the Cerat fauna from a variety of places about the globe (Belize, Norway, Canada, etc.). And we have found species novum in a variety of these locations.
3. What do you have in mind as to a study of the Dips?
4. Have you already been running light traps, sticky traps, emergence traps, etc.?
5. Do you already have the alcoholic material with Cerats?
6. Has the material been collected generally from about the island, and collected about the calendar?
7. Is there any support for trip(s) from U.S. to collect on Guana Island/ BVI?
8. I have retired from Georgia Southern University, and am Prof. Emeritus of Biology, Dec. 2004. And Jim Keirans retired from Georgia Southern University in June 2005.
9. I began this September, an appointment in the School of Medicine, Mercer University.
10. Please let me know what you have in mind re: the Cerats of Guana Island, BVI?
Best regards,
Dan Hagan
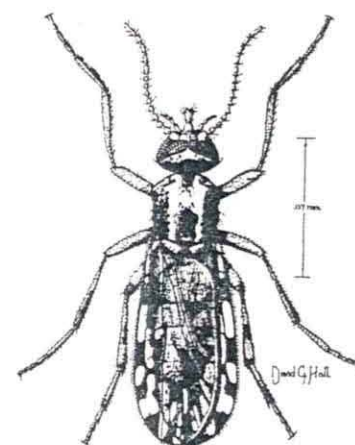
Ceratopogónidae

A Punkie

Dr. Daniel V. Hagan, PhD   email: dhagan@georgiasouthern.edu
Prof. Emeritus of Biology
  275 Audubon Place   H: 478-475-4226
  Macon, GA 31210   C: 478-319-6095

POST-SALADOID CULTURAL CHANGES ON GUANA ISLAND, BVI

By Elizabeth Righter

ABSTRACT

Among cultural changes that appear to have occurred following the Saladoid period in the US and British Virgin Islands are: break up of large Saladoid villages, continuing use of some village sites and settlement of numerous additional smaller sites in areas that were not previously inhabited, changes in ceramic styles and shifts in dietary remains. Causes of changes, which are debated among archaeologists, include a combination of one or more factors such as population increase, social change, internal cultural break down, influences from Puerto Rico and west, Caribbean-wide environmental catastrophe, and introduction of new people from South America.

This paper discusses a small post-Saladoid (or early Ostionoid) site on a small off shore cay of Tortola in the British Virgin Islands; and seeks to explore whether midden deposition patterns and content can a.) identify cultural change, and b.) provide clues to the causes of some changes.

1 March 2006

POST-SALADOID CULTURAL CHANGES ON GUANA ISLAND, BVI

By Elizabeth Righter

This paper presents an introduction to our investigations of a prehistoric site on Guana Island-a ca. 800-acre hilly island off the northeast coast of Tortola in the British Virgin Islands (3 slides:1-3). Subsuface prehistoric material has been recovered over an area of about 11 hectares or about 4 acres situated at the eastern end of about 43 hectares of flat ground that extends inland from White Bay Beach, below hills to the northwest and northeast (1slide-4). On its southwestern boundary, the prehistoric site is located about 100 meters inland from the beach and extends parallel to it for a distance of about 200 meters northwest from the center of the garden area. In a northeasterly direction the site extends between about 100 and 200 meters inland from the shore of White Bay Beach (2 slides-5&6). An extensive midden located along the northeast edge of the site provided the data upon which this paper is based (3 slides=7-9).

Guana Island is a privately owned mostly undeveloped island with a resort nestled in the saddle of a hill northwest of the prehistoric site (same slide). Currently a ca. 3-hectare salt pond is located on flat land inland from the shore, northwest of the prehistoric site (same slide and overview of pond=10). This pond is usually hypersaline, except during heavy rains when it is diluted, or after hurricanes which can flush the pond out completely so that it contains fresh water for a few days. The pond, originally a dense mangrove swamp, is fed by a ghut that originally flowed from the hill southeast of the garden, across the garden and the northeast corner of the prehistoric site into the pond area.

