# Guana Report for 2003

## by James Lazell



Photos by Gad Perry

Worm-eating Warbler:
new for the BVI?

# The Conservation Agency

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21 April 2004

Dr. Henry Jarecki
10 Timber Trail
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Dear Henry:

Scientists month on Guana in 2003 began early, in September, with the BBC team and the iguana restoration project. The resulting video for their series "Animal Sanctuary" was a great success -- as was the initial phase of the Norman Island iguana restoration: we relocated 12 *Cyclura* (*Iguana*) *pinguis* there. We are full of hope.

There was a second TV recording session for The Travel Channel -- also featuring Guana's 'guanas. I thought this too was very good publicity. But lots of real science got done too. A quick summary of what follows:

## Cover Story

The Worm-eating Warbler is a classic example of a neotropical migrant: it breeds and nests in North America -- from Connecticut and Illinois to Georgia and Missouri -- and migrates each year to winter in the tropics. The books say "West Indies" (and Central America) but I have not found an actual documented (observers/photo with date and locality) record for the BVI. This species prefers real forest for nesting and is declining throughout its range as forests are cleared and fragmented. It does about half its feeding on the ground, where it is unique in manipulating cover items, like fallen leaves, to get its vermiform prey. It should be looked for by birders from

November to April because, if it actually overwinters on Guana then, the island constitutes an important resource for its survival. (Data from Morse, D.H., 1989, *American Warblers*. Harvard U. Press.)

Next comes a dollop of practical science: what to do about termites. Then follows a treatise on more arcane aspects of termite societies. Next, Dr. Richard Levins, of Harvard, or one of his students, at least, seems to have been a pioneer scientist on Guana, going back to the sixties. I have not received any response to my letter and need to follow up -- at least getting the referenced thesis. The Valentines provide and update, and there are brief notes on other invertebrates.

Plants got shorted in 2003: our botanical team failed to appear. Only work on the melon family plants -- Cucurbitaceae -- continues, with publication in sight (they say). After a bit of news, I provide three book reviews I hope will be of interest, tending as they do to explain why some of us so love reptiles.

There are two major anole lizard contributions this year, one published (Gad, Kate, *et al.*) and one from Greg Mayer's grad student (at last!). The latter is tedious (as theses tend to be) but of great interest to me because it documents aspects of the genetics of speciation. Then comes a frog note (frogs are not reptiles, of course), followed by Clint's excellent bird report (birds *are* reptiles, of course).

Enjoy! All the Best!

Skip

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# Research Report:
# Effective, Efficient, and Environmentally Friendly Technologies for Detection and Control of Structural Pest Termites on Guana Island

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**Background**: Of the 2600+ species of termites worldwide, all are important as decomposers that recycle plant nutrients in ecosystems, and about 10% are also significant structural pests (because humans construct buildings out of termite food). Several of the 98 species of termites in the West Indies are aggressive structural pests, including at least 3 species on Guana Island (the arboreal termite *Nasutitermes acajutlae*, the drywood termite *Incisitermes* sp., and the subterranean termite *Heterotermes* sp.)

Conventional approaches to control of these pest termites involves heavy use of pesticides, either injected into soil (aimed at arboreal or subterranean termites) or applied locally or as a fumigant against drywood termites. There have been recent advances in the efficacy of termiticides (e.g. commercialization of nonrepellent chemistries such as Termidor® or Premise®), but the fact remains that large amounts of a widely broadcast chemical are required to control these structural pests.

Novel approaches to termite detection and control, especially technologies that specifically target termites and minimize pesticide use, are of key interest as alternatives to traditional pesticide applications. Termite baits have emerged as a promising tool for effective, environmentally-sound structural pest management. If a cellulose matrix is impregnated with a slow-acting insect growth regulator or low risk pesticide, it can serve as a delivery system to suppress the population of an entire termite colony. When worker termites find the bait, the will eat it, ingest toxin, and – when they return to their colony – spread the pesticide to nestmates through social food flow. Thus, over time, as foragers feed at the bait they will directly and indirectly act to spread the toxicant throughout their colony, ultimately suppressing the population.

Effective termite baits have been developed and are in heavy use against subterranean termites in the United States (genera *Reticulitermes* and *Coptotermes*), but those bait designs and matrices have proved relatively unappealing to subterranean termites in the Caribbean (*Heterotermes*). Effective baits targeting drywood termites have not been developed for any species. The British Virgin Islands thus offers an excellent opportunity for testing new, environmentally sensitive technologies for termite pest management.

In this research I explore utility of a "preferred food source" for termites (trade name Summon™; FMC Corporation) as a bait matrix for detection and control of arboreal, drywood, and subterranean termites on Guana Island. Summon™has no termiticide; this study is merely to explore termite interest in the matrix. The manufacturer plans to release a version of Summon™ that will be impregnated with a tasteless insect growth regulator. If pest termites in the British Virgin Islands are shown to readily eat

Summon™, we can proceed to trials using that new version that will be poisonous to insects (if, of course, there is interest and permission to test this technology in the BVI).

**Objectives**: The objective of my 2004 work was to test whether the "preferred food source" designed for termites (trade name Summon™) was readily consumed by the structural pest species on Guana Island, i.e. the arboreal termite *Nasutitermes acajutlae*, the drywood termite *Incisitermes* sp., and the subterranean termite *Heterotermes* sp. If found attractive to these termites, the matrix might serve as a delivery system for insect growth regulators, thereby offering an environmentally safe technology for colony control.

As a side project, I tested the electronic TermaTrac unit, a system designed to pick up feeding and movement sounds of termites within wood and therefore detect active infestations of cryptic drywood termites.

**Experiment and Results**: All pest termite species were offered Summon™ disks in both laboratory and field settings on Guana Island (see photographs at end of this report). In all cases, worker and soldier termites immediately swarmed the surface of the Summon™ disk, quickly beginning to feed and in some cases completely consuming the disk within 24 hours (see photos). When disks in the field were placed adjacent to established foraging tunnels, termites encased Summon™ material and in each case fully consumed it within 24 hours.

I tested the TermaTrak unit in collaboration with James Egelhoff (BVI Pest Control) on Virgin Gorda, and I also tested it on both subterranean and drywood termite infestations on Guana Island. The unit is highly sensitive and did an excellent job of detecting active infestations, with the caveat that the equipment has to be held precisely still, and may therefore not be practical for assessing activity on high ceilings, beams, etc. The only other limitation is that the range of the TermaTrak is only right under the monitor, so the unit has to be moved and activated at many locations to precisely map an infestion.

**Conclusions**: The composition of the Summon™ matrix appears to be highly promising as a bait delivery system for pest species on Guana Island and the British Virgin Islands. Up to this point no wood or matrix had been found that was so rapidly appealing to termites; structural woods currently used in commercialized baits are occasionally eaten but have not been found to be of high interest to termites already foraging on another food source.

Several limitations of the TermaTrak unit are described above, but those constraints aside, I was extremely impressed with the precision of the technology, and feel that it has many useful applications in determining the exact location of active termites.

**Future Research**: I have been told by the FMC Corporation that they are developing a Summon™ bait that will be made of the same matrix I tested on Guana, impregnated with an insect growth regulator. They anticipate that I will be able to test that bait in the BVI in 2006, assuming that all permissions are approved and arranged. This could offer a highly effective, precisely targeted, environmentally friendly technology for termite control in the Caribbean, a goal that has thus far evaded achievement.

Galleries and damage caused to ceiling of living room, main house, Guana Island by the subterranean termite *Heterotermes* sp.

Termite tunnels built on concrete by subterranean termites, connecting the soil to wood

Summon™ disk placed 24 hrs earlier at the base of the foundation of the cistern on Guana Island. Subterranean termites discovered the disk quickly, covered it with their gallery material, and are consuming it.

Subterranean termites from Guana actively consuming Summon™ disk (above); fully consumed (below).

Summon™ disk inserted into an arboreal termite trail on Guana Island (left); fully consumed 24 hours later (right).

Astute *Anolis* lizard observes that termites are attracted to the Summon™ disc placed on the tree; the predator lurks, dashing down occasionally to grab termites off of the Summon™ "platter".

Drywood termites (*Incisitermes* sp.; collected on Virgin Gorda) beginning to consume a Summon™ disk.


TermaTrak unit, borrowed from Protec Corporation, as a device to detect and locate active termite infestations (positioned here on top of an infestation in a bookshelf in the living room, main house, Guana Island).

**Wenhua Lu**

**From:** "Barbara Thorne" <bthorne@umd.edu>
**To:** <rogsimi@hotmail.com>
**Cc:** <hq@theconservationagency.org>
**Sent:** Thursday, October 16, 2003 2:10 PM
**Subject:** termite issues

Hi Roger,

Finally I have a chance to send my comments and recommendations regarding the termite infestations in Guana Island Club buildings.

Firstly, I contacted the company that sells that "Termatrac" unit that I brought and tested on the island. I asked for their price and said that I would put them in contact with you, but no response yet. I know that their reps travel a lot, so maybe they're swamped right now, but I'll forward you the message when I hear from them.

I was very impressed with the sensitivity and accuracy of that machine. It's usefulness will depend on the type of termite you're trying to find.

There are 3 types of structural pest termites on Guana: the nasutes that have the big tree nests and gzillions of workers and soldiers in the wider, conspicuous 'runways'; "Heterotermes" which have the narrower tunnels and connections into the soil (hence are termed "subterranean termites" and are targeted with a soil soak or injection application) - that's the type of termite infesting the beams and ceiling we looked at in the 'living room' of the main building; and drywood termites such as are in the small white cabinet that the photo albums sit on.

For nasutes and the subterranean termites, you can usually tell where they are by the presence of their tell-tale runways. If you scrape those open and termites come pouring out or the tunnels are rebuilt, you know the infestation is active. The Termatrac unit could be helpful in telling you exactly where the termites are feeding and whether a treated infestation is still active, but the main strength of the Termatrac machine is in diagnosing drywood termite infestations. Drywood termites are the sneakiest. They do not build tunnels on the outside of the wood, and they do not need a connection to the ground. The whole colony could be in one beam or stud or piece of furniture, cryptically hiding while chomping away and doing damage. Drywood termites are the type that are typically treated by fumigation. Since drywood termites don't display any visible signs of their presence, except sometimes piles of dry frass pushed out from very small holes, they are usually the hardest to detect, and the Termatrac machine was designed with them in mind (although it works on all termites).

Limitations of the Termatrac are that it gets confused by steel (e.g. rebar) and aluminum siding, although the latter is obviously not a problem on Guana. Also, to get a good reading the whole unit, including the cord, has to be held very still. That's relatively easy on a piece of furniture or down low, but if you're interested in a reading from up on a ladder with the probe placed against a ceiling beam, it's really hard to keep the cord as still as it needs to be, at least that was my experience.

Anyway, that's my spin on the Termatrac, for what it's worth. It's the best termite detection unit I've seen so far, if you need one, and the case is definitely ultra cool.

Ok - on to treatment recommendations...

I think you should proceed with your plan to treat the entire main house with a

conventional soil drench treatment. The termiticide "Termidor" (active ingredient = pesticide = fipronil) has had very impressive results, and as we discussed, is more "forgiving" or "goof proof" in the sense that when applied as well as possible but maybe not perfectly due to rocks or other unavoidable features that prevent achieving an absolutely complete barrier, it tends to work well anyway. Now that the roofs and ceilings have been replaced in part of the building, you want to ensure their protection, so to experiment with the 'living room' beams at this point does not make sense. Also, that appears to be a fairly monstrous infestation, so best to deal with it soundly. We aim to keep termites fat, sassy, and happy in their natural environment where they do all sorts of good things like recycle nutrients and aerate soils, but the gloves come off when they get into buildings.

The place that an experiment could be done would be on that white cabinet with the photo albums. I detected active termites (probably drywood termites, although you should move the piece of furniture just a bit to make sure that termites are not coming up from a crack in floor below) on the far right side of the top of that cabinet, and on the right vertical side panel. If you'd like, a pest management professional could drill a small hole (from underneath or inside so it would not be visible) and inject some Termidor directly into the drywood termite gallery system. I gave Jim Egelhoff an injector to do that type of application; if you choose his firm to do the work he would probably be willing to give that a try. I think that Jim is responsible, ethical, and on top of the latest technologies, but you know the players and their proposals best, so that all has to be your decision.

IF you decide to try this on the white cabinet and IF you want to use Termidor, I can see what I can do about trying to get some free Termidor for that job (which realistically would mean some chemical towards the exterior soil drench treatment too since the cabinet job would require so little volume). In short, the company that manufactures Termidor (BASF) is interested in tests on drywood termites, and they told me that they could remunerate with free pesticide. Thus no guarantees, but I could certainly try. I think that that would be accomplished through the distributor that handles the pest management company's account (likely in Puerto Rico or Miami). Best for you to decide if you want to give this a try and who will do your main job, then link me with that firm and I'll try to arrange for some Termidor to help with your bottom line.

Aside from the Termatrac explorations I also tested a matrix that is designed to be an especially preferred food for termites, the idea being that that matrix could then be impregnated with a pesticide and form an effective termite bait for direct, efficient delivery of termiticide to the target audience. Those experiments worked very well - nasutes, Heterotermes, and drywoods all ate the matrix as if it was candy. Tune in next year; I hope to have that approach (which is very environmentally friendly) in gear for field experiments if you're interested.

In the meantime, let me know if you have any questions, and we'll take the rest of it a step at a time (I'll get back to you about the Termatrac price and contact information if you decide you want to buy one, and you'll get back to me if you decide to try Termidor against drywood termites.).

Thanks for everything, keep up the excellent work, and best wishes always,

Barbara

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**Wenhua Lu**

**From:** "Barbara Thorne" <bthorne@umd.edu>
**To:** <wenhua@etal.uri.edu>; <hq@theconservationagency.org>
**Sent:** Monday, November 03, 2003 9:03 AM
**Attach:** Thorne PNAS 10.28.03.pdf
**Subject:** Hi Wenhua and Skip

Hi Wenhua and Skip,

I hope that you both had a terrific stay on Guana. It was great to see you early in the month - I had a great time and got significant things done on 3 different projects. Coming back to reality was tough this time; field work is such a pleasant and productive escape from budget and administrative hassles. Thanks for all that you do to make Scientists' Month possible!

Skip, did you ever hear from or get a chance to visit Jim Egelhoff? I've emailed him about several things, including getting a location for that gecko he collected on Virgin Gorda for you, but I haven't heard anything back. I assume he's just super busy getting his exhibits open (due to debut yesterday, 11/2), plus his "day job".

← Yes! Got all the info....

FYI, attached is my version of Skip's book, i.e. a nearly career-long synopsis of work and perspective, although in this case much much shorter than the awesome treatise on Islands. This work reflects my latest spin on the evolution of eusociality in termites, the question that got me interested in that group 25+ years ago.

Keep me posted on the book publication progress, thanks again for everything on Guana, and yes we'll send in some pages for the notebook that Roger is compiling on the scientists' work...

Best wishes to you both,
Barbara

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# Evolution of eusociality and the soldier caste in termites: Influence of intraspecific competition and accelerated inheritance

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Edited by Bert Hölldobler, University of Wurzburg, Wurzburg, Germany, and approved August 25, 2003 (received for review June 9, 2003)

**We present new hypotheses and report experimental evidence for powerful selective forces impelling the evolution of both eusociality and the soldier caste in termites. Termite ancestors likely had a nesting and developmental life history similar to that of the living family Termopsidae, in which foraging does not occur outside the host wood, and nonsoldier helpers retain lifelong options for differentiation into reproductives. A local neighborhood of families that live exclusively within a limited resource results in interactions between conspecific colonies, high mortality of founding reproductives, and opportunities for accelerated inheritance of the nest and population by offspring that differentiate into nondispersing neotenic reproductives. In addition, fertile reproductive soldiers, a type of neotenic previously considered rare and docile, frequently develop in this intraspecific competitive context. They can be highly aggressive in subsequent interactions, supporting the hypothesis that intercolonial battles influenced the evolution of modern sterile termite soldier weaponry and behaviors.**

The origin and maintenance of eusociality, cooperative societies composed mainly of subfertile or sterile members, are evolutionary paradoxes, because they seem to conflict with Darwin's concept of reproductive self interest (1–5). Progress in understanding the evolution of eusociality was incisively advanced by Hamilton's (2) theory of kin selection as applied to Hymenoptera (ant, bee, and wasp) societies. Female Hymenoptera are diploid and males haploid, a circumstance that affects relatedness, control of sex ratio, and aspects of genetic structure that, in combination with various ecological features, may promote eusocial evolution (3, 6–9). Highly social thrips are similarly haplodiploid (10), but eusociality also occurs in all termites and some other fully diploid animal species, including aphids (11), beetles (12), shrimp (13, 14), and naked mole rats (15). Kin selection is less potent in these groups that lack relatedness asymmetries between sexes and generations, indicating that other factors must also be important in explaining eusociality (3, 4, 14, 16, 17). Previous nonmutually exclusive theories regarding the evolution of eusociality in termites (4, 17–22) have been constructive but indecisive; the current consensus is that termite eusocial evolution was driven by a suite of selective forces (4, 17, 21, 23).