In 1986, Dr. Michael Gibbons of the University of Massachusetts in Boston found evidence of prehistoric use of caves on the northeast hill above the flat at Guana (slide-11). He dated the remains to between 0 and AD 500 and considered them to be indications of Archaic rather than Saladoid people (Lazell, in press). A second occupation is represented by the post-Saladoid prehistoric site identified on the flat. No radiocarbon dates have been obtained for the latter prehistoric site, but diagnostic ceramic sherds of both the Monserrate and Santa Elena styles (2 slides-12 & 13) would place the site sometime after ca. AD 750 and before AD 1200. No classic Cedrosan Saladoid sherds or ceramics of the late Ostionoid or Esperanza style have been recovered.

Written records upon which to base a history of possible post-depositional disturbances to the prehistoric site are scarce. A Quaker family is known to have lived at the resort property between 1743 and 1759. Recovered nineteenth century artifacts indicate occupation beyond that

time. After purchase by the Bigelows in 1935 the property underwent many reconstructive changes. It is possible but not likely, that construction of a tennis court and rough golf course on the flat removed a portion of the prehistoric site. Since scraping of the site to determine site structure would not be appropriate for a number of environmental and resort reasons, a plethora of possible unknown disturbances to the site must be determined from archaeological investigation.

A lack of well kept records and a shortage of carefully and systematically conducted archaeological investigations increase the challenges of archaeological investigation in the BVI. Among known prehistoric sites in the BVI are Belmont Grove and a number of sites on Tortola, a site on Jost van Dyke, the Gun Creek site in Virgin Gorda and a site found by this investigator and Louis Potter on Greater Camanoe island. A site at the Beef Island airport on Tortola is the closest to Guana Island (1 slide-14).

For years, potsherds had been unearthed in the garden area at Guana. Little attention was paid to the artifacts until, in 1980, Lazell began his biological work on Guana Island. Recognizing the value of the prehistoric remains, and that data recovery might yield information about native flora and fauna, important to his own research, he contacted me in 1987 to assist with investigation of a probable site on the flat.

In 1987-88, a program of subsurface shovel testing was conducted by myself, Elaine Acevado and Isabelle Rubin on the White Bay flat. Recovered faunal material was taken to the University of Massachusetts in Boston for analysis under supervision of Dr. Gibbons. Unfortunately Dr. Gibbon's lab was vandalized and the material was lost. Although no proveniences were noted, monk seal, fish, and turtle bone had been identified in the remains recovered from shovel testing..

After a fifteen-year hiatus, systematic investigation of the subject prehistoric site was again undertaken in 2003. An area formerly used as a garden and allowed to grow fallow, had been cleared and scraped to a depth of about 20 cm to accommodate a donkey pen. After the scraping, ceramic sherds were exposed on the ground surface (1 slide-15).

With the assistance of Ellen MacClean, Robert Pederson, Wenhua Lazell and others about 2 cubic meters of the midden were excavated in 2003.. The midden, in dark sandy loam soil varied between 38 and 43 cm in thickness. While excavating, it was not possible to discern levels or strata (1 slide - 16). The midden, therefore, was excavated as one stratum, but in 10 cm increments. Slight changes in soil color, interpreted as strata, only became evident in wall profiles after excavation of units. Since two historic sherds were found in the first 10 centimeters of the excavation, as a precautionary measure, the first 10 centimeters of the midden were considered to be potentially disturbed

Ceramics recovered from the midden will be analyzed by Dr. Emily Lundberg, while phytolithic analysis is underway by Dolores Piperno of the Smithsonian Institute in Panama. Faunal bone is being analyzed by Dr. David Steadman of the University of Florida in Gainesville. Shells were identified and counted by the author.

Of a total of 416 shells, *Cittarium pica* shells (most of which have been altered and probably were not brought to the midden by hermit crabs) comprised 38%, land snails made up 16%, *Codakia orbicularis* comprised 4% and juvenile *Strombus gigas* comprised 2.4%. Segments of *Acropera cervicornus* (coral) comprised 3% of shell and coral combined.

Hermit crab claws and a few claws of gecarcinid land crabs were recovered from 18 levels and test pits.. Such findings correspond well with Elizabeth Wing's (1995:108) findings of increased *Cittarium pica*, small inedible land snails and hermit crabs in many post-Saladoid sites of the Caribbean islands. Wing correlates diminished *Cardisoma guanhumi* and Gecarcinus sp remains with overexploitation during the Saladoid period. At Guana, and at a number of other early Ostionoid sites in the US and BVI, human occupation was not preceded by Saladoid habitation or known human exploitation. Although habitat for *Cardisoma guanhumi* crabs apparently was present at Guana, these animals were not well represented in the post-Saladoid midden. There may be some other explanation. Since land crab consumption apparently also was not significant in the Archaic period, crab consumption may have been linked to certain Saladoid feasting ceremonies. These did not occur at the early Ostionoid site on Guana Island.

Fauna observed in the midden remains included fish in all units and levels. Hutia were well represented and there was a small amount of sea turtle. Bird bones consisted of Sula sp, *Puffinus lherminieri*, *Anas bahamensis*, and Columba sp. Iguana was present in one level of one excavated unit of the midden.

✗ Sula: Booby

One Oliva bead and one spindle whorl were recovered along with three small *Strombus gigas* celts and a number of Strombus whorl tips that may or may not have been zemi preforms (1 slide-17). Two possible ball belt or stone collar fragments also were recovered from the midden (1 slide-18).

We observed many differences between the late Salad midden at Tutu and the early Ostionoid midden at Guana Island. Among these were differences in soil color and consistency, disposal patterns, and contents of the midden..

It was to be expected that soils of the two sites would differ, but combined with refuse distribution and contents, the midden at Guana was very different from late Saladoid midden at Tutu. At Tutu the late Saladoid food refuse deposit was thick and consisted primarily of remains of large land crabs (1 slide-from Tutu-19), along with large sherds of decorated or fine plain ware. It was possible to observe discrete disposal episodes---as if waste from a specific feast or ceremony or even meal were being discarded. At Guana, midden soils were heavy, moist and dark with refuse densely deposited throughout the midden's thickness. Large land crab claws were absent, and it was not possible to discern episodes of disposal .

In the late Tutu Saladoid midden, in addition to large sherds of decorated or finely crafted ceramics, items of personal adornment such as discoidal shell beads and a shell pendant, and ceremonial objects such as stone idol insets and zemis believed to be related to ancestor worship were recovered. These were finely crafted items of personal value or religious significance which required skill and time to manufacture. The Guana midden primarily contained food remains and sherds of a variety of broken ceramic vessels, most of which were

poorly finished. One Monserrate sherd (1 slide-20) was mended indicating some significance attached to the vessel. A number of flat bottomed vessels in a wide range of craftsmanship and plates with finished interiors also were present in the Guana midden.

Characteristics of the Guana midden indicate initial settlement by a small group of possibly related people who utilized new food resources, made pottery belonging to a new series and did not discard recognized ceremonial items with the food refuse The Guana site appears to have been one among a number of late Saladoid or early Ostionoid pioneer settlements that, in the US and British Virgin Islands, usually were located on offshore cays or in previously unoccupied or remote areas. These settlements made use of new or under-exploited food resources. However, much more work needs to be accomplished in order to determine the size and configuration of the Guana site; and to verify its continuous occupancy The presence of a spindle whorl and shell celts suggests that the site was a settlement, however, the number of structures present at any one time and continuous early to mid-Ostionoid occupancy are not yet established. So far we have not found any burials or definite post holes. It is possible that the site was utilized periodically over a long period of time for a specific purpose.