Many hypotheses regarding how and why eusociality evolved in phylogenetically diverse animals, including termites, profitably focus on shared aspects of biology and ecology, identifying commonalities in selective regimes that apparently favored social evolution (3, 4, 14, 17, 24). The chance for nest inheritance, either by unrelated helpers or through philopatric reproduction (succession to a breeding position within the natal group), is considered a fundamental element of many theories on the evolution of both eusociality (3, 4, 14, 17, 25, 26) and cooperative breeding in insects, fish, birds, and mammals (27–29). Incentives for helping without inheritance occur through kin selection if offspring can sufficiently enhance reproduction by their parents or relatives; however, opportunities for inheritance can further promote helping behaviors and life history modifications. Moreover, the survival and fitness payoffs of inheriting an established nest and resource area may well exceed those realized by dispersing offspring in solitary species, thus favoring helpers that remain as "hopeful reproductives" (30) in social units, even if that means delaying or forgoing reproduction. In philopatry, a system of serial reproductive inheritance by kin, all individuals in the group also gain inclusive fitness benefits. The inheritance hypothesis, productively applied to the evolution of altruistic behavior in other social groups, was logically extended to termites, supported by the fact that philopatric reproduction is common through helper differentiation into secondary (replacement or supplementary) reproductives on death or senescence of established reproductives (4, 17, 26). An apparent problem with this application to termites, however, is that founding kings and queens are long-lived in captivity and in some field colonies (31), even in relatively primitive taxa (32), suggesting that early orphaning of helpers (or originally, nonhelpers) and thus opportunities for inheritance might be rare in the young families that must have characterized the evolutionary transition to termite eusociality (23). Founder life spans may have been shorter in prototermites than in modern groups (4), but timing of inheritance opportunities would be important if parents survived past the time their first offspring could reach sexual maturity and if remaining in the natal nest resulted in progeny delaying or forgoing direct reproduction. Demise of founding reproductives might also occur through predation or parasitism, but it is difficult to imagine preferential attack of the king and/or queen while leaving healthy helpers capable of assuming reproductive roles in a robust colony. Thus the question remained, why would early brood offspring remain as helpers in prototermite families if their probability of inheriting the nest resource and assuming reproductive status depended on the apparently unlikely circumstance of death of a parent but not the whole colony?

All of the >2,600 living species of termites are eusocial, and solitary ancestors are sufficiently distant to obscure prototermite selective regimes. No "stepping-stone" intermediate taxa exist for comparative study of transitional states from solitary to social to eusocial species; we must instead draw evolutionary inferences based on theoretical constructs and/or the biology and socioecology of the most primitive living lineages. Modern species belonging to even the most basal groups represent blended assemblages of primitive and derived traits. Recent phylogenetic analyses differ in topology but include the same three families as the most basal living termite clades: Mastotermitidae, Hodotermitidae, and Termopsidae (33). Although living Mastotermitidae and Hodotermitidae retain pleisiomorphic morphological characters, their social organization appears to be derived. Among extant taxa, Termopsidae are widely viewed as the closest available approximation to ancestral termites in socio-

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Abbreviation: mRS, male reproductive soldier.

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Fig. 1. Natural chambers of young *Zootermopsis* colonies revealed under the bark of a Ponderosa pine tree; note close proximity of neighboring families and therefore high probability of interactions in the field.

ecological features such as colony size, social organization, nesting biology, and caste polyphenism (4, 17, 22, 34–37). Termopsid colonies live and feed exclusively within a single log, facing eventual resource limitation (38, 39). Fertile alates, produced seasonally, are the only colony members to leave their natal wood. They fly to find mates and nest under the bark of a recently dead tree to found new colonies. Tens or hundreds of typically monogamous "royal pairs" (king and queen) may settle synchronously in the same piece of wood, frequently using beetle holes to colonize a tree trunk at an appropriate stage of decay. As they consume the wood, initially preferring the soft, narrow, nutrient-rich decaying phloem layer under bark, they create nest chambers, often in close proximity to neighboring colonies (Fig. 1) (32, 40).

Development is exceptionally flexible in Termopsidae, with all individuals except soldiers and reproductives capable of molting into any other caste (4). Two types of nondispersing secondary reproductives can develop to replace or supplement the founding king and queen. Neotenic reproductives typically differentiate in response to the death or senescence of reproductives; multiple males and females may persist in the same colony (41). Fertile reproductive soldiers (soldier neotenics), individuals of either sex with soldier-like heads and neotenic gonad development, have been recorded in six species of termopsids (4, 42, 43). Soldiers of all other families of modern termites, as well as normal soldiers within the Termopsidae, are sterile,[†] and the significance of reproductive soldiers has heretofore been obscure.

Using the termopsid species *Zootermopsis nevadensis* (Hagen), we test the Accelerated Inheritance hypothesis that intraspecific interactions, occurring as families grow and meet within a limited resource, can result in high mortality of established reproductives and opportunities for helpers to differentiate into reproductives. We further demonstrate that these interactions frequently prompt production of reproductive soldiers, a caste unique to primitive termites, and previously considered rare, incidental, and docile. We show that reproductive soldiers can be highly aggressive in intercolony interactions, supporting the postulate that they represent an evolutionary precursor to the sterile soldiers characteristic of modern termite societies.

[†]In the most primitive living Termopsid, *Archotermopsis wroughtoni* Desneux, gonads of all soldiers are as well developed as in alates (44), but their fertility is not established.

## Methods

**Intercolony Interactions.** To simulate the circumstance of intraspecific competition in which growing colonies meet one another within their natural shared food and nesting resource, we arranged interactions between 14- to 16-month-old complete *Z. nevadensis* colonies of similar sizes. The protocol for interactions between colonies was to provide a Tygon tube connection between Petri plates containing each colony and their nest material (detailed methods described as even-age interactions in ref. 32). We report on data from 57 such interactions, each involving two similarly sized colonies containing their founding king and queen and an average of 46.2 (±2.69 SE; median 43) "workers"[‡] and soldiers (range of one to five soldiers). Seventeen unmanipulated colonies were monitored as controls. Time intervals are reported from the time of the interaction. Statistical comparisons were made by using $\chi^2$ analyses. Each of the six replicates of interactions between already interacted, merged "colonies" and simple families originated from colonies 16 months old at the time of the first interaction. Results from 14 interactions between colonies ≈1 yr apart in age are also discussed (the protocol is described as uneven interactions in ref. 32). Colonies used in all experiments were bred from alates that emerged from colonies collected near Placerville, CA (El Dorado County) and were thus complete families and social units (rearing methods described in ref. 32).

**Intracolony Reproductive Soldier Behavior.** We observed laboratory colonies (complete families) ranging in age from 1 to 2 years and containing 100–300 individuals in 15-cm-diameter Petri dishes covered with red Plexiglas. One of the colonies had a queen, and the other two had three and four female neotenics; all had a male reproductive soldier (mRS) and two to five normal soldiers. Position data were recorded at 30-min intervals for 10 h on 4 days.

**Interactions Between Colonies Containing Reproductive Soldiers.** Data are reported on 11 paired interactions involving 22 complete families that had not been involved in a previous interaction. The experimental set-up was identical to that described above for intraspecific interactions. One treatment involved six replicates of interactions between similarly sized colonies headed by a king and queen and colonies containing a mRS and a queen. The second treatment, five replicates, involved meetings between two similarly sized colonies that each contained a mRS and reproductive females (female neotenics were in 9 of 10 colonies; 3 also contained a queen). Behavioral observations were made for 2 h after the beginning of an interaction. We defined aggression as a continuum of behaviors ranging from mandible flaring (mild agonism) to biting.

## Results and Discussion

**Intraspecific Interactions: Opportunities for Accelerated Inheritance.** In the interactions between complete 14- to 16-month-old colonies, staged as an experimental test of this Accelerated Inheritance hypothesis, termites typically explore the connection between the two families immediately. Agonistic behavior ensues, directed primarily at reproductives, although some other individuals on one or both sides may be injured or killed. Workers, soldiers, kings, and queens all act as aggressors toward reproductives during intercolony interactions. In 72 interindividual attacks witnessed during the first 2 h of 18 interactions, reproductives were aggressors in 22 (30.5%) of the strikes, and

[‡]In formal termite terminology, Termopsidae do not have true workers because those individuals retain substantial developmental plasticity (35); however, in the functional sense that they help and work within the colony, we refer to them as helpers or workers in this paper.

in all but one of those cases (95.4%), the target was another king or queen. Workers attacked reproductives in 35/72 = 48.6% of observed cases, and soldiers were the aggressors in the remaining 15/72 = 20.8% of attacks.

Twenty-four hours after the interaction, at least one of the four original kings or queens was killed in 94.7% of the 57 replicates, and at least one king and queen survived in 87.7% of the conjoined colonies. Of the 26 interactions in which we know the colony of origin of the reproductives due to identifying paint marks, 92% of the king and queen pairs that survived were from the same original colony. At 24 h after an interaction, all castes from both original colonies appear to intermingle freely in the newly merged "colony." At 6 months after the interactions, at least one king and queen persisted in 61.1% of interactions compared with 94.1% of 17 unmanipulated controls; a king and queen survived in 46.3% of interaction colonies at 12 months (88.2% of controls).

A remarkable result of intercolony interactions is that reproductive soldiers appeared in 58% of merged colonies within the first 3 months and in 66.7% of them within the first year (never within control colonies within 12 months). Normal neotenic reproductives differentiated in 40.4% of interaction colonies within the first year but in only two (11.8%) control colonies, both of which had lost one or both founding reproductives. One or both types of secondary reproductives, normal neotenics or reproductive soldiers, occurred in 41/57 = 71.9% of interaction colonies within the first 12 months, and in 27/41 = 65.8% of those cases, one or more of these secondaries appeared when at least one king and queen remained alive in the combined colony. Secondary reproductives occurred only rarely in isolated control colonies within the same time period (2/17 = 11.8%) and never when both original reproductives were healthy (Fig. 2). The higher mortality of founding reproductives in groups involved in intraspecific interactions vs. controls ($P < 0.0001$) and development of secondary reproductives in a statistically greater proportion of such colonies in comparison to controls ($P < 0.0001$; Fig. 2*c*, D and d) are critical contrasts in support of the Accelerated Inheritance hypothesis. Molecular genetic analyses are in progress to determine the colony of origin of reproductives that differentiate after interactions and the long-term genetic structure of the growing populations. Experiments regarding colony recognition cues and how they are adjusted to facilitate "merging" within interaction colonies are also underway.

Survivorship of founding reproductives is reduced after intercolony interactions, and even if they live, the inhibition that normally restrains development of secondary reproductives in the presence of a functional king and queen (41) is muted, allowing helpers a chance to molt into fertile replacement or supplementary reproductives. This is not simply a phenomenon of weakened inhibitory control by kings and queens due to larger colony size. In long-term rearing of control colonies, families that retained a viable king and queen never developed a secondary reproductive ($n = 12$, up to 7 years of age, colony population sizes exceeding 1,000 individuals). In control colonies that lost the king or queen, a neotenic or reproductive soldier of the same sex as the surviving primary developed only once in 7 years; a male neotenic was recorded in a census immediately before the death of the king.

The merged associations that result from interactions appear to function as stable groups and are much larger units than same-aged nuclear family neighbors. Six months after the original interaction, we arranged meetings ($n = 6$) between those merged colonies and simple family colonies of the same age. The previously interacted colonies were now at least twice as large as unmanipulated families. In all replicates, the second series of interactions resulted in death of all reproductives in the smaller simple family colonies within 24 h, whereas only one of 15 reproductives (a female neotenic) died in the merged colonies.

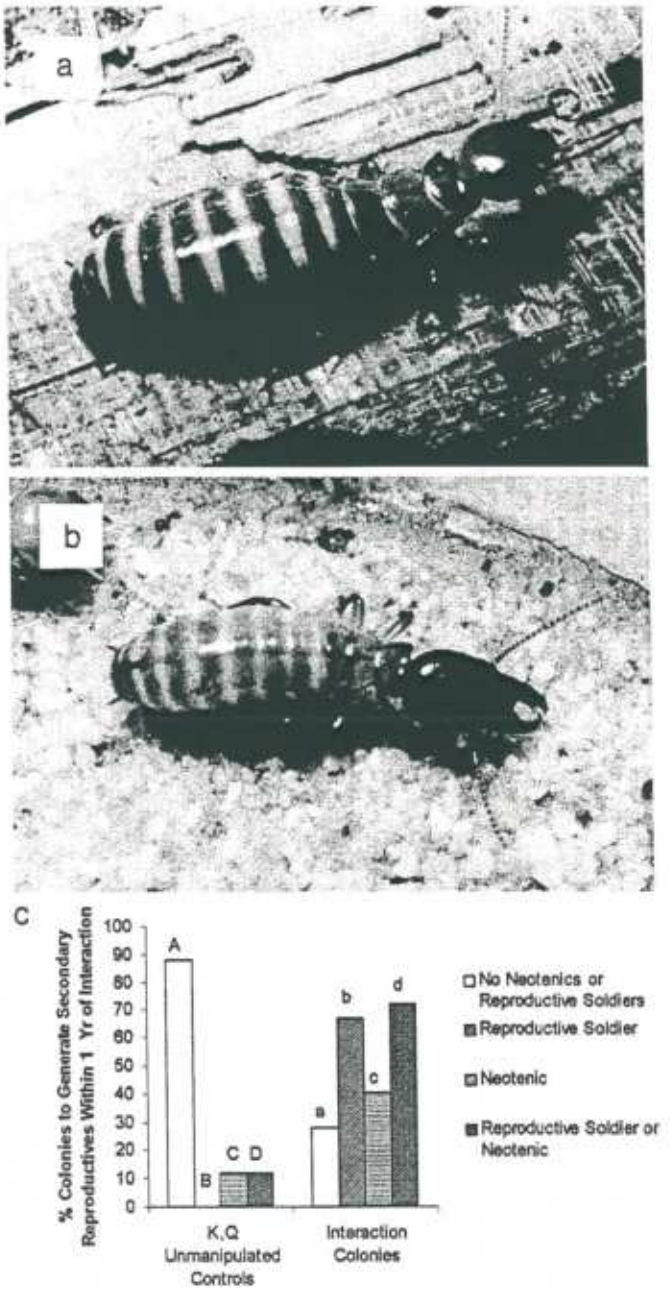


Fig. 2. Occurrence of secondary reproductives in control vs. interaction colonies. Normal neotenic (*a*), reproductive soldier (soldier neotenic) (*b*), and secondary reproductives (neotenics and/or reproductive soldiers) (*c*) differentiate significantly more frequently after intercolony interactions than in control colonies (and only in controls in which founding reproductives died). Compare columns with the same letters; if case differs, they are significantly different. $\chi^2$ levels of significance: Aa, $P < 0.0001$; Bb, $P < 0.0001$; Cc, $P < 0.05$; Dd, $P < 0.0001$.

This demonstrates a competitive advantage due to size conferred on groups resulting from interactions [note parallels to brood raiding and associated behaviors in ants, e.g., Adams and Tschinkel (45), although those examples involve no philopatry or colony inheritance].