Accumulated evidence indicates that in the northern Caribbean, significant cultural changes took place either at the end of the Saladoid period or at the beginning of the Ostionoid. Exact dates for observed cultural changes are either unknown or not consistent throughout the Greater Antilles including the Virgins. Changes in diet may have been gradual and there is no clear break in the trend from late Saladoid to Monserrate pottery (Curet, Torres and Rodriguez 2004:63). Nevertheless, it is inescapable that at the time of these changes, many new settlements were being established in the US and British Virgin Islands and there must have been an impetus for this. In some cases, such as at Tutu, Saladoid villages previously stable for some time appear to have been abandoned or sparsely settled. There does not appear to be a post-Saladoid component at the Cedrosan Saladoid Main Street site in St Thomas or at several late Saladoid sites in St. Croix. The situation is similar in the BVI, where first settlement of sites such as Belmont Grove in Tortola, Gun Creek in Virgin Gorda and Guana Island was during a late Saladoid or early post-Saladoid period, Taken all together, this was a time of change and the changes are evident not only in the establishment of a new settlement on Guana island, but also in the nature of the midden and its remains.

.. 	The hypothesis presented here is that, as in eastern Puerto Rico, Saladoid villages in the Virgin Islands remained stable for some time. Population was able to grow when sustained in the large Saladoid village setting; but something occurred which upset the balance and required many inhabitants to change their habits and seek subsistence elsewhere. In an admittedly Darwinian model, the catalyst may have been a Caribbeanwide disease among one or more important food resources. Dramatically reduced food supplies might lead to a break down of established villages and traditions and a need for establishment of a number of smaller settlements seeking new food resources in previously unexploited areas. In such a scenario, subsistence may, at first, have been an overriding concern, with less emphasis on ceremonial life and craftsmanship.

Another explanation that "fits" the data would attribute cultural changes to increased socio-political complexity. A sharing of late Ostionoid culture between the Virgins and eastern

Puerto Rico has been demonstrated by Righter, Wild and Lundberg (2004), and it is likely that marked socio-political changes, including shifting power structures and population centers during the Ostionoid period in eastern and southern Puerto Rico (Curet, Torres and Rodriguez 2004: 64) were reflected in the US and British Virgin Islands. Thus another explanation might be that in the US and British Virgin Islands increasing social complexity and expansion resulted in retention of some population centers, along with establishment of a number of related pioneering or satellite settlements. In either scenario, it is likely that after a period of cultural change, and perhaps disruption, re-organization under reigning caciques, led to a hierarchy of settlements, renewed ceremonial vigor, stability and a population increase which culminated in the Taino chiefdoms of eastern Puerto Rico and the Virgins.

ZOARCHEOLOGY
To Elizabeth Wing

At 10:52 AM 6/12/2005 -0400, you wrote:
>Dear Liz: I know you are retired but I don't know where else to turn. As
>you know I have been doing some excavating of a midden on Guana Island.
>This is very interesting because so little is known of the BVI and also it
>appears to be an early Ostionoid site.
>
>I met David Steadman at an IACA Congress and he looked at the bones that
>we excavated, but he said he really only knew birds, which was fine. But
>there are other bones there too and we would like to know what they
>are. There is something he identified as agouti and iguana. To my
>knowledge the only agouti-like animal on the northern Caribbean islands is
>the hutia but we don't know for sure. Also the type of iguana, if it can
>be discerned, is of interest. I wonder if you know anyone who might be
>able to help David or take over identification of the other
>animals? (fish are a problem because there are so many of them and it
>takes a long time to do them, so we are just calling them "fish" for now.
>There are not many other bones but we will hopefully get more.

>We don't have any money for analysis at this point, although after we know
>a bit more, I may be able to get a grant. I am writing a paper for the
>Trinidad IACA Congress—are you coming? — and I wondered if you could
>tell me the common name of Anas bahamensis? I have tried and tried to
>contact David (and invite him and Ann to Guana) but can not elicit a reply.
>
>Thanks for your help. Best wishes, Elizabeth Righter (Holly)
>
>PS I find your crab paper fascinating and have been trying to think about
>what might have caused a change in diet from the Saladoid to the
>Ostionoid. I am particularly puzzled by the "new" sites where a previous
>exploitation of C, guanhumi could not have taken place. I wonder if crabs
>were associated with specific ceremonies of the Saladoid people and for
>some reason these ceremonies were not practiced at most newly settled
>Ostionoid sites. Also I was thinking that about 9 Cittarium shells (which
>look like a lot in a midden) are barely a meal, while a large crab is more
>substantial. I wonder if we over-perceive the importance of Cittarium
>pica--not so much relative to other shell fish but in terms of their
>actual food value. It would seem that fish were the mainstay of the people
>at Guana.

From: "Elizabeth S. Wing" <ewing@flmnh.ufl.edu>
To: <Bobbfly@aol.com>
Sent: Tuesday, June 14, 2005 1:18 PM
Subject: Re: Queries

Dear Holly:

What you have to say about the animal remains you are excavating is most enticing. But I am having a great deal of trouble just getting a little bitty chapter written and must not take on anything else though it would be fun. Easy question first the duck's common name is the white-cheeked pintail. The iguana may well by Cyclura a very common component of thos faunas. The agouti-like animal could be the remains of the actual agouti Dasyprocta introduced from the Lesser Antilles. One such animal was identified from a site on Vieques Island but the specimen is now missing. It is the only record of agouti in the Virgin Islands. The hutia Isolobodon portoricensis is a fairly common component of Virgin Island site introduced from islands to the west. It would really be a shame not to work on the fish remains. It should be heavy duty reef fishes with snapper and parrotfishes predominating and if it is not that would be very interesting as a departure from the norm in the VI. I just went down to the lab to ask Irv if he had any students looking for a project and he does not furthermore he has more on his plate than he can manage. Betsy Reitz is coming on monday for a months time I will ask her if she is looking for a project.

I do not plan to go to the IACA meetings. Too bad really but I have so much to do here. I will let you know if I find someone to help you out. Have a good meeting in Trinidad.

Best Regards, Liz

Date Sent: Tuesday, April 04, 2006 4:04 PM

From: Bobbfly@aol.com : HOLLY RIGHTER

Hi: I am sorry you can't come to our wedding, but China sure has its appeal (did I tell you I am taking Chinese brush painting and in the process learning something about China?) I wondered if you had any idea when Guana would be this year. What I would like to do is find more bones, scrape (we can get the help of the Guana island grass crew) and make a GOOD contour map of the site. I think Bob can come again and Josh. If there is room and IF he agrees, I would also like a mapmaker friend of mine to come for a couple of days and bring his equipment (not sure how long) and make a very good contour map of the site (he can also map the ruins if you wish).

What I am thinking now is that the midden has been subjected to periodic flooding (lessons learned from last years' inundations), which has mixed some of the material and also made some of it weather worn, which makes bone i.d. difficult. If you remember, the bones that Michael had were from shovel test pitting, not the midden.

SO I think we would get the best specimens from more 1 or 2-meter squares carefully excavated where remains are (like the pit we started last year with Tom's help and we got rained out). If we do some scraping it should tell us if there was a settlement there (it should be near the midden) and then if we do some selective excavation in the site, we might recover better specimens.

Let me know what you think of this plan and also of the availability of space for everyone. Bob and I can share a room and Josh could stay with the map maker (at least for a little while). Do you want Ellen back again? She is good company.