Disparity in colony size has a similar effect on the outcome of interactions between nuclear families of different ages, with consequences for colonists of the same resource in different years or same-aged colonies with slower growth rates. Of 14

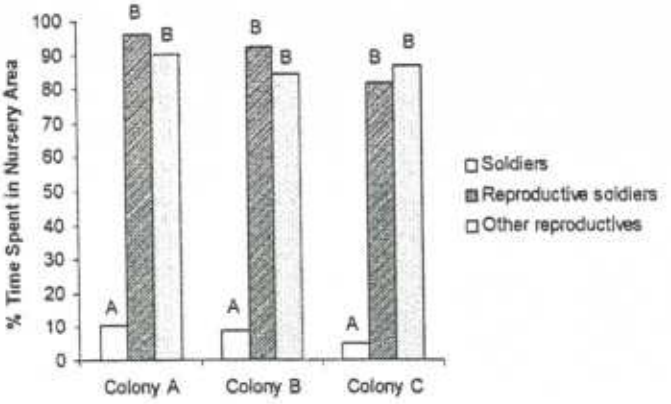


Fig. 3. Time spent in nursery area of the nest by normal soldiers and reproductives. Normal soldiers rarely associate with eggs and dependent brood in the nursery; in this regard, reproductive soldiers behave like other reproductives. Compare columns with the same letters; if case differs, they are significantly different ($\chi^2$ analysis; $P < 0.0001$).

interactions involving meetings between 14- and 19-month-old colonies and 3- to 4-month-old incipient colonies (each 40–62% the size of the older colony), all primary reproductives in the younger colonies were killed, but only a single king died among founders of the larger colonies. All of the younger colonies were decimated in entirety by the larger families, i.e., no "merging" occurred, as was typical in the more evenly sized interactions described above. Pairs and extremely young colonies often use protective tactics to avoid contact with other colonies. In 8 of the 14 replicates, the smaller 3-month-old colonies invested in building "fecal fortresses" as reinforcement barriers to intrusion by the larger colony, forcing a delay but not altering the outcome of interaction between neighbors. Thus successful early colonists in a resource gain an advantage over founders that colonize in subsequent years, nest usurpation by younger pairs or their incipient colonies is unlikely, and chance of survival is precarious if the resource is already relatively densely settled (although predators or pathogens may eliminate entire colonies, opening uncontested resource space for later arrivals).

The ecological circumstance of intraspecific termite families growing and expanding within a limited resource ensures meetings among young colonies. Among similarly sized colonies, such as a cohort of neighboring families initiated the same season, intercolony interactions often precipitate death of founding reproductives, opportunities for nest inheritance by offspring helpers, and resulting large merged colonies that have a competitive advantage in future interactions. This dynamic therefore accounts for a missing link in the logic of theories of the evolution of eusociality based on philopatric reproduction: how even the earliest brood in a family might have had opportunities for reproductive inheritance and therefore further incentive to remain as helpers, even in an insect in which parents have the potential to be long lived. Once alleles for offspring to help in the parental nest and delay or forgo dispersal spread, the selective landscape was in place for potential evolution of caste polyphenism and ultimately the evolution of sterile castes (3, 4, 30).

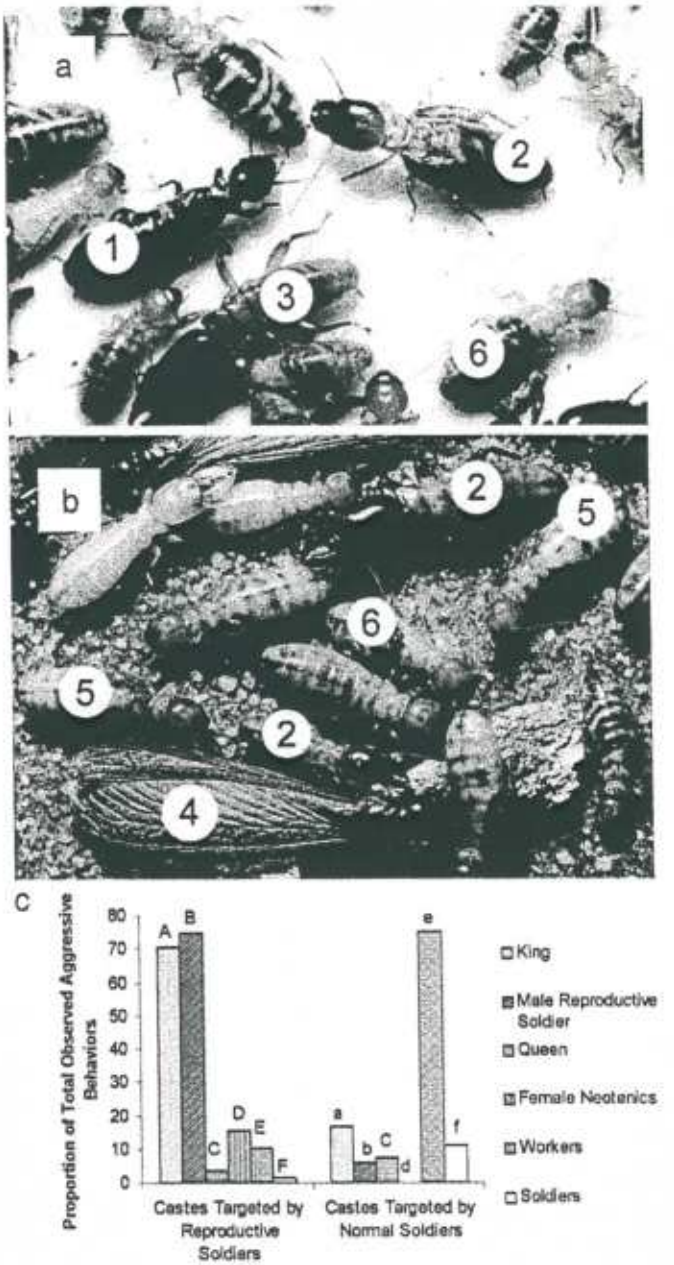


Fig. 4. Differential targeting of castes by reproductive soldiers vs. normal soldiers. (*a* and *b*) Castes and polyphenism within colonies. Number indicates caste: 1, queen; 2, reproductive soldier; 3, normal soldier; 4, alate; 5, nymph; and 6, worker. (*c*) mRSs selectively attack other male reproductives during intercolony interactions (treatments I and II combined, thus categories are context-dependent and proportions do not add to 100). $\chi^2$ levels of significance: Aa, $P < 0.001$; Bb, $P < 0.0001$; Cc, not significant; Dd, no attacks by normal soldiers on female neotenics; Ee, $P < 0.0001$; Ff, $P < 0.05$. Compare columns with the same letters; if case differs, they are significantly different ($P \leq 0.05$).

**Evolution of the Soldier Caste.** Intraspecific interactions between dampwood termite colonies have also facilitated insights regarding evolution of the soldier caste in termites. It is unknown whether modern sterile termite soldiers evolved as a defensive caste or from neotenic reproductives similar to modern reproductive soldiers. Myles (26, 42) postulated that reproductive soldiers are ancestral to modern sterile soldiers, having "weapons" selected in response to intracolonial competition among neotenics with the soldiers' role in colony defense as a secondary adaptation. Roisin (43) questioned this hypothesis, noting that there was no evidence of reproductive soldier aggression against any individual. Our data demonstrate a context in which reproductive soldiers frequently develop (i.e., after intraspecific interactions) and support that within their own colonies, reproductive soldiers are docile. We have never seen a reproductive soldier behave aggressively toward a nestmate and, like other

reproductives, they remain with eggs and brood while normal soldiers patrol away from the nursery (Fig. 3).

Although reproductive soldiers are passive within their own colonies, we documented multiple cases of reproductive soldier aggression during intraspecific interactions. We set up two interaction treatments, the first ($n$ = 6) between families headed by a king and queen and colonies containing a mRS and a queen. The second treatment ($n$ = 5) involved meetings between two colonies that each contained a mRS and one or more reproductive females (female neotenics were in nine of 10 colonies; three also contained a queen). None of the colonies used in this series of experiments had been involved in previous interactions.

As in the experimental interactions described above, we observed marked mortality of established reproductives as a result of interactions involving mRSs: 45.8% of the 12 reproductives in treatment I and 34.8% of the 46 reproductives in treatment II were killed within the first 24 h after interaction. In 10 of 11 cases (91%, combined treatments), some or all reproductives from one colony were killed during the interaction, whereas all reproductives from the opposing colony survived in the merged colony. In all 11 cases (both treatments), only one of the two male reproductives originally present in the interaction remained in the merged colony after 24 h.

We observed aggression toward noncolony members by 5 of 6 (83.3%) mRSs in the queen–mRS/queen–king treatment and 7 of 10 (70%) mRSs in the female–mRS/female–mRS treatment. Notably, the four reproductive soldiers not observed as aggressive were never seen in the same colony chamber as the other male reproductive, hence their reaction in that context could not be assessed. No aggressive acts by mRSs were aimed toward colony mates in any trial. All five aggressive mRSs from the first treatment were observed directing aggressive behaviors toward the opposing king and occasionally members of other castes. Strong aggressive behaviors (biting and/or killing another termite) were correlated with mRS survivorship in the queen–mRS/queen–king treatment. All four reproductive soldiers observed displaying strong aggression did so toward the opposing king and survived to 24 h after the interaction, whereas the remaining two relatively passive mRSs died within 24 h of the interaction. mRSs directed most of their biting and lunging behaviors toward male reproductives in the other colony, a significant difference from targets of aggression by normal soldiers (Fig. 4).

Therefore, instead of soldier morphology and agonistic behavior evolving as an adaptation to intracolonial aggression among neotenics (26, 42), our study supports the new hypothesis that termite soldier weaponry and aggressive behaviors evolved in the context of intercolonial fighting among reproductives with modern sterile soldiers and their roles in colony defense having evolved secondarily. Fertile "soldiers" in the basal family Termopsidae, found commonly on our *Zootermopsis* collecting trips, are retained in extant species due to nesting biology (coincidence of food and nest/habitat) and intraspecific competition. A similar sequence of reproductive forms with soldier-like morphologies and behaviors evolving prior to sterile soldiers apparently occurred in social aphids (11) and thrips (46); ant soldiers also appear derived from reproductives (47).

Thus the same ecological context, intraspecific interactions between colonies nesting within a limited resource, may have influenced the evolution of both eusociality and the soldier caste in termites. The hypothesis of Accelerated Inheritance fortifies the theory that the evolution of termite eusociality was promoted by a suite of ecological conditions providing advantages to family living and long-term helping behavior by offspring that retain remarkable developmental plasticity. Those offspring gain inclusive fitness advantages by helping to produce fertile siblings. They also benefit from opportunities to become replacement reproductives as their young families grow, meet, and interact with neighboring colonies, often resulting in early death of established reproductives. Helping behavior by offspring that forgo or delay risky dispersal options, even if in a young family, results in their being profitably situated to become a reproductive upon the death of founding reproductives. After interactions, they would belong to a larger association, conferring a competitive advantage in future intraspecific meetings.

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# The Conservation Agency

*Exploration, Education, and Research*

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July 22, 2003

Dr. Richard Levins
HSPH, Dept. Population and International Health
665 Huntington Ave., Room 1109
Boston, MA 02115

Dear Dr. Levins:

There is no reason at all that you should remember me. I have been in and out of Harvard since 1959 and am still on the staff of MCZ. You might remember Greg Mayer, reprint enclosed, a Lewontin student.

I have just completed and submitted a ponderous tome -- Island: Fact and Theory in Nature -- to Academic Press. There will, of course, be the usual frantic updates and minor revisions before it gets printed. The center stage of the book is Guana Island, BVI. There are n-dimensional wings and backstages. It is my book about everything.

Of course, you have your paragraph -- in the section called "The Cast," in the lists of Diptera. Apparently your 1969 (Am. Nat. 103:483-499) paper is the first published record of an insect on Guana (*D. simulans*). Scott Miller, USNM (also Harvard) got me that reference. In your paragraph readers are reminded that you are also the first of Levins and Heatwole (1973), Levins and Lewontin (1985), and the second of Heatwole *et al.* (1981), which are cited elsewhere.

That was about all until Hoffmann *et al.* (2003. Science 301: 100-102): what's that: "thermal acclimation and heat resistance?" Endangered species. Isolates. Global warming... Your paragraph may become a short story.

Did you actually get to spend time on Guana Island? Want to? We have the whole island for October each year. The accommodations are posh, the food is generally excellent, the beer, soda pop, and fruit juices are all free, and wine is served with dinner. If you can get to Beef Island airport we will pick you up and bring you over. Barbara ("Bambi" when at Harvard) Thorne and a U. MD entomological team will be there 1-8 October. Entomologist Barry Valentine will be there all month, as will my wife, Wenhua Lu, who is an argroentomologist, systematist, and biogeographer (you can find us in *Natural History*, 1996, 105(1): 35-39: "The voyage of the beetle").

I am currently in the field, in a trailer, in SE Oklahoma (where Wenhua is researching watermelon pests, etc., for OSU). I am not online, but my email is received

HQ@theconservationagency.org

I can fax you back. Or, snail mail still works fine: P.O. Box 86, Lane, OK 74555. If you are interested in Guana, I can mail you my book on a CD-Rom. Your critique would be most welcome -- no matter how devastating (my math is deplorable)

With best wishes,

Skip Lazell

James Lazell, Ph.D.

J Mol Evol (1997) 45:232–237

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# *Adh* Nucleotide Variation in *Drosophila willistoni:* High Replacement Polymorphism in an Electrophoretically Monomorphic Protein

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**Abstract.** *Drosophila willistoni* was the subject of intensive allozyme studies and the locus coding for alcohol dehydrogenase (*Adh*) was found to be virtually monomorphic. DNA sequence analysis of 18 alleles throughout the distribution of the species has revealed six replacement polymorphisms. The ratio of replacement to silent polymorphisms is higher in *D. willistoni* than in any other *Drosophila* species studied for *Adh* nucleotide variation. Also in contrast to other species, the variation in introns and noncoding DNA is about the same as in the coding region. We speculate that both these differences indicate *D. willistoni* has historically had a small population size possibly related to Pleistocene refugia in the Neotropics.

**Key words:** *Drosophila willistoni* — DNA polymorphism — Alcohol dehydrogenase

## Introduction

The Alcohol dehydrogenase (*Adh*) locus in *Drosophila* has served as a paradigm for molecular population genetics since Kreitman's (1983) path-breaking work. DNA sequence variation at the *Adh* locus has been studied extensively within *D. melanogaster* (Kreitman 1983; Hudson et al. 1987; Kreitman and Hudson 1991; McDonald and Kreitman 1991; Laurie et al. 1991) and *D. pseudoobscura* (Schaeffer and Miller 1991, 1992a, 1993), and to a lesser extent in other species of the *melanogaster* subgroup (McDonald and Kreitman 1991). These species represent two of the three major lineages in subgenus *Sophophora*. Here we add information on *Adh* nucleotide polymorphism in the third major lineage, the *willistoni* group. Previously, we showed that *Adh* in the *willistoni* group displayed some unusual features: the loss of an intron and a significant shift in codon usage (Anderson et al. 1993).

Species of the *Drosophila willistoni* group have been analyzed extensively for electrophoretic variation in soluble enzymes in natural populations (reviewed in Ayala 1975). Variation at the *Adh* locus was surveyed in several of these studies. ADH was one of the least polymorphic enzymes analyzed in the *willistoni* group, and was electrophoretically monomorphic in several populations of *D. willistoni, D. paulistorum,* and *D. tropicalis.* In *D. willistoni,* a total of four different alleles were detected in a sample size of nearly 5000 alleles, although the most common allele had a frequency of about 0.994. Thus, by virtually all criteria, this protein can be considered electrophoretically monomorphic.

Here we present the nucleotide sequence variation in 18 *D. willistoni Adh* alleles, sampled from strains that represent 10 populations distributed throughout the geographic range of this species. These data reveal unusual patterns of nucleotide polymorphism in coding versus noncoding regions, and a high level of replacement polymorphism.

## Materials and Methods

Drosophila *Stocks. Strains of Drosophila* were obtained from several sources. Collection sites and sources of *Drosophila* are listed in Table 1. Each strain is an isofemale line. The strains sampled in this study

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**Table 1.** Strains of *D. willistoni* used in this study, their original collection sites, and sources from which they were obtained

| Strain | Abbreviation | Collection site | Source | GenBank accession no. |
|---|---|---|---|---|
| W4L[a] | wil4L | Aguas do los Rios, Brazil | J. Powell | L08648 |
| W3L[a] | wil3L | Aguas do los Rios, Brazil | J. Powell | U95253 |
| A57[a] | wilA5 | Aguas do los Rios, Brazil | J. Powell | U95254 |
| 0811.0 | wil0 | Santa Maria, Nicaragua | Bowling Green[b] | U95251 |
| 0811.4 | wil4 | Cuernavaca, Mexico | Bowling Green | U95252 |
| Belize II | wilB2 | Belize | F. Ayala | U95256 |
| Belize VI | wilB6 | Belize | F. Ayala | U95257 |
| Caño Mora | wilC | Caño Mora, Costa Rica | F. Ayala | U95258 |
| Atlixco | wilA | Atlixco, Mexico | F. Ayala | U95255 |
| Guana | wilG2 | Guana Island | P. Chabora | U95259 |
| Lima | wilL | Lima, Peru | F. Ayala | U95260 |
| Manaus 1 | wilM1 | Manaus, Brazil | V. Valente | U95261 |
| Manaus 2 | wilM2 | Manaus, Brazil | V. Valente | U95262 |
| Manaus 3 | wilM3 | Manaus, Brazil | V. Valente | U95263 |
| Manaus 4 | wilM4 | Manaus, Brazil | V. Valente | U95264 |
| PA 1 | wilPA1 | Porto Alegre, Brazil | V. Valente | U95265 |
| PA 2 | wilPA2 | Porto Alegre, Brazil | V. Valente | U95266 |
| PA 3 | wilPA3 | Porto Alegre, Brazil | V. Valente | U95267 |

[a] Isofemale line; obtained as a genomic DNA preparation from C. Anderson
[b] National *Drosophila* Species Resource Center, Bowling Green State University, Bowling Green, Ohio, USA

basically represent the geographic range of this species, ranging from Mexico to Porto Alegre, Brazil (on the Uruguay border), and from Lima, Peru in western South America to eastern Brazil, and one Caribbean strain. Strains of *D. willistoni* from Lima, Peru have previously been identified as a subspecies, *D. willistoni quechua*, based on sterility in male hybrids (Ayala 1973). However, crosses of the Lima strain we studied to known *D. w. willistoni* in our lab produced fertile $F_1$ males (J. Powell and J. Gleason, unpublished observations).

*PCR Amplification.* Total genomic DNA was prepared using standard phenol–chloroform extraction followed by proteinase K digestion (Werman et al. 1990). A 1.3-kilobase (kb) region of the *Adh* gene was amplified by polymerase chain reaction (PCR) from each strain using oligonucleotide primers identical to highly conserved regions 5′ and 3′ to the *Adh* coding region of the *D. willistoni* W4L genomic clone (Anderson et al. 1993). Oligonucleotide synthesis was done using an Applied Biosystems 391 DNA synthesizer. All of the oligonucleotide primers used in PCR and sequencing reactions are listed in Table 2. PCR conditions were optimized for these primers, and the following conditions were used. One μg template DNA, 300 ng of each primer, 1 unit Taq polymerase (Perkin Elmer Cetus), and 100 μmol of each dNTP were used per reaction, in a total volume of 100 μl. The reaction buffer was the same as that in the GeneAmp kit (Perkin Elmer Cetus) except for the $MgCl_2$ concentration (50 mM KCl, 10 mM Tris-Cl, pH 8.3, 30 mM $MgCl_2$, 0.1% gelatin). Forty cycles of amplification were carried out using a Perkin Elmer Cetus DNA Thermal Cycler under the following conditions: 94°C, 1 min; 50° or 55°C, 1 min; 72°C, 1 min. PCR products were checked by electrophoresis on 2% agarose gels.

*Cloning and Sequencing of PCR Products.* Unpurified PCR products were ligated into pCR1000 and pCRII vectors (Invitrogen) and transformed into INVαF′ (Invitrogen) or DH5α (BRL) competent cells. Plasmid DNA for sequencing was obtained from either minipreparations done with the Magic Minipreps DNA purification kit (Promega) or by large-scale plasmid preps, done by the polyethylene glycol/LiCl precipitation method described by Sambrook et al. (1989). DNA sequencing was done by the dideoxy chain-termination method (Sanger et al. 1977) using Sequenase (USB). The sequences of primers used in these experiments are shown in Table 2. M13 forward and reverse primers were used as well, and were obtained from New England Biolabs. The 5XINT primer was obtained from C. Anderson. Both strands of DNA were sequenced for all strains of *D. willistoni*, but an independent PCR product was sequenced only for those strains that had amino acid replacement changes. Sequences were aligned by eye.

**Table 2.** Sequences of oligonucleotide primers used for PCR amplification and DNA sequencing

| Primer | Sequence (5′–3′) | Use |
|---|---|---|
| ADH 1 | TTAGTTGAGAAGAGAAGAGCC | PCR amplification |
| ADH 2 | CGATTATCAAATCAGCCTTC | PCR amplification |
| B3 | TCTGTGACCGGTTTCAATGC | sequencing |
| B4 | GGCCTTAACAAAGTTCTGGG | sequencing |
| B5 | AACATCTTGGTGGCCGG | sequencing |
| 5XINT | ACAGCAATGGTAGCTC | sequencing |

## Results

A PCR amplified product of approximately 1.3 kb of the *Adh* region was obtained for each of the strains listed in Table 1. These PCR reactions always yielded a very strong single band on an agarose gel. This region included the complete coding sequence of 759 bp, as well as the 214-bp noncoding 5′ DNA, a single 67 to 68-bp intron, and 127-bp 3′ noncoding DNA. Both strands of one PCR product were sequenced for each allele in the present study, unless there was an amino acid change, in which case an additional PCR product was cloned and sequenced. There were no sequence differences between independent PCR products for these strains. The com-

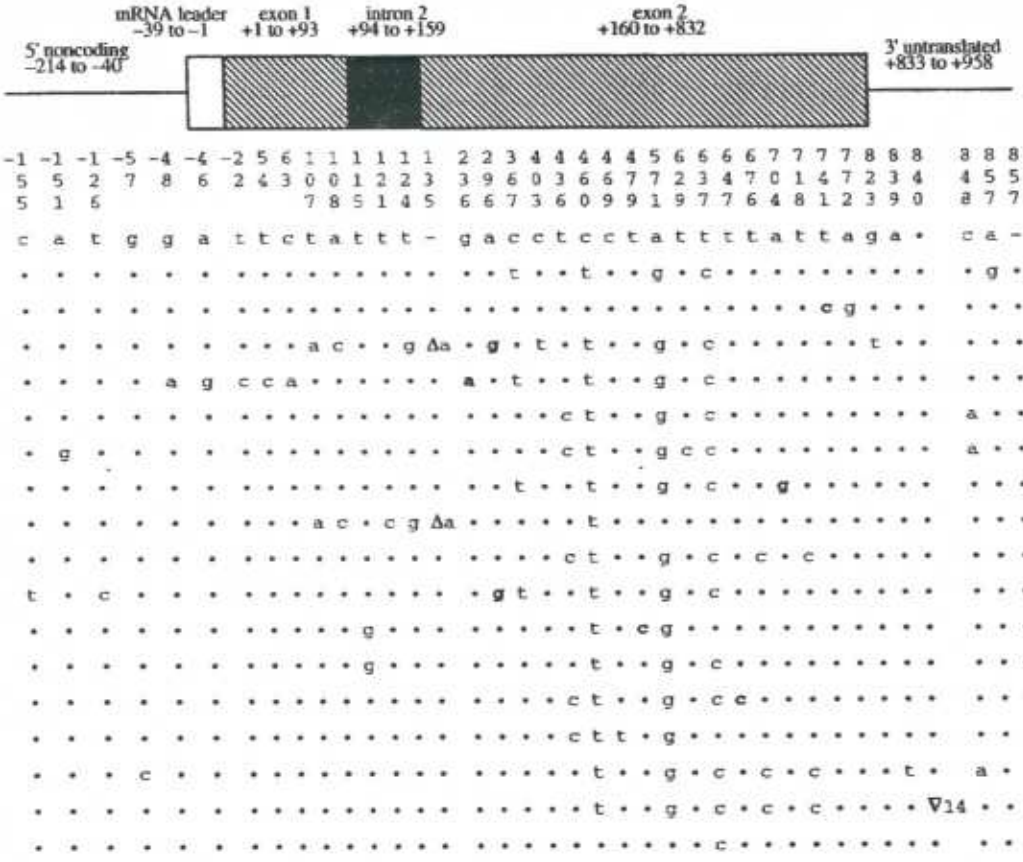


**Fig. 1.** Polymorphic sites in the 18 *D. willistoni Adh* alleles. The wil4 sequence is used as the reference sequence. Δ/∇ refer to indels. Replacement polymorphisms are indicated in bold type. Strain abbreviations are as listed in Table 1.

plete nucleotide sequence and deduced amino acid sequence of the wil4 *Adh* gene was presented in Anderson et al. (1993) and will not be repeated here. Comparison of these strains to other species of the *willistoni* group as well as the GenBank accession numbers are in Gleason et al. (submitted).

Figure 1 illustrates the polymorphic sites detected in the 18 *D. willistoni Adh* alleles. A total of 35 nucleotide substitutions and four single nucleotide indels were found. In the noncoding DNA, introns had the highest percentage of polymorphic sites (7.46%), with the 5′ region having 3.27%, and 3′ region 2.36%. In the coding region, there were 20 nucleotide polymorphisms, 14 of which were synonymous. Tables 3 and 4 summarize the nature of these polymorphisms. Twelve of 14 synonymous polymorphisms are transitions, a not unexpected result given the structure of the genetic code. The frequency of synonymous polymorphism for each codon redundancy class is not significantly different from random expectations ($G^* = 4.48$, 3 d.f., $p > 0.1$). Surprisingly, many of the synonymous polymorphisms are shared across strains from widely separated localities.

Table 4 summarizes the replacement polymorphism. The predicted charge at pH 7.0 of each polymorphic protein was determined, and none differed from that of wil4 ADH. Hydrophilicity profiles and predictions of

**Table 3.** Summary of polymorphic synonymous codons in the 18 *D. willistoni Adh* alleles. Sites are labeled as in Figure 1

| Site | Change in NT sequence | Amino acid encoded | Codon redundancy group | Transition or transversion |
|---|---|---|---|---|
| 54 | GGT-GGC | Gly | fourfold | Transition |
| 63 | ACC-ACA | Thr | fourfold | Transversion |
| 367 | ATC-ATT | Ile | threefold | Transition |
| 403 | GTC-GTT | Val | fourfold | Transition |
| 436 | GAT-GAC | Asp | twofold | Transition |
| 460 | GGC-GGT | Gly | fourfold | Transition |
| 469 | TGC-TGT | Cys | twofold | Transition |
| 571 | TTA-TTG | Leu | sixfold | Transition |
| 629 | TTG-CTG | Leu | sixfold | Transition |
| 637 | CAT-CAC | His | twofold | Transition |
| 678 | GCT-GCC | Ala | fourfold | Transition |
| 718 | TGT-TGC | Cys | twofold | Transition |
| 772 | AAA-AAG | Lys | twofold | Transition |
| 823 | TCG-TCT | Ser | fourfold[a] | Transversion |

[a] Serine cannot be analyzed as a sixfold redundant codon, because substitutions in two codon positions must occur between some synonymous codons. Serine codons are thus divided between the two- and fourfold redundant groups

**Table 4.** Summary of amino acid replacement polymorphisms in the *D. willistoni Adh* alleles

| Strain | Amino acid change | Change in nucleotide sequence | Position in nucleotide sequence | Position in primary amino acid sequence |
|---|---|---|---|---|
| wil4 | Val to Ile | GTT to ATT | 236 | 57 |
| wil0 | Thr to Ala | ACC to GCC | 296 | 77 |
| WilL | | | | |
| wilM1 | Ser to Pro | TCT to CCT | 479 | 138 |
| wilM3 | Ser to Pro | TCC to CCC | 647 | 194 |
| wilC | Thr to Ala | ACA to GCA | 704 | 213 |
| wilA5 | Ile to Thr | ATT to ACT | 741 | 225 |

secondary structure were obtained for each of the polymorphic proteins as well as for wil4 ADH, using the MacVector program (IBI) (data not shown). None of the amino acid replacements caused a major shift in these properties. Thus our results are consistent with an inability of electrophoresis to detect these polymorphisms.

We performed two statistical tests to determine how well the data conform to expectations of the neutral theory. One is that of McDonald and Kreitman (1991) which tests whether the ratio of silent to replacement polymorphisms are significantly different from that ratio between species. We used *D. nebulosa* as the comparison species because it has sufficient divergence to provide a reasonable sample of changes, yet is sufficiently closely related that saturation has not occurred (Gleason et al. submitted). The silent:replacement ratio for polymorphisms is 14:6 and for interspecific differences is 54:9. A G-test yielded a value of 2.19, 1 d.f., $p > 0.1$. Thus by this test we cannot reject the hypothesis of neutrality.

The other test is the Tajima (1989) test which tests how well the average observed number of nucleotides differences between alleles ($\pi$) corresponds to the average number of segregating nucleotides per site predicted by a panmictic neutral model at equilibrium ($\theta$). For the coding region, the test statistic $D = -1.21$, $p = 0.1$. For the noncoding region, $D = -1.69$, $0.1 > p > 0.05$. Thus in each case the D is negative, but not quite statistically significant.

## Discussion

As mentioned in the Introduction, virtually no electrophoretically detectable variation was observed in allozyme studies of *D. willistoni Adh*, despite a very large sample size. Thus it was a surprise to find six replacement polymorphisms in a sample of only 18 alleles. None of the detected polymorphisms cause a change in charge, although several of these changes involve polar/nonpolar R groups, which potentially could have affected electrophoretic mobility. We performed electrophoresis on the strains with amino acid substitutions using cellulose acetate strips, and detected no mobility differences (G. Allegrucci and J.R. Powell, unpublished data). Ayala (1975) and his coworkers used starch gels. It is possible that a method that can detect conformational changes may detect mobility differences. It is unlikely that size differences could be detected as the predicted molecular weights of the ADH molecule for these alleles differ at most by 0.16%

Table 5 compares our results with previous nucleotide polymorphism studies on *Drosophila Adh*. Most notable is the uncharacteristically high ratio of replacement to silent polymorphism in *D. willistoni*. This implies there may be reduced selective constraints on this gene in *D. willistoni* compared to the previously studied species. Consistent with this is the fact that the *D. willistoni Adh* gene has lower codon usage bias, having an "effective number of codons" (Wright 1990) of 45.1 compared to 31.4 for *D. melanogaster Adh*. However, the level of ADH enzyme activity in the *willistoni* group species is about the same as for the Slow allele in *D. melanogaster* and *D. simulans* and is about the mean for species breeding in rotting fruit (Merçot et al. 1994). Unless there is a significant difference in specific activity of the enzyme, it would seem that the level of expression is not the cause of the relaxation of selection. Considering the similar ecology of *willistoni* and *melanogaster* groups (tropical fruit breeders) it would be surprising if the level of expression of *Adh* would be greatly different.

Generally, *D. melanogaster* has proportionately more replacement polymorphisms than does *D. simulans* (Moriyama and Powell, 1996). This has been attributed to the possibility that the effective population size of *D. melanogaster* is smaller than that of *D. simulans*. This makes selection against mildly deleterious mutations less effective, and implies that most replacement polymorphisms are slightly deleterious. If this is the explanation for differences in the proportion of replacement to silent polymorphisms among species, then this implies that the effective population size of *D. willistoni* is even less than that of *D. melanogaster*. This may seem improbable given the high densities that have been measured for *D. willistoni* (Burla et al. 1950) and the extremely large range of the species. Doubtless today, the census size of *D. willistoni* is extremely large. This suggests that perhaps *D. willistoni* had a smaller population size in its recent history and has only recently expanded to its present size. One indication of an expanding population is a negative Tajima's D statistic because expansion of a small population is analogous to a selective sweep (Tajima 1993; Braverman et al. 1995). For these data, this statistic is negative but not quite significantly so (see Results). The only other study of nucleotide polymorphism in *D. willistoni* is for the *period* locus where all the Tajima's Ds were also negative, but not significant (Gleason and Powell, 1997). Another possible indication of a small effective population size is the unusually

**Table 5.** Comparison of level of *Adh* polymorphism among *Drosophila* species

| Species | Number of alleles | Coding DNA | | | | Noncoding | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Silent | Replacement | $\pi^a$ | $\theta^b$ | $\pi$ | $\theta$ | |
| *willistoni* | 18 | 14 | 6 | 0.0053 | 0.0076 | 0.0057 | 0.0104 | This paper |
| *melanogaster* | 15 | 16 | 3 | 0.0081 | 0.0076 | 0.0191 | 0.0170 | Laurie et al. 1991 |
| *simulans* | 5 | 11 | 0 | 0.0068 | 0.0069 | 0.0271 | 0.0272 | McDonald and Kreitman 1991 |
| *pseudoobscura* | 107 | 38 | 1 | 0.0040 | 0.0098 | 0.0202 | 0.0315 | Schaeffer and Miller 1992b, 1993 |

[a] Observed average proportion of nucleotide differences between alleles

[b] Predicted average number of nucleotides segregating per site under a panmictic neutral model at equilibrium (Tajima 1993)

Note: Values are taken from Moriyama and Powell (1996)

low polymorphism in adjacent noncoding DNA (Table 5). Nucleotide variation in noncoding regions in all other species is two to five times that of coding regions, but in *D. willistoni* it is about equal. Reduced selection in the coding region and insufficient time since the expansion for mutation to generate equilibrium amounts of (nearly?) neutral variation in the noncoding region could account for this pattern. There are also several polymorphic sites shared among strains from throughout the range of this species, which is also consistent with an expansion of a recent small population.