Hope all is fine in China (fine China, ho ho) love, Holly

Date Sent: Wednesday, April 05, 2006 9:37 AM

From: Bobbfly@aol.com

Hi Skip: I talked to Travis on email and he says he can do the mapping (it might be useful to you also) but he needs to bring an assistant, Nathan (this might be his son). It might mean staggering Travis and son with Bob and Josh. I would need to be there for both Travis and the Josh/Bob unit. Maybe Ellen could be my roomate when Travis is there. I will find out how long it will take Travis to do the job--not long I would think. If this will not work out, let me know and I can let Travis know--maybe another year. Travis will bring the necessary equipment also. love, Holly
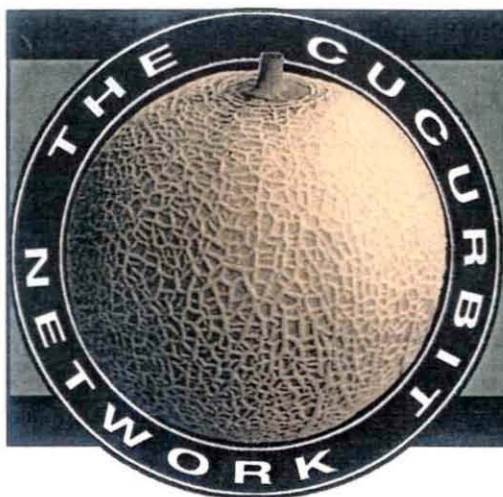
The Cucurbit Network News

Cucurbit Profiles

The Elusive American Melonleaf

by Angela Davis, with Rudy O'Reilly, Jr., & James Lazell

Two years ago, I traveled to the Caribbean looking for wild cucurbits. I had hopes of documenting the first *Cayaponia americana* (Lam.) Cogn. (American melonleaf) on Guana Island, a conservation-oriented resort in the British Virgin Islands. Cucurbits are, after all, my main interest, and collecting germplasm is an important function of species preservation, not to mention, part of my job as a scientist with the United States Department of Agriculture (USDA) in Lane, Oklahoma. I thought my chances of discovery and seed procurement were good because a similar species, *C. racemosa* (P. Mill.) Cogn. (mountain melonleaf), had been found previously on Guana Island.

My quest began with several days of searching the Guanas Mountains on my own—to no avail. Then, on one clear tropical morning, I set out in the field with Rudy O'Reilly, Jr., (District Conservationist, USDA, Natural Resources Conservation Service, St. Croix) who was looking for a rare cactus species. We battled the thick brush, endured the sultry heat, and fended off hoards of chiggers. We were rewarded at the end of our third arduous day with the first documented finding of American melonleaf on Guana Island. Rudy unfortunately did not find his species of interest.

Two days later, while I was convalescing from my assault in the field by blood-thirsty chiggers, an entomologist searching for beetles in the brush on a steep slope some 10 m from the island bungalows stumbled across a beautiful and quite impressive American melonleaf specimen. Rudy and I are convinced that our previous efforts were not in vain, although the discovery of the species so close to civilization—and by someone else!—was a reminder of the vagaries of plant exploration.

Cayaponia—Continued from page 1

American melonleaf is a New World native known by several common names, including bejuco de torero, which is Spanish for "bull-fighter vine". It has reportedly been used as a remedy for scabies. This splendid species occupies open, moist habitats at lower and middle elevations from Cuba to the Lesser Antilles.

Mountain melonleaf, which is found in Florida, the Caribbean, and South America, is best distinguished from American melonleaf by looking at their flower structure. Both species are monoecious, bearing male and female flowers on the same vine. However, American melonleaf flowers have a calyx that is 6–9 mm long with lanceolate lobes, whereas mountain melonleaf's calyx is 3–4 mm long with triangular lobes.

American melonleaf is a strong climber that can reach heights of 10 m. The angular, lon-
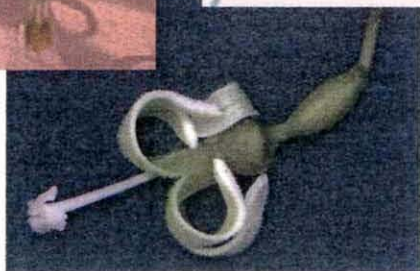
Above: A plant grown by the author, which produced the male flower (far left), female flower, and fruit shown to the left as well as the tuberous roots shown to the right.

gitudinally-ribbed stem may be glabrous or puberulent, and has swollen nodes where new stems may form. The vine is a vigorous perennial producing many lateral branches and one three-branched tendril per node. The leaves can be 5–20 cm long and wide, and one plant can sport leaves of multiple sizes and shape—from almost heart-shaped to pentalobate. This and the dark green glossy foliage make the plant very attractive, even though the flowers and

fruits are small and simple. The green berries, which are produced in abundance, turn burnt orange when dry, often stay on the vine, and contain few seeds.