Is it reasonable to invoke a small effective population in a species so numerous today? It is generally thought that during times of glaciation, the Neotropics experienced a cooling and drying period (Haffer 1969; Colinvaux 1993). The appropriate habitat for *D. willistoni* could have decreased considerably. Spassky et al. (1970) felt that the distributions of the semispecies of the very closely related *D. paulistorum* complex could be accounted for by Pleistocene refugia.

Seemingly inconsistent with a recent small effective population is the high allozyme polymorphism in *D. willistoni* (Ayala 1975), among the 25% highest in species of *Drosophila* (Powell 1975). However, a decoupling between nucleotide and allozyme polymorphism has been observed in *Drosophila* (Begun and Aquadro 1993) as well as in other organisms (Karl and Avise 1992). The case of *D. melanogaster* may be analogous to *D. willistoni*. In *D. melanogaster*, the ancestral African populations have considerably more nucleotide variation as compared to human commensal populations throughout the world, but the allozyme variation is very similar for all populations examined (Begun and Aquadro 1993). It is possible that only a few individual colonists were the origin of the human commensal populations. This history has apparently reduced nucleotide polymorphism while not affecting appreciably the allozyme polymorphism.

A potential alternative explanation is related to suppression of recombination due to the high inversion polymorphism in *D. willistoni*. If the level of recombination is very low, then natural selection is less effective at detecting single nucleotide variants which may be slightly deleterious. However, *Adh* in *D. willistoni* is on the IIR chromosome (Rohde et al. 1995) which is the least polymorphic arm for inversions. In a sample of 57 populations from throughout the range, an average of 60% of all individuals are karyotypically homozygous for the IIR (Dobzhansky 1957; da Cunha et al. 1959). The two inversions which would contain *Adh* within their breakpoints (C and D) are quite rare with heterokaryotypes for these inversions averaging only 9% in the 57 populations. Thus there is little reason to suspect that inversion polymorphism is greatly affecting recombination at this particular locus.

Whatever the explanation for the pattern of *Adh* nucleotide polymorphism in *D. willistoni*, it is instructive to note that the *D. willistoni* group has consistently yielded different molecular evolutionary patterns, especially when compared to *D. melanogaster*. It has a significantly different codon usage (Anderson et al. 1993; Powell and Gleason 1996), its *Adh* gene has lost an intron (Anderson et al. 1993), and in the *period* locus a region that is highly polymorphic in *D. melanogaster* (Costa et al. 1991, 1992) is virtually monomorphic in the *willistoni* group (Gleason and Powell 1997). Thus generalizing about molecular evolutionary patterns and processes (in as much as processes can be deduced from patterns) from a single species (*D. melanogaster*) to *Drosophila* is risky at best.

*Acknowledgments.* We thank E.N. Moriyama for statistical assistance, A. Calderon-Urrea for oligonucleotide synthesis, C. Anderson for DNA samples, and the individuals listed in Table 1 for kindly sharing their *D. willistoni* stocks. This study was supported by a NSF Dissertation Improvement Grant (DEB9122899) to ECG and NSF grant DEB 9318836 to JRP. ECG was supported by a PHS Training Grant.

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**Wenhua Lu**

**From:** "Scott Miller" <miller.scott@nmnh.si.edu>
**To:** "Wenhua Lu" <wenhua@etal.uri.edu>
**Sent:** Saturday, August 23, 2003 9:53 PM
**Subject:** Guana flies again

This probably has records from Guana. She was a Levins student. Evidently it was never published.

>Title: Geographic variation in insular populations of
> Drosophila /
>Author(s): Pressick, Mary Lou.
>Year: 1968
>Description: 105, [38] leaves : ill., charts, map.
>Language: English
>SUBJECT(S)
>Descriptor: Ecology.
>Note(s): Typescript./ Includes bibliographical references
> (leaves 100-105)./ Dissertation: Thesis
> (M.S.)--Universidad de
> Puerto Rico, 1968.

I will try to get a copy of this....

Skip

# The Valentine Report

Guana Island Non-Marine Invertebrate Survey
Progress Report: Insecta

PROTURA (1 family:1 genus:1 species
COLLEMBOLA (5:?:13+)
- Isotomidae (2 species)
- Entomobryidae (3)
- Hypogastruiidae (1)
- Onychiuridae (2-3)
- Sminthuridae (3)

DIPLURA (2:3:3)
- Campodeidae (1)
- Japygidae (2)

MICROCORYPHIA (?:?:2)
THYSANURA (1:?:2)
- Lepismatidae (2)

ODONATA (separate survey)
BLATTARIA (4:15:19)
- Blaberidae (4)
- Blatellidae (6)
- Blattidae (3)
- Polyphagidae (1)
- Unknown nymphs (5)

ISOPTERA (3:7:8)
- Kalotermitidae (4)
- Rhinotermitidae (1)
- Termitidae (3)

MANTODEA (1:1:1)
- Mantidae (1)

PHASMIDA (2:3:4)
- Heteronemiidae (1)
- Phasmatidae (3)

ORTHOPTERA (4:14:23)
- Acrididae (3)
- Gryllacrididae (1)
- Gryllidae (14)
- Tettigoniidae (5)

DERMAPTERA (1:1:1)
EMBIOPTERA (1:1:1)
PSOCOPTERA (?:?:10)
MALLOPHAGA (1:1:1)
HEMIPTERA:Heteroptera (20:?:40)
- Alydidae (3)
- Anthocoridae (2)
- Coreidae (1)
- Corixidae (1)
- Cydnidae (1)
- Enicocephalidae (1)
- Hebridae (1)
- Largidae (1)
- Lygaeidae (2)
- Miridae (3)
- Nabidae (1)
- Pentatomidae (6)
- Phymatidae (1)
- Reduviidae (2)
- Saldidae (1)
- Scutellaridae (3)
- Thyreocoridae (1)
- Tingidae (2)

HEMIPTERA:Anchenorrhyncha (13:?:40+)
- Aetalionidae
- Cicadidae (1)
- Cicadellidae (many)
- Membracidae (1)

- Acanaloniidae (1)
- Achilidae (3)
- Cixiidae (4)
- Delphacidae (8)
- Derbidae (1)
- Flatidae (4)
- Issidae (2)
- Kinnaridae (2)
- Torpiduchidae (2)

HEMIPTERA:Sternorrhyncha (?:?:?)
- Aphididae (?)
- Ortheziidae (1)
- Other scale insects (?)

THYSANOPTERA (?:?:5)

NEUROPTERA (5:9:11)
- Ascalaphidae (2)
- Chrysopidae (3)
- Coniopterygidae (1)
- Mantispidae (1)
- Myrmeleontidae (4)

COLEOPTERA (56:288+:412+)
- see separate list

LEPIDOPTERA (30:?:?)
- see separate list

DIPTERA (35+:?:90+)
- Anisopodidae
- Ceratopogonidae
- Chironomidae
- Culicidae
- Mycetophilidae
- Scatopsidae

- Asilidae (3)
- Bombyliidae (2)
- Dolichopodidae
- Empedidae
- Leptogasteridae
- Stratiomyidae
- Tabanidae
- Therevidae

- Anthomyidae
- Calliphoridae
- Clusiidae
- Drosophilidae
- Ephydridae
- Hippoboscidae (1)
- Micropezidae
- Muscidae
- Nycterobiidae (1)
- Otitidae
- Phoridae
- Pipunculidae (3)
- Sarcophagidae
- Streblidae (1)
- Syrphidae
- Tachinidae
- 5 undetermined families

HYMENOPTERA (3:85:129)

Braconidae (4)
Ichneumonidae (2)
Chalcidae (3)
Elasmidae (1)
Encyrtidae (1)
Eucharitidae (1)
Eulophidae (1)
Eupelmidae (2)
Enrytomidae (1)
Pteromalidae (2)
Signiphoridae (1)
Trichogrammatidae (1)
Eucoilidae (2)
Figitidae (1)
Diapriidae (3)
Platygasteridae (1)
Scelionidae (10)
Ceraphronidae (1)
Evaniidae (3)

Bethylidae (8)
Dryinidae (1)
Scoliidae (2)
Tiphiidae (1)
Mutillidae (1)
Pompilidae (5)
Vespidae (3)
Sphecidae (18)
Formicidae (30)
Anthophoridae (7)
Apidae (1)
Colletidae (1)
Halictidae (5)
Megachilidae (4)

Totals: 22 orders, 261+ families, 1,140+ species

Major help in the field and many specimens were provided by our daughter Susan Valentine-Cooper, Wei-Ping Liao, Wenhua Lu, and Roy Snelling. Also, Angela Davis. Lianna Jarecki, Kate LeVering, Clive Petrovic, Fred Sibley and Tom Willard collected and helped in many ways. All of the other October science month participants helped us and are gratefully acknowledged.

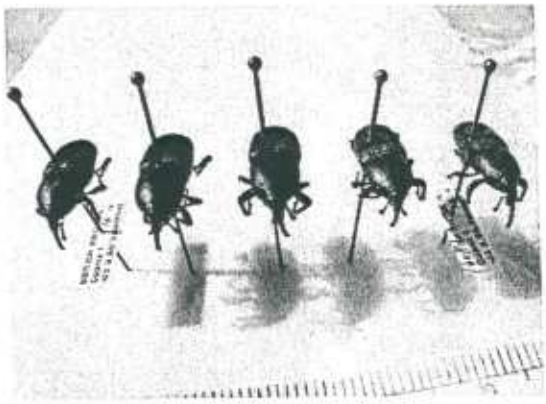
Identifications are our responsibility, but we have profited and used the knowledge of Michael Ivie (Coleoptera), Norman Johnson (Hymenoptera), Lucianna Musetti (Hymenoptera), David Rosenthal (Collembola), Louis Roth (Blattaria), Justin Runyon (Diptera), Fred Sibley (Odonata), Roy Snelling (Hymenoptera), and Lionel Stange (Neuroptera). All were generous with their time and expertise.

Barry & Buena Valentine
November, 2003

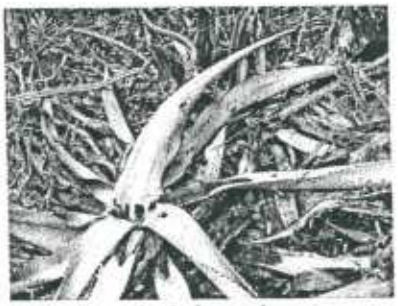
# Why did the beetle cross the sea?

Guana Island is a remarkably diverse place. More species are known from it than from any island of comparable, and even much greater, size. So it isn't that surprising to find a new species here: it happens during science month almost every year. Usually, it is a happy find.

But a few years ago, an unwelcome addition occurred. We noticed that the century plants were dying, and discovered that a new species of beetle, Agave Weevil, has just arrived on Guana. Normally, this pest is found in South America and in the Deserts of North America.



A healthy agave



Damaged, dying plant.

Dead husk

Since then, work has focused on two objectives. First we have been trying to find out how bad the impact of the beetle is and to try to identify means of preventing it from happening. Second, we have used information on their present whereabouts in the region to try and identify how they got to the BVI in the first place, so that additional pests can be blocked.

The work is led by Dr. Gad Perry from Texas Tech University, with the help of Dr. Skip Lazell (the conservation Agency) and Dr. Barry Valentine (Ohio State University).

Dr. Perry

Wasps & Ants

**Wenhua Lu**

**From:** "Roy Snelling" <rsnellin@nhm.org>
**To:** <wenhua@etal.uri.edu>
**Sent:** Saturday, March 27, 2004 4:01 PM
**Subject:** miscellaneous

Dear Wenhua:

The beetles did arrive in good condition and the loan records are straight. As I mentioned when we talked, our current policy is to loan in 1 year increments. I think it is silly, but so far have been unable to enlighten the Registrar ("but, it's policy"!).

Psorthaspis gloria will be in print again, this time properly reproduced in glorious color! I will have a small paper on the Spider Wasps of Puerto Rico and the British Virgin Islands published later this year in the Journal of the Kansas Entomological Society. I will send a reprint after it is published.

My plans for this fall have been changed. My funding for the work in Kenya has dried up (at least for the remainder of this year). So, I would be available for Scientists Month on Guana if the offer still holds. That would give me an opportunity to finish up the ant work there. There is an elusive little beast out on Long Man's Point that I got last time, but only a couple of minor workers; I need the major workers in order to identify the species. There are also a couple of things that should be there that I have not yet collected and there is a problem about the chemistry of one of the species I sampled years ago, so I need to get more specimens for a new assay of the venom chemistry.

Best wishes to you and Skip!

Roy

Roy R. Snelling
Natural History Museum
of Los Angeles County
900 Exposition Boulevard
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FAX 213-746-2999
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http://www.notesfromunderground.org

Whipscorpion

**Wenhua Lu**

**From:** <Cokendolpher@aol.com>
**To:** <hq@theconservationagency.org>
**Cc:** <lorenzo@amnh.org>
**Sent:** Wednesday, November 19, 2003 2:43 PM
**Subject:** amblypygid of Guana Island

Hi Skip,
The amblypygid you sent from Guana Island, British Virgin Islands, arrived safely. Because you stated that you also sent a specimen to Lorenzo, I am cc him on this message. The specimen is a female Phrynus longipes (Pocock). This species has been reported (misidentified) as Tarantula palmata in some earlier literature and thus probably the reason Petrunkevitch labeled some samples as such in the YPM. The latest revision on this group is by Quintero, D., 1981. The amblypygid genus Phrynus in the Americas (Amblypygi, Phryniidae). Journal of Arachnology, 9:117-166. Another paper that deals with some new species from further west (Mona Island, west) is: Armas, L. F. de & A. Perez-Gonzalez, 2001. Los amblypigidos de Republica Dominicana (Arachnida, Amblypygi). Revista Iberica de Aracnologia, 3:47-66.

Your specimen will at first key to one of the new species from Dominican Republic, but upon counting segments on the other leg tibia IV it goes back to longipes. I am not certain of the value of the tibial segments when they vary in a single individual. In other counts, your specimen goes to longipes. Longipes has been recorded by Quintero from St. John, St. Croix, St. Thomas in the Virgin Islands. I am not sure what to make of your smaller Phrynus from the island? Could they be smaller longipes? Quintero does not record an another Phrynus from the Virgin Islands. Size in amblypygids is a problem because they can molt for up to 9 years after reaching adulthood. I have not seen a good study that looked at size and age in these beast.

Thanks for the specimen.

Regards, James

James Cokendolpher
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Natural Science Research Lab.
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phone 1-806-742-2307

**From:** <lorenzo@amnh.org>
**To:** <Cokendolpher@aol.com>
**Cc:** <hq@theconservationagency.org>
**Sent:** Thursday, November 20, 2003 11:29 AM
**Subject:** Re: amblypygid of Guana Island

Jim,

Thanks for the ID (Skip, apologies for not getting back to you on this). It's mighty large for a Phrynus. How reliable is the diagnosis of the genus?

Would it be OK if I sent you some schizomids to identify sometime?

cheers,
Lorenzo

# Cucurbits

**Wenhua Lu**

**From:** "Lisa DeCesare" <ldecesar@oeb.harvard.edu>
**To:** <wenhua@etal.uri.edu>
**Cc:** "Lisa DeCesare" <ldecesar@oeb.harvard.edu>
**Sent:** Wednesday, November 19, 2003 11:48 AM
**Subject:** Dr. Lazell's query

Hi there. Dr. Lazelle called us at the Botany Libraries this morning with a question about the plant Doyerea emeto-cathartica. Here is what I found. It was lots of fun, the references for this plant were wrong so I must thank you. We were able to find the entry, correct the citation information and send the changes on to the International Plant Name Index. Now that database will be updated with the correct information.

I apologize if this is unclear or too much info. Also, I am away from my desk and the pine e-mail system I am using does not allow me to underline or italicize titles or names so this might add a little to the confusion. Please let me know if you need further clarification.

Lisa DeCesare
ldecesar@oeb.harvard.edu

The original citation is:

Cucurbitaceae Doyerea emeto-cathartica Grosourdy
It was published by D. Renato de Grosourdy in his El Medico Botanico Criollo (1864).

Grosourdy was a very well respected doctor and researcher, from what I learned from one of our proffesors here. The plant is listed in part 1, tome 2 on page 338. The description lists nothing about the plants curative properties.

However, the second part of the work lists the plants by ailment/properties. The section heading where this plant is listed is "Emeto-catarticos" which refers I think to your author's idea of diarrhea. I had someone translate it for me (the work is in Spanish) and it reads "this name refers to substances where ... action of vomiting ... constant purging"

I copied both sections if you are interested in reading them. This also may be why your author drops that "h" in catarthicos at times. He may be confused because while the "h" exists in the botanical name it does not exist in the medical term. Or he may be just sloppy :-)

I hope this makes sense so far.