I visited this grand cucurbit outside the island bungalow several times before my departure from the island just to admire it. What an attractive plant, growing in full sun, spreading across multiple trees. Seeds from this vine have now been deposited in the USDA germplasm bank, and of course I have an American melonleaf draped in a corner of my greenhouse in Lane, OK, producing seeds for the next generation.

Suggested Reading

Acevedo-Rodrigues, P. 1996. Flora of St. John, U.S. Virgin Islands. Memoirs of the New York Botanical Garden 78:193–194.

Bourdy, G., Chavez de Michel, L.R., and Roca-Coulthard, A. 2003. Pharmacopoeia in a shamanistic society: the Izoceno-Guarani (Bolivian Chaco). Journal of Ethnopharmacology 91:189–208.

Kraus, F. 1991. Biodiversity conservation on Guana Island, British Virgin Islands. Pp. 76–87 in Proceedings of the Regional Symposium on Public and Private Cooperation in National Park Development. B.V.I. National Parks Trust, Road Town, Tortola.

Liogier, A.H. 1997. Cucurbitaceae. Pp. 195–196 in Descriptive Flora of Puerto Rico and Adjacent Islands. Universidad de Puerto Rico, San Juan.

The Still-Virgin Island

On Guana Island, wildlife replaces nightlife, and the guests keep coming back for more.

BY LAWRENCE MILLMAN

WHEN I TOLD MY FRIENDS THAT I WAS GOING TO GUANA ISLAND, they were not overly envious. One of them quipped, "Harvesting guano, are you?" Another declared, "Better you than me, mate." • They were, I don't doubt, thinking of the Guano Islands of Peru, desolate, sunbaked specks of land primarily composed of bird droppings. My friends can be forgiven their ignorance, for Guana, a hilly, rugged island in the British Virgin Islands, is not among the better known Caribbean destinations. It has no resort action, unless you consider a

game of croquet resort action. And rather than nightlife, it offers wildlife — according to biologist James "Skip" Lazell, an authority on Guana — possibly the richest fauna of any island its size in the world. This richness is due mostly to the fact that Homo sapiens, a species known throughout history for its destructive habits, has left Guana more or less alone.

On my first day on the island, I climbed to its highest point, Sugarloaf mountain (elevation: 806 feet), and saw what appeared to be a dinosaur basking on the summit. Later, near the jasmine-scented Sunset Terrace of the island's only facility, the Guana Island resort, I encountered another of these giant reptiles dining on papaya handouts, sneaked to them by guests.

Actually, the reptiles in question are stout iguanas (*Cyclura pinguis*), an endangered species that can grow to a length of more than 6 feet and weigh upward of 70

pounds. Between 100 to 200 of these docile monsters inhabit the 850-acre island (and a few more live on nearby Necker, Norman and Anegada). In fact, Guana derives its name from a local rock formation that looks exactly like a giant iguana.

Guana does take you back in time, although perhaps not as far back as the age of dinosaurs. The resort's seven whitewashed cottages suggest an earlier, more refined era, and their rattan-furnished interiors contain nothing so blatantly modern as a TV, telephone or even a clock. With its telescope, stucco walls and library of old books, the living room in the main house is similar to an English-country parlor circa 1860. It also has a self-serve bar, and in keeping with the general ambience, I mixed myself an old-fashioned.

The island goes to sleep early. The loudest sound I heard at night was not a boom box or a faulty muffler, but

Named for a rock formation that looks like its giant, stout iguanas, Guana is a wildlife haven with more flora and fauna than most Caribbean islands its size. Luckily this natural setting comes with a cocktail hour and seven beaches.

the male piping frog advertising for a mate. Indeed, it was the only sound I heard at night.