Okay the original naming of the plant occured in 1864 (see above) and the re-naming occured in 1891 (see below). The new name was:

Cucurbitaceae Corallocarpus emetocatharticus (Grosourdy) Cogn.
This was first published in Bulletin de la Societe Royale de Botanique de Belgique (1891). It was published in an article titled "Cucurbitaceae" by Celestin Alfred Cogniaux, a Belgian botanist.

Both names include the "h" in emetocatharica (1st citation) or emetocatharticus (2nd citation) it is only the ending that changes.

Please let me know if I wasn't clear. Also we are all in agreement here that Doyerea was PROBABLY named for the person that collected the plant for Grosourdy (maybe someone named Doyer?) but we are not positive.

Let me know if you need any more info.

Lisa D.
ldecesar@oeb.harvard.edu


A. Male flowers. B. Female flowers

**Wenhua Lu**

**From:** "Lisa Decesare" <ldecesar@oeb.harvard.edu>
**To:** <wenhua@etal.uri.edu>
**Sent:** Thursday, November 20, 2003 11:49 AM
**Subject:** Doyerea

X-Sender: gandhi@oeb.harvard.edu
X-Mailer: QUALCOMM Windows Eudora Version 5.1
Date: Thu, 20 Nov 2003 12:36:14 -0500
To: ldecesar@oeb.harvard.edu
From: KGandhi <gandhi@oeb.harvard.edu>
Subject: Doyerea

Presently, the USDA PLANTS database accepts *Doyerea emetocathartica* Gros. as the correct name and treats *Corallocarpus emetocatharticus* (Gros.) Cogn. as a synonym of the former.

Flora Venezuela (5(1): 35. 1992) also accepts Doyerea.

Jeffrey (Kew Bull. 33: 348. 1978) was doubtful whether Doyerea desrves a generic status; perhaps, for this reason, some botanists merge Doyerea under Corallocarpus. But the latter is an Old World genus.

I will go along with accepting Doyera.

Kanchi

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**Wenhua Lu**

**From:** "Rudy.OReilly" <Rudy.O_Reilly@pr.usda.gov>
**To:** "'Angela Davis'" <adavis-usda@lane-ag.org>
**Cc:** <wenhua@etal.uri.edu>
**Sent:** Friday, November 21, 2003 10:19 AM
**Subject:** Doyerea

Hi Angie

The letter Wenhua forwarded from Lisa DeCesare explains the date issue very well. To add to the confusion there are two more references noted in Dr. Liogier's publication. In 1879 (Bull. U.S. Nat. Mus. 13:55) Baron Eggers aslo published this species as Anguria glomerata. The species was re-named Corallocarpus glomeratus in 1881 by Cog. (Cogniaux) in DC., Mon. Phan. 3:658. I'm not sure if this is the same Cogniaux of the 1891 publication of Corallocarpus emetocatharticus. The most current references I have all select Doyerea as the preferred name.

Pedro Acevedo's publication makes note of the watery sap produced by the stems. I thought I gave you copies of thes in Guana. If you need them again I'll be happy to fax them to you. I'll need your fax number again. Please use my full name in the publication (Rudy G. O'Reilly, Jr.). If there is anything else, please let me know.

Rudy

**Angela Davis**

**From:** CucurbitNetwork@netscape.net
**Sent:** Wednesday, December 03, 2003 6:06 PM
**To:** adavis-usda@lane-ag.org
**Subject:** TCN article

Angie,

Thanks for the articles and pics. Your submission was so complete!

I am interested in using your articles. The only things is that I already requested two 2004 profiles articles from two other scientists. However, I'm not sure that one of them will come through. If not, then your submission will fill in that spot.

When I figure out which issue to put your articles in, I'll contact you a few months before the issue is due out with edited versions of the articles for your approval.

Thanks again. It's good to see that you are staying so active with exotic cukes.

Take Care, Deena

--
The Cucurbit Network
P.O. Box 560483
Miami, FL 33256
U.S.A.

FIG. 2.—MELON, AN EDIBLE MEMBER OF THE GOURD FAMILY, SHOWING FLOWERS AND FRUIT (PEPO) AND GENERAL HABIT OF GROWTH.

# News Notes & Correspondence

## GUANA ISLAND SCIENTIFIC PROGRAMME CARRIES OUT IMPORTANT RESEARCH.

An international team of scientists, led by Dr. James Lazell, President of the Rhode Island USA based Conservation Agency, has completed a month of research on Guana Island.

Every year in October, Guana Island hosts a scientific research programme. Scientists are invited to the island to conduct their studies in the areas of natural history, ecology, conservation biology, systematics and biogeography. Their results are relevant to natural resource preservation and species conservation in the BVI.

In an interview with The Island Sun Tuesday, Dr. Lazell, who is also Director of the Guana Island Wildlife Sanctuary, explained that the sanctuary on Guana Island was established in 1980 and the programme featured a month of activities focusing on plants and animals.
Dr. Lazell pointed out that a large number of species had been wiped out thousands of years ago, e.g. a guinea pig like rodent domesticated by the Amerindians, a chicken like bird called the flightless rail, parrots and the stout iguana.

"The first that occurred to me from the inception was the need to do an inventory of the island," according to Dr. Lazell. "There were two counts which had to be done, one was the presence/absence and then the second was to get an idea of the population and that was not an easy thing to do, so we had to engage a cadre of experts."

He said there were scientists working on the different species of lizards, of which they found eight; snakes 3; bats 4; butterflies 31; spiders 150; land crabs 10; grasshoppers 4 or 5 and others.

"As we develop this list, other people work on the ecological physiology of the animals, like water balance," he continued. "We also look at energetics, that is how fast they can run, how much time they spend basking in the sun and their diets what do they eat and who eats them."
In addition, the scientists examine the molecular biology of the animals by tagging them with micro chips and monitoring their activities from year to year to see whether they've grown and whether they've lived in the same place or moved around.

The focus of the research programme expanded in 1990 to include the marine environment, with the activities directed by Ms. Lianna Jarecki, daughter of the Guana Island resort owners Dr. Henry and Gloria Jarecki of the Falconwood Foundation that supports the research programme. ← Now Dr.
The research is not only confined to Guana Island. The scientists go island hopping as well to collect specimen and this year, they found an unidentified species of the whipscorpion, which they have taken back for analysis at the Harvard University museum.

This year, the team of scientists were drawn from China, the U.S., St. Croix, Dominica and Romania and included botanists, herpetologists, entomologists and archaeologists.
While here, they conducted a lecture and symposium at the H. Lavity Stoutt Community College (HLSCC). The symposium highlighted talks and photographs on Raptors in the BVI, The Mysteries of Distress Calling, Invader Plants, Body Size and Habitat in BVI Lizards and Opportunities for Study and Research at Texas Tech.

Dr. Lazell said the HLSCC provided strong support to the programme. He also mentioned that every year, the scientists play host to a number of students who

take part in the science fair and also youth from the Adventist Pathfinders.

"It's an outreach to let the community know what we're doing and some of what's unique about the territory," he said. "Unfortunately, we don't get as big an audience as we would like at the lectures, which really aren't technical at all, we try to avoid using scientific names, we're really just re-telling the story."

Local News

The team of scientists left the territory Wednesday. The research programme resumes next year October.

# Landmarks

THE COLLEGE OF AGRICULTURAL SCIENCES & NATURAL RESOURCES | 2004




LANDMARKS VOL. 18-2004

*Landmarks magazine is a publication of the College of Agricultural Sciences and Natural Resources at Texas Tech University. It is published yearly and sent to alumni and friends of the college.*

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# Range, Wildlife, & Fisheries Management

Some of the most urgent natural resource management issues in the world today involve the tropics. This fall semester, our students experienced first-hand. Dr. Gad Perry, a new faculty member in the Department of Range, Wildlife, and Fisheries Management, taught a semester-long Tropical Ecology course for the first time. As part of the class, seven graduate students spent 10 days on a private nature preserve in the British Virgin Islands during the month of October. The students benefit from extensive financial assistance from The Conservation Agency, the Falconwood Foundation, and the Texas Tech Office of International Affairs. Together, these cover most of their expenses, allowing them to participate in an otherwise prohibitively expensive program. During their stay, the students will get a hands-on experience of some of the natural resource management issues affecting the tropics, and engage in a class-designed research project. They will also present some of their work at an annual research symposium, held at the BVI community college, during the visit. This will also be an opportunity to promote Texas Tech for both undergraduate and graduate studies. The students were accompanied by Dr. Perry, as well as Dr. Kate LeVering, an Adjunct Professor in the department.

**Wenhua Lu**

**From:** <Rafe_Boulon@nps.gov>
**To:** <HQ@theconservationagency.org>
**Sent:** Tuesday, October 28, 2003 6:14 AM
**Subject:** Thank you

Skip:

I want to thank you for inviting us to Guana Island this past weekend. It was a real treat. I have heard bits and pieces over the years about Guana and some of the work going on there. I was very glad to finally have the opportunity to visit and see all the things that are going on. It is all pretty exciting. I did finally get to see several pinguis (although usually heading in the opposite direction in a flurry of legs and tail). Did watch one eating a worker's left-over lunch. It would be pretty exciting to establish a colony in the USVI on a cay. Will look into National Park Service restrictions and give some thought to a suitable island and the hurdles we would have to overcome to do it. I am all for it however.

Thank you again and if there is anything I can help with please let me know.

Rafe

---

Rafe Boulon, Chief

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**Wenhua Lu**

**From:** "Arijana Barun" <abarun@utk.edu>
**To:** <wenhua@etal.uri.edu>
**Sent:** Sunday, March 14, 2004 8:41 PM
**Subject:** coming to Guana 2004

Hi Skip and Wenhua,

I talked to Dan Simberloff, my family, Bob Henderson and Gad Perry and have decided to come to Guana this year for 10 days if there is place for me. Bob, Gad and myself decided to start experiments on tail luring in Alsophis portoricensis. I have a list of literature but have not decided yet exactly on the experiment, many hypothesis but have not decided with which one to go. Gad, Bob and myself will decide what to do and since they will be there also, it could turn out to be a nice project.
We are working on publishing paper for Journal of Herpetology on research we did with A. portoricensis for the past two years.

On completely different matter. I need tissue samples of mongoose from Chinese mainland but would prefer from Huianan island (I think I have misspelled name of the island. I will check it tomorrow in my office). Do you know somebody in the museum or field person who would catch them for me? Dan will pay for samples. We need 20 male and 20 female samples approximately. I am not sure I told you about this study but we are working on genetics of mongoose in introduced and native range. We already have samples from Bangladesh and Pakistan for native range but apparently mongoose are much bigger on this Chinese island and mainland.

Thank you.
Ari
P.S. I will NOT bring my daughter with me to Guana.

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BIOGEOGRAPHY OF THE WEST INDIES. PATTERNS AND PERSPECTIVES. 2nd ed. Charles A. Woods and Florence E. Sergile, eds. 2001. CRC Press, Boca Raton, Florida. ISBN 0-8493-2001-1. 582 p. $139.95 (cloth).—This is a remarkable book, far more a second volume of *Biogeography of the West Indies* (Woods, 1989) than a second edition. Only three of the 27 chapters are revisions of material in the 1989 volume; two of those are extensive updates. Only 15 of the 582 pages are explicitly or exclusively herpetological: Hass, Maxson, and Hedges' molecular clock work on nine family level groups: Bufonidae, Hylidae, Amphisbaenidae, Anguidae, Iguanidae, Teiidae, Colubridae, Tropidophidae, and Typhlopidae. However, seven more chapters—134 pages—significantly involve reptiles or amphibians or both.

Among these, first and foremost is Hedges' overview which draws heavily on amphibians, reptiles, and mammals. Hedges finds little evidence to support land barge or land bridge hypotheses, including the novel Iturralde-Vinent and MacPhee (1999) "landspan" of "GAARlandia" (named for the Greater Antilles and Aves Ridge). Instead the highly disparate dates of Antillean radiation initiations—exemplified in the herpetological chapter noted above—indicate

classic overwater waif dispersal and colonization. If the GAARlandia landspan existed, one may conclude that it was of brief tenure and never more than a filter bridge of islands (as Iturralde-Vinent and MacPhee admit as possible), not a land bridge. It may have served to get sloths from South America to the Antilles, but they had to get back again later across water if the phylogeny developed by White and MacPhee is correct. The only herpetological radiation whose initiation time seems to fit GAARlandia is the genus *Bufo*, if South American *Bufo granulosus* indeed represents the sister group.

The last vestige of good evidence for continental connection of any Antillean island comes from Jamaica where Portell, Donovan, and Domning report on an Eocene site with a rhinoceros (*Hyrachyus* sp.) and, among other things, an iguanid lizard, a pelomedusoid pleurodiran turtle, and a crocodilian (? *Charactosuchus kugleri*). Their reconstruction of the relevant geology and geography indicates that Jamaica at that time was the eastern end of a Chortis Block-Nicaragua Rise peninsula, not a drifting land barge.

The divergence time data generated by Hass, Maxson, and Hedges add to the growing accumulation previously published and cited coming out of Hedges' lab. Among their more interesting results are that the several named hylid frog genera date from about 58 mya initial divergence and are polyphyletic ("*Osteopilus*") or paraphyletic (*Hyla*). *Hyla heilprini* had to be disassociated from the rest of the radiation because its divergence time might make its lineage older than the Antilles or even the breakup of Gondwanaland. Figuring out where *H. heilprini* came from will be a fascinating job.

Similarly, the amphisbaenas mostly seem to have initial divergence at approximately 55 mya, but *Amphisbaena gonavensis* seems much older—at approximately 86 mya rivaling Florida's *Rhineura*, calling into question the family and generic status of the latter. The Cuban form "*Cadea*" *blanoides* fits into the radiation in the Antilles and notably postdates the divergence of the main group from *Amphisbaena alba* of South America.

The iguanid genus *Leiocephalus* goes back about 26 mya and appears closest to North American crotaphytines, not South American tropidurines, adding further evidence to the view that partition of Iguanidae sensu lato is untenable (Lazell, 1992; Macey et al., 1997). The Antillean teiid lizard radiation has been separated from South American *Ameiva ameiva* about as long as from some *Cnemidophorus*. The xenodontine colubrid snake radiation, with a suite of Antillean "genera," seems to be very young, diverging approximately 13 mya.

The snakes long known as *Tropidophis haetianus* have been separated on Jamaica and Hispaniola much longer than Jamaican *Tropidophis* have been separated from Cuban species: approximately 16 versus 6 mya. Thus, *Tropidophis jamaicensis* emerges as a valid full species. The typhlopid snakes seem to have been separated from Philippines *Typhlops* approximately 58 mya.

McNab discusses adaptations to island life demonstrated by members of the fauna, noting especially the replacement of large endotherms by reptiles like iguanas and tortoises. The chapter on ticks by de la Cruz features many species with amphibian and reptile hosts. He notes again the peculiar host and geographic distribution of the iguana tick *Amblyomma antillarum* I have called attention to (Lazell, 1989).

Wing's chapter on zoarcheology includes data on squamate reptiles from 17 of 19 samples from 13 sites and marine turtles from 13. Interestingly, Wing reports no tortoise remains at all. Remarkably, from the Tutu site on St. Thomas, Virgin Islands, Wing extrapolates more than 2000 kg of iguanas were consumed in a short time centered on 560 yBP (approximately 1440 A.D.); earlier a similar biomass was consumed at Maisabel on the north coast of Puerto Rico around 1850 yBP. She tabulates these iguanas variously as "Iguanidae" or "*Cyclura*," but I believe all of these would have been *Cyclura pinguis*, the stout iguana native and endemic to the Puerto Rico Bank. It is little wonder that this magnificent species has been reduced to a few hundred individuals on three (of more than a hundred) islands (Mitchell, 2000).

A final chapter by Sergile and Woods describes a decade of conservation efforts in Haiti on Hispaniola. They note the incredible biodiversity of this island (e.g., 54 of the Antilles' 126 frogs are endemic; they do not give figures for reptiles, but both are no doubt higher) and the terrible conflicts with nature brought on by poverty and overpopulation. Although most herpetologists are acutely aware of the crisis our study organisms (and we ourselves) face, Haiti is a fearsome exemplar. It is encouraging that Sergile and Woods can report "Efforts to promote the conservation of biodiversity and to encourage sustainable development have been successful in Haiti." I wish there was more explicit recognition of the role of overpopulation and some real steps to redress it. As the author-editors state "Unfortunately, . . . there are lessons to be learned about failure, as well as about

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remarkable success" throughout the region and the world.