So how did Guana escape the mania for development that has beset so many other Caribbean islands?

For one thing, it's always been a pri-

lovers — not the types to leave heavy footprints on the environment.

When the current owners, Henry and Gloria Jarecki, purchased the island in 1975 they kept most of it as a wildlife haven and decided to restrict the number of guests to no

than 400 species), reef fish (more than 100 species) and a blind legless lizard called an amphisbaena.

Many of Guana's birds are migratory species, as are its guests: The same ones seem to flock to the island every year. A couple I met from Connecticut were celebrating their 26th straight year here. The husband, a radiologist, was a history buff, and he told me about the two Quaker families, the Parkes and the Lakes, who grew cotton on Guana in the 18th century and built a stone wall down

Where the Wild Things Are

Guana Vacation Guana's only resort consists of seven guest cottages and the main building. The cottages start at $595 (low season) and go up to $1,850 for the secluded North Beach cottage, all meals and transfers from Tortola included. 800-544-8262, *www.guana.com.*

What to Do? Once you have hiked all the trails and lazed on the seven beaches, there is tennis, snorkeling, fishing, windsurfing and, if you really must, Wi-Fi Internet access. And did we mention the self-serve bar?

vate island and thus outside the radar of resort-minded developers. For another, its owners have always been eager to keep it in a largely natural state. Beth and Louis Bigelow, well-to-do New Englanders who owned the island from 1935 to 1975, were nature

more than 34 at a time, for crowds of people might disturb the locals — i.e., the fauna. In the past, the resort has been closed in September and October to everyone except scientists studying this fauna — not just iguanas but also moths (Guana has more

the island's center to separate their respective properties.

This story inspired me to visit the extremely ruinous ruins of the Lake family's plantation house. And since it was quite hot, I continued walking until I reached one of Guana's seven

beaches, Bigelow Beach, where I went for a swim. There wasn't a soul in sight except a brown pelican.

The next day I met Liao Wei-Ping, the island's orchardist, who recommended Grand Ghut. He wasn't suggesting that I distend my belly with extra helpings of rich cuisine (more seafood thermidor! more lime mousse!) from the resort's kitchen, but rather that I investigate the Grand Ghut area — "ghut" is the B.V.I. term for ravine.

Liao himself joined me on this trip. Half a mile from the resort, we scrambled up boulders to a small, cavelike amphitheater and watched a colony of fruit bats cool themselves with a palpitating motion of their wings. After a while, the bats grew weary of our voyeurism, and the entire colony took to the air with a series of exasperated squeaks.

As we hiked, large soldier crabs —

not the most agile of creatures — kept falling out of trees and hitting the ground with dull thuds. What if one of these plummeting crustaceans landed on me? I imagined the following newspaper headline: "Man Brained by Crab on World's Most Peaceful Island."

Just as we reached Grand Ghut, a brownish snake reared up and spread its neck laterally like a cobra … quite a good performance considering it was no more than a yard long.

"There are no poisonous snakes on Guana," said Liao. "Actually, there's no poisonous anything."

Soon I began wondering whether I had in fact been knocked senseless by one of the crabs, for I seemed to be in some sort of Caribbean never-never land. In front of me was an undulating green coastline with neither houses nor roads on it; ancient loblolly and tamarind trees rose up all

around me; and a powder blue lizard skittered past my feet before disappearing into the bush.

My reverie was interrupted by Liao's voice. "Beautiful bird," he said, pointing to a bridled quail dove. I gazed at the quail dove. Its rather portly shape and unhurried manner made me think that it wasn't only us guests who were enjoying the good life on Guana.

Later I found myself sitting on White Bay Beach, a half-mile expanse of sand usually empty of people. But then I saw an inflatable dinghy on the beach and several people from a yacht anchored offshore relaxing in the afternoon sun. I fought back an urge to wander over to them and find out what had happened in the world — What leaders deposed? Wars declared? Volcanoes erupted? — since I'd stepped off it. ◆