Despite its price, this is a book very much worth having for anyone interested in the West Indian biota. The 45 contributors have provided stimulating, highly informative reading, and the editors have done a careful job. There are relatively few typos (I suspect "elude" for allude on p. 21 is a lapsus). The first 22 pages, including contributors addresses (infuriatingly without zip codes, but with e-mail), contents, and map are unnumbered. There is a very useful 22-page index. For those of us with interests extending farther than herps, herein is a menagerie of butterflies and beetles, spiders and bats and parrots, and those weird mammals, the sloths, hutias, and insectivores. There is meteorological and human history, and a rich and (for me) surprising lot of detail about some of the great characters of natural history: James Bond, Eric Ekman, Hermano Alain (a.k.a. Alain Liogier), David Weatherbee, Glover Allen, Harold Anthony, Thomas Barbour, W. D. Matthew, William Abbott, David Klingener, and Ernest Williams—in Charles Woods' introductory chapter.

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James Lazell, *The Conservation Agency, 6 Swinburne Street, Jamestown, Rhode Island 02835; E-mail: hq@theconservationagency.org.*

Sea level at Wurm glacial maximum (solid line) and land areas during the Sangamon Interglacial (stippled).

The Caribbean Basin and West Indies in the recent past. From: Lazell, J. 1989. Wildlife of the Florida Keys, Island Press.

# BOOKS et al.

ECOLOGY

## Reflections on Lizard Diversity

Martin J. Whiting

Ranging in size from a 0.5-g gecko to the 250-kg Komodo "dragon"; sometimes possessing jewel-like coloration or spectacular ornamentation such as horns and crests; moving by running, gliding, swimming, or crawling; lizards occupy an array of niches in habitats that include snow-capped mountains, Galapagos beaches, deserts, and tropical rainforests. To escape from predators, members of this paraphyletic group (their clade also includes snakes) employ such bizarre strategies as squirting blood from the eyes, leaving patches of skin in the predator's grasp, and shedding the tail, which wriggles rapidly while its owner flees. Lizards epitomize diversity, and thus Eric Pianka and Laurie Vitt's *Lizards: Windows to the Evolution of Diversity* is appropriately titled.

Pianka (a professor at the University of Texas, Austin) and Vitt (a professor at the University of Oklahoma) belong to the breed of field biologists who spend months at a time away from home and e-mail, living out of a tent or field vehicle, in sometimes inhospitable conditions. Their combined experience encompasses research in the rainforests of Central and South America as well as the deserts of North America, southern Africa, and Australia. *Lizards* represents a synthesis of their lives' work, and it provides the rare opportunity for a comparison of independently evolved lizard faunas, particularly in the context of life histories—the area of expertise of both authors.

**Trying to impress.** Many lizards, such as this Amazonian *Enyalioides palpebralis*, use open-mouth displays to appear more threatening than they actually are.

Divided into three sections (lizard lifestyles, lizard diversity, and a synthesis), the book has an unorthodox format, which I found appealing. Rather than beginning with a traditional detailed evolutionary history and following with lengthy accounts of the major lizard taxonomic groups, the authors keep discussion of taxonomy and phylogenetics to a minimum. The first section of the book focuses on what lizards do rather than what they are. In the second section, Pianka and Vitt explore the biology of the major clades of lizards in six easy reading chapters that often focus on morphological and behavioral innovations (e.g., the toe pads of geckos). The result is a welcome departure from the classic approach of pages of dry taxonomic accounts. Nonetheless, readers would have been better served had the authors offered an explanation of why they favored the classification (portrayed in a cladogram of extant lizards that appears in the first chapter) they use to organize their discussions. In the absence of any reference to an original source or the underlying data, one cannot determine whether this is a well-supported phylogeny or simply the authors' best estimate of lizard relationships. This uncertainty is unfortunate, given that many students may follow the same arrangement simply because the book is current and its authorship authoritative.

A book of this nature can serve as a great source of ideas for graduate student projects. For example, the authors mention that horned lizards (*Phrynosoma*) squirt blood from the suborbital sinus when they are seized by foxes or coyotes. The blood is distasteful and prompts the canid to release the lizard (*1*). However, neither the identity nor the source of the chemical compounds in the blood that cause this response are known. Given findings from studies of other taxa, such as frogs that obtain toxic skin chemicals from the ants they eat, an obvious avenue of inquiry is the lizard's diet—horned lizards are, after all, ant specialists, and ants are famous for their production of chemical cocktails. Other questions worthy of further investigation abound in the authors' discussions.

**Lizards**
**Windows to the Evolution of Diversity**
*by Eric R. Pianka and Laurie J. Vitt*

University of California Press, Berkeley, 2003. 347 pp. $45, £29.95. ISBN 0-520-23401-4. Organisms and the Environment.

As one expects of any book that attempts to provide a comprehensive yet concise account for a broad audience, readers immersed in particular aspects of lizard biology are likely to identify points of disagreement. For example, Pianka and Vitt state that chameleons are capable of catching flies in midair. I may be putting my head on the chopping block, but I have great difficulty believing this claim. In my experience, chameleons take several seconds to both focus on a prey item and obtain just the right body posture before flinging out their tongue. The authors also state that "sperm competition has yet to be explored in any lizard"; although it is true that sperm competition in lizards is largely unstudied, Pianka and Vitt have inadvertently ignored several important studies by Mats Olsson and co-workers that examine multiple matings in sand lizards (*2*, *3*). One such study demonstrated that female sand lizards select sperm from more distantly related individuals (*4*). Such selection can be particularly important in systems in which matings with close relatives are likely (sand lizard females are promiscuous).

Wide-ranging books such as this are also handicapped by spatial constraints in that the coverage of any given topic must necessarily be limited. The authors mention that lizards have been deemed model organisms for a number of questions in biology (and particularly in ecology), but they do not discuss some of the really important studies that would have bolstered this statement. For example, Barry Sinervo's team used an allometric engineering analysis of side-blotched lizards (*Uta stansburiana*) to test basic tenets of life history evolution, including the relation between the size and number of eggs (*5*). In this same species, the mating behavior of males conforms to a paper-rock-scissors game in which no single throat form dominates all strategies (throat forms correlate with fixed strategies such as territory defense, mate guarding, and sneaking copulations) (*6*). Several recent papers by Sinervo and Kelly Zamudio—some too recent to have been included in the book—underscore the importance of lizards in contributing to our understanding of sexual selection (*7*). And, to offer one more example, recent work by Manuel Leal and Leo Fleishman (*8*) identifies an additional resource axis for ecologists to consider, that of the light environment. The spatial separation of two anole species seems to be based on properties of their visual systems and the

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amount of ultraviolet reflectance in their dewlaps (throat fans used for signaling); the conspicuousness of the dewlaps is tied to the levels of ultraviolet radiation in the anoles' respective microhabitats.

The book's abundant high-quality color photographs (which show lizards in their natural habitats and depict various behaviors) highlight the spectacular diversity of lizards. Readers will also enjoy the authors' anecdotes from their field experiences, presented in a series of sidebars that add a more personal dimension to the book. Momentarily transporting one to far-flung places, these stories offer snapshots of such moments as gecko hunting at night in Kalahari dunes where hungry lions roam. Besides providing interesting reading, these accounts may inspire future biology students to spend sufficient time in the field to come to grips with the systems on which they work.

Field biologists unable to get out of the office or lab will find that Pianka and Vitt provide them a welcome reminder of the value and pleasures of managing such an escape. All readers will find *Lizards* an excellent introduction to the ecology of an interesting and scientifically rewarding group of animals.

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# BOOK REVIEWS

*Copeia*, 2003(4), pp. 917–920


LIZARD SOCIAL BEHAVIOR. Stanley F. Fox, J. Kelly McCoy, and Troy A. Baird, eds. 2003. Johns Hopkins University Press, Baltimore, Maryland; London. ISBN 0-8018-6893-9 438 p. $89.95 (hardcover).—When I read this book, a nonscaly colleague passed by and asked: "So, tell me, can lizards really be social?" At that point I knew this book was very well conceived! Not only are lizards indeed social, they have actually served as important model systems for the understanding of the evolution of sociality as the editors nicely illustrate in their introduction. In fact, the main aim of this contribution, which emanated from a 1999 herpetologist's symposium, was to highlight exactly where lizard behavioral ecologists contributed to the study of adaptive variation in social behavior. As such, the book certainly will continue the great tradition of the previous three volumes on lizard ecology. The book has been a while in the making; almost all the literature citations are from before 2001. I would have loved to have the excellent citation section earlier as a reference tool. I also particularly appreciated the beautiful color plates showing that lizards are indeed more beautiful than even the most decorated birds. It would have been helpful, however, to directly link each picture to the studies of the respective chapters.

The book is organized into three sections covering variation among individuals, among populations, and among species. Each of the sections is introduced by an eminent ecologist who is not a die-hard herpetologist, and each does a great job in putting lizard ecology into a broader perspective. Peter Marler highlights that lizards are indeed good model organisms because they allow us to understand why animal social behavior is so variable. Gordon Orians emphasizes the importance of studying variation in a natural setting. Again lizards seem to be almost perfect systems, because they are conspicuous and tractable, even in the "messy world of nature." George Barlow, introducing the *variation among species*, reminds fury, feathery, or arthropod colleagues that lizards allow for the understanding of social behavior in a world where kinship does not play a major role.

The individual chapters are valuable contributions to our understanding of lizard social behavior, but some are better than others in generalizing beyond the taxon studied. In particular, Martin Whiting, Kenneth Nagy, and Philip Bateman do a superb job in summarizing the evidence for costs of social status-signaling badges, from arthropods to amphibians, fish, lizards, birds, and mammals. Similarly excellent is their table on the current theoretical models for the maintenance of signaling systems, divided into costly or cost-free categories. Kelly Zamudio and Barry Sinervo, in discussing the ecological and social contexts for the evolution of alternative mating strategies, nicely highlight general characteristics of the environment at which competition and dispersal events occur. They suggest that the social context of lizards, such as the potential for mate monopoly, may determine the evolution of alternative mating strategies. Diana Hews and Vanessa Quinn carry this line of thought further by asking seven highly stimulating questions about the endocrinological causation of species differences in sexually dichromatic signals. Their experiments with masculinized females and feminized males will appeal to biologists of all disciplines who may never see *Sceloporus undulatus* or *Urosaurus ornatus* in the wild. In a similarly elegant way, Jonathan Losos, Marguerite Butler, and Thomas Schoener discuss how habitat use can affect the differences in body size between males and females. Their summary of 36 years of published data is impressive.

The remainder of the chapters focus more on the presentation of exciting new empirical data, for example for social behavior in collared lizards (Troy Baird, Dusti Tinamus, and Chris Sloan), antipredatory defense (William Cooper), or the potential for polygyny (Kelly McCoy, Troy Baird, and Stanley Fox). Three pleasantly data-rich chapters exploit differences in lizards among altitudes (Stanley Fox and Paul Shipman) or islands, trying to explain the evolution of mating systems and morphology (Masami Hasegawa in the Japanese Izu islands and Paul Stone, Howard Snell, and Heidi Snell in the Galapagos archipelago). The remaining chapter is devoted to a beautiful field study on iguanid mating systems (Paul Gier).

With varying success each chapter attempts to generalize beyond studies on particular lizard species. In each of these sections, I would have liked to see a similarly broad approach as mastered by Marler, Orians, and Barlow to put lizard studies in perspective with ecological studies

on other organisms. As a stimulus for the next book on lizard ecology, I would suggest also including a final discussion on "Where we stand and where to go next," a chapter that could not only be a healthy exercise for the contributors of the symposium, but also an aid to a new generation of lizard aficionados to continue the great traditions of Stan Rand (1954), Eric Pianka (1966), and Ray Huey, among others.

*Lizard Social Behavior* should indeed be read by my colleague who smilingly doubted that lizards are social animals, but it would have been easier for me to entice him to do so had the focus of the book been broadened to appeal even more to other disciplines.

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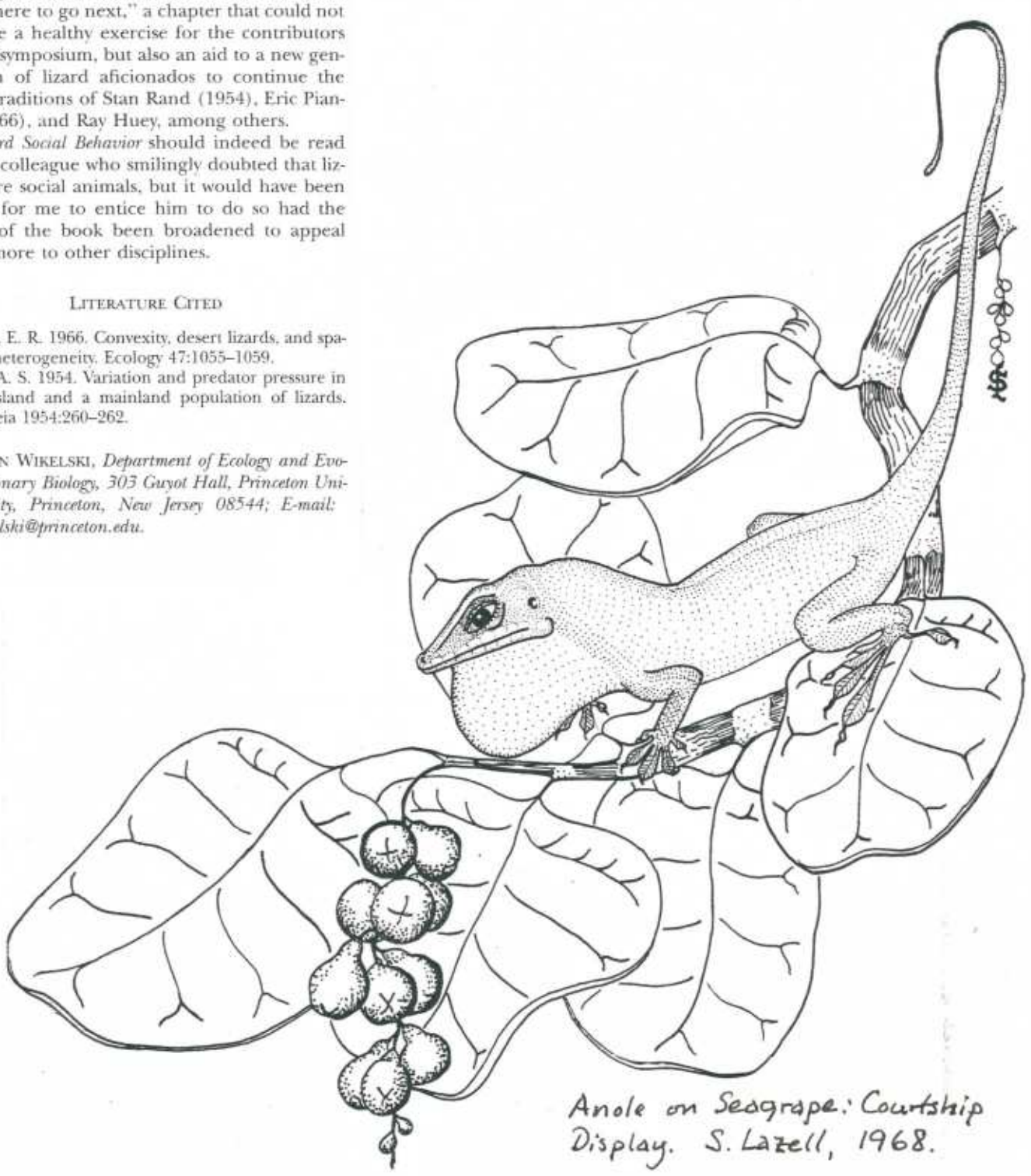

Anole on Seagrape: Courtship Display. S. Lazell, 1968.

***CYCLURA PINGUIS*** (Stout Iguana, Anegada Rock Iguana). **JUVENILE PREDATION**. *Cyclura pinguis* was repatriated to Guana Island, British Virgin Islands, in 1986. Its population has since expanded considerably (Lazell 2002. Ecol. Restor. 20:179–185), and sightings, especially of hatchlings, have become increasingly common. Nevertheless, the ecology of this critically endangered species remains poorly understood. Since 2000, our regular observations of recently hatched iguanas in early October have afforded the opportunity to better understand their ecology. In particular, predation on *C. pinguis* by other than exotic predators (feral cats; Mitchell 2000. *In* Reading and Miller [eds.], Endangered Animals: A Reference Guide to Conflicting Issues, pp. 22–27. Greenwood Press, Westport, Connecticut) has not been recorded. Here, we describe three predation events on juvenile *C. pinguis* involving the native fauna of Guana Island (18°38'N, 65°25'W).

Two observations involved the locally abundant Puerto Rican racer, *Alsophis portoricensis*. On 12 October 2001 at 1530 h, we were told by Guana Hotel staff that an adult snake had been seen attempting to swallow a juvenile iguana and had the head of the lizard in its mouth. When we arrived, the snake was gone, and the juvenile iguana (106 mm SVL), already dead, had been left by the snake. The sighting occurred on a road, in an area containing both ornamental and native scrub flora. The dead iguana was preserved, tagged, and catalogued (JL F-4890), but remains in the British Virgin Islands because of CITES regulations.

We made the second observation on 9 October 2002, in an area characterized by ornamental vegetation in the Guana Hotel area. At ca. 1840 h, the snake was coiled around the midsection of the lizard and biting the lizard's chest. The lizard (ca. 100 mm SVL) was still struggling weakly, but appeared to have already been envenomated. We took photographs and video footage of the ingestion process, which was completed at ca. 2040 h. The snake, subsequently caught, measured 68 cm SVL (tail length = 35 cm). *Alsophis portoricensis* primarily preys on lizards (Henderson and Sajdak 1996. *In* Powell and Henderson [eds.], Contributions to West Indian Herpetology: A Tribute to Albert Schwartz, pp. 327–338. SSAR, Ithaca, New York); these are the first records of iguanas in its diet.



We observed a third predation event on 8 October 2002 around 1530 h. A female kestrel (*Falco sparverius*), locally abundant on Guana (Lazell 1996. Guana Island. The Conservation Agency, Jamestown, Rhode Island, USA. 20 pp.), was observed feeding on a freshly killed juvenile iguana (ca. 110 mm SVL), which it had carried up to a tree perch. We recorded the feeding process, which began at the head of the lizard, for ca. 15 minutes with photographs and videotape. At the end of this period, the bird flew off with the partially consumed lizard in its talons. Much of the head was gone by this time, but the body and tail of the lizard remained mostly untouched.

We thank Christine Matthias, Susan Slater, and Roy Snelling for helping us locate these events, the staff of Guana Island for technical assistance, and Henry and Gloria Jarecki for access to Guana Island. The Conservation Agency through a grant from the Falconwood Foundation provided financial support. This is manuscript T-9-969 of the College of Agricultural Sciences and Natural Resources, Texas Tech University.

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IGUANA NOTES

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***CYCLURA PINGUIS*** (Stout Iguana, Anegada Rock Iguana). **JUVENILE BEHAVIOR.** Following its repatriation in 1986, *Cyclura pinguis* is flourishing on Guana Island, British Virgin Islands (Lazell 2002. Ecol. Restor. 20:179–185). Recently hatched juvenile iguanas are now frequently encountered in early October, but their behavior remains unstudied. As part of a census effort, the marking of iguanas during October 2002 afforded the opportunity to follow animals and individually identify them. Here, we provide preliminary observations on agonistic behaviors of juvenile *C. pinguis* from Guana Island.

During the month-long study, a white water-based latex paint was used to individually mark iguanas throughout the 340-ha island. Sightings occurred throughout Guana Island (18°38'N, 65°25'W), though most were near the hotel area (for additional information on Guana see Lazell 1996. Guana Island. The Conservation Agency, Jamestown, Rhode Island, 20 pp.). We observed no obvious adverse effects from the marking. Recently hatched individuals were repeatedly sighted, typically within a 10-m radius of previous sightings. Overall, we observed 13 marked and an unknown number of unmarked juvenile *C. pinguis* during the month. Many of them were observed on multiple occasions, resulting in over 35 sightings. All observations were made between 0800 and 1700 h.

On four separate occasions, a juvenile *C. pinguis* was seen displaying aggressive behaviors towards two other juveniles seen in the same area. Agonistic behaviors were varied. Head bobs and push-ups were seen in all interactions and were the most common aggressive behaviors observed. In one case, biting occurred during the display stage. During one lengthier interaction that lasted > 10 min, we also observed full apposition (the lizards were parallel to one another with their heads pointing in the same direction) and lateral compression of the bodies such that the side facing the other lizard appeared larger than normal. Chasing typically followed up to 10 min of displays, lasted less than 1 min, and covered less than 5 m. The loser left the area following each interaction, though the distance it traveled is not known. In the case of one pair of lizards, the lizard subsequently returned and interactions were observed daily for three days. In the other case, we never saw the loser again following the interaction.

*Cyclura pinguis* adults are known to avoid the centers of activity of conspecifics (Mitchell 1999. *In* Alberts [ed.], West Indian Iguanas: Status Survey and Conservation Action Plan, pp. 45–70. IUCN/SSC West Indian Iguana Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK) and display aggression towards both adults and juveniles that approach them (N. Mitchell, unpubl. observ.). Our observations imply that juvenile *C. pinguis* begin establishing territories early in life, and that aggressive encounters may be important in determining the boundaries of these nascent territories.

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*CYCLURA PINGUIS* (Anegada or Stout Iguana). **TIMING OF REPRODUCTION and HATCHLING SIZE**. The Stout Iguana, originally found throughout the Greater Puerto-Rico Bank, today survives only in the British Virgin Islands (BVI; Binns 2003. Iguana 10(2):39-48). Perhaps the densest extant population is the one restored to Guana Island, BVI, now numbering about 100 (Perry and Mitchell 2003. Iguana 10(2):49). Little is known about the life history of this critically endangered species. Binns (*op. cit.*) reported that some females were heavily gravid in late July. L. Jarecki and F. Kraus (pers. com.) found iguana eggs accidentally dug up on the Guana beach in late September or October 1991, and those hatched three days after being found. Roger Miller, manager of Guana Island, reported (in litt., 27 June 2003) seeing an aggregation of nesting females on the beach in late June. We regularly encounter hatchlings on Guana in October, allowing for incidental observations of behavior and ecology (Perry et al. 2003. Herpetol. Rev. 34:367; LeVering and Perry 2003. Herpetol. Rev. 34:367-368). In October 2003 we systematically captured, measured, and released a total of 33 animals, of which 29 were hatchlings. Twenty five juveniles, as well as all four adults, were also uniquely marked with PIT tags for ongoing study. We used a ruler to measure snout-vent length (SVL) and found that mean SVL for the 29 juveniles was 111 mm (SD = 9.0 mm). For a sub-sample of 21, we also used a Pesola scale to measure mass to ± 1 g. Mean mass for these juveniles was 59 g (SD = 8.0 g). For the 21 animals for which both parameters were measured, mass was positively and significantly related to SVL (linear regression; mass = -50.2 + 0.953*SVL, $R^2 = 0.394$, $p = 0.002$).

Animal size was significantly positively correlated with capture date (N = 29, Pearson $r = 0.538$, $p = 0.003$). Captures of juveniles occurred during three time blocks: 2

October (N = 8), 11-19 October (N = 13) and 23-25 October (N = 8). SVLs measured during each of these blocks were (mean and SD, in mm): 103 ± 11.6, 114 ± 5.0, and 116± 5.7, respectively. These numbers and observations suggest that, in 2003, hatching peaked on or shortly prior to 1 October, following an incubation period of about three months. This is consistent with emergence patterns we have observed on Guana over the last five years, as well as the absence of reports from residents regarding hatchling sightings at other times of the year. In addition, our observations suggest that growth occurred during the study period. However, it is impossible to estimate growth rate for individuals, since each animal was only measured once.

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To: Dr. Gad Perry



**Title:** Restoration of the Stout Iguana (*Cyclura pinguis*) on the Puerto Rico Bank: a twenty-year perspective

**Medium:** Paper

**Authors:**

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**Subject area covered**: Restoration of endangered species

**Abstract**: The Stout Iguana, originally found throughout the Greater Puerto Rico Bank, today survives only in the British Virgin Islands (BVI). The last naturally surviving population is found on Anegada. Animals from that population were moved to Guana Island in 1984 and have since bred up to about 100 individuals. Vigorous reproduction has allowed us to collect considerable biological information about this little-known and critically endangered lizard, and about its role in the local ecosystem. ~~Reproduction~~ Oviposition occurs in July and ~~and~~ with hatching in September and October. Juveniles quickly disperse throughout the island. They engage in agonistic behaviors to establish areas in which they can be repeatedly found for at least several weeks. The diet is primarily herbivorous, but animals of all sizes will occasionally take animal food. Both adults and juveniles spend much time on the ground, but will also climb on a regular basis. Natural predators on

juveniles include both kestrels and snakes. Where food is plentiful, survivors appear to grow rapidly, reaching reproductive status in their fourth year.

In contrast with this restored population, iguanas on Anegada suffer from multiple threats, including habitat loss to development, competition for food with feral ungulates, and predation by feral cats. Consequently, the Anegada population appears to be rapidly declining. A head-starting operation is of limited help, because the authorities will not release animals on any other island and appropriate conditions are not available on Anegada. The expanding Guana population has recently served as the stock for efforts to establish the iguana on several other islands in the BVI. At least one of these, on Necker Island, appears to be expanding as well. However, genetic diversity is low and it would be preferable if head-started Anegada animals could also be used in this effort.

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# Locomotor performance and social dominance in male *Anolis cristatellus*

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The proximal mechanisms determining social dominance are not well understood. We used the highly territorial lizard *A. cristatellus* to test two main hypotheses: (1) that male social dominance is associated with locomotor abilities; (2) that locomotor abilities (maximal performance), as measured in the laboratory, are correlated with behaviour in the field. In the field, we recorded locomotor behaviours and assertion displays, then characterized microhabitat use and thermal relations. In the laboratory, we measured maximum sprint running speed, endurance and morphometric characters, and assessed dominance by pairing males of similar body size in an experimental arena. In 72 of 77 interactions, one lizard (the 'winner') was unequivocally determined to be dominant over the other (the 'loser'). Winners performed more assertion displays than losers before capture and also had higher endurance in laboratory tests. Although contestants were matched for snout–vent length, winners had significantly deeper and wider heads. However, we found no significant differences in field locomotor behaviours, perch or thermal characteristics, head length, or maximal sprint speed. Our findings support those of previous studies, and extend them in several ways. This is the first demonstration that assertion displays in the field are related to both locomotor performance and laboratory-assessed social dominance. Locomotor performance may directly affect social dominance by allowing some males to perform better in dyadic interactions. Alternatively, both locomotor performance and social dominance may be linked to a common underlying mechanism, such as variation in hormone levels, which are known to affect aggression, locomotor performance and morphology.



The influence of behavioural choices and repertoires on the outcome of agonistic interactions has received considerable attention in recent years (e.g. Andersson 1994; Jenssen & Nunez 1998). However, the behavioural options available to an individual may be constrained by its whole-organism performance abilities and, in turn, by the underlying morphological and physiological traits that determine those abilities (e.g. Arnold 1983; Bennett 1983; Garland et al. 1990a; Garland & Losos 1994; Irschick & Garland 2001). Therefore, the relationship between physiological performance abilities and success in agonistic interactions has also begun to receive attention (Garland et al. 1990b; Chappell et al. 1999; Hammond et al. 2000; Robson & Miles 2000).

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Physiological performance most affects individual fitness when it directly impacts survival or reproduction (Pough 1989; Bennett & Huey 1990). Locomotor performance, the focus of the current study, can directly affect foraging abilities, predator avoidance and reproductive success (references in Garland & Losos 1994; Clobert et al. 2000; Irschick & Garland 2001). We focus on the potential impact of locomotor performance on the ability of male lizards to acquire and control territory. A large number of studies have shown that controlling a territory can have a major effect on Darwinian fitness (reviews in Dewsbury 1982; Ellis 1995), and should thus be under strong selection. However, the processes by which a territory is established remain unclear (Maynard Smith 1982; Stamps & Krishnan 1995, 1998).

Lizards are common models in both behavioural ecology (e.g. Huey et al. 1983; Vitt & Pianka 1994) and locomotor biology (e.g. Gleeson & Harrison 1988; Losos & Sinervo 1989; Garland & Losos 1994; Irschick & Losos 1998; Autumn et al. 1999; Irschick & Jayne 1999; Zani 2000; Irschick & Garland 2001; Vanhooydonck et al.



0003–3472/03/$30.00/0

2001). Interspecific comparisons demonstrate that locomotor performance is related to morphological traits (Bonine & Garland 1999 and references therein) and to activity patterns in the field (Garland 1999).

Many lizard species are territorial, and male dominance is generally considered important in controlling a territory (e.g. Stamps 1997; Sinervo & Lively 1996; Jenssen & Nunez 1998; Stamps & Krishnan 1998). In lizards, two studies have found that locomotor performance ability is positively related to the ability of male lizards to establish social dominance in experimental arenas (Garland et al. 1990b; Robson & Miles 2000). In the field, males with larger territories are likely to enjoy higher fitness, as demonstrated by Haenel et al. (2003a, b) and suggested by Sinervo & Lively (1996). Thus, locomotor performance may be important in determining the Darwinian fitness of male lizards (Stamps 1994). However, the generality of these findings is unclear. First, in the study of Garland et al. (1990b), only speed was a significant predictor of dominance, whereas Robson & Miles (2000) found that both endurance and sprint speed were positive predictors of the outcome of experimental encounters. Second, the species examined, *Sceloporus occidentalis* and *Urosaurus ornatus*, are rather closely related (Reeder & Wiens 1996). Finally, the relationship between laboratory and field measures of dominance is unknown (Robson & Miles 2000).

The goal of the present study was to expand upon previous studies of the relationship between locomotor performance, field behaviour and social dominance. To explore the generality of previous findings, we focused on *A. cristatellus*, a member of an iguanian clade that is only distantly related to *Sceloporus* and *Urosaurus* (Pough et al. 2001). The genus *Anolis* has received much attention, including several studies of factors related to agonistic interactions, such as body size (Tokarz 1985), communication (McMann 1993) and territoriality (Stamps & Krishnan 1995, 1998; Jenssen & Nunez 1998). *Anolis cristatellus* is common on the Greater Puerto Rico Bank (Lazell 1983; Schwartz & Henderson 1991). Only males possess the large body and tail crests that give the species its scientific name (Rivero 1978). Adult male body length is about 1.4 times greater than adult female body length (Fitch 1981). Males defend a territory typically consisting of several trees. Adults of both sexes, as well as the juveniles, show frequent aggression and territoriality, although males are especially combative (MacLean 1982). In *S. occidentalis* and *U. ornatus*, agonistic interactions typically last only a few minutes (Garland et al. 1990b; Robson & Miles 2000). In contrast, male–male agonistic interactions in *A. cristatellus* may last up to an hour, and often leave participants visibly exhausted (G. Perry and K. LeVering, unpublished observations). Such interactions involve pushup displays, dewlap extensions, body compression, biting, head butting, tail lashing and extensive chasing (Ortiz & Jenssen 1982). Interactions tend to be long, rather than rapid. Because agonistic interactions are potentially physiologically taxing, we expected that locomotor performance would be positively correlated with social dominance in this species. More specifically, we hypothesized that social dominance in *A. cristatellus* would be more closely related to endurance than to sprint speed. We also predicted that field behaviours would be positively correlated with locomotor performance and social dominance gauged in the laboratory.

## METHODS

To maximize comparability with previous studies, we replicated the methodologies of Garland et al. (1990b) and Robson & Miles (2000) to the extent allowed by differences in the biology of the species. The methods used by McMann (1993) to study dyadic encounters in *A. carolinensis* were used whenever possible. Prior to collection, we conducted behavioural observations of a subsample of randomly chosen individuals. We collected the lizards immediately afterwards. Locomotor performance was measured during the following days, and morphometric measurements were taken at their completion. On the final testing day, prior to release, we measured dominance in an experimental interaction.

### Study Animals

In some *Anolis* species, aggression is much more common during the reproductive season (Jenssen et al. 1995). We therefore conducted our work during October, when *A. cristatellus* are reproductively active and aggression is common in all segments of the population. In 1999 and 2000, we collected 224 male *A. cristatellus* for study. Animals were collected by noosing and immediately transported to the field laboratory on Guana Island, British Virgin Islands. Prior to collection, data on field behaviours (see below) were collected for a subsample of 50 adult males.

Of the 224 males collected, 175 exceeded the minimum snout–vent length (SVL) of 47 mm at which secondary sexual characters first appear (Philobosian 1975; G. Perry & K. R. LeVering, unpublished data). Smaller animals were released, and adult males were individually housed in large plastic bags containing wet paper towels to restore moisture lost in transit. Individuals remained isolated for less than 18 h prior to testing.

In the laboratory, each animal was first tested for sprint speed, then for endurance, and finally for social dominance (see below). Morphometric data were obtained following locomotor testing but prior to dominance testing. Depending on the size of the animal, mass was measured to the nearest 0.1 or 0.5 g using a 10-g or 30-g Pesola scale, respectively. Snout–vent length was measured to the nearest 1 mm using a metric ruler. Tail condition can affect the outcome of agonistic interactions (Fox & Rostker 1982; Fox et al. 1990; Martín & Salvador 1993). We therefore measured tail length and the regenerated length of the tail if broken, tail crest length and crest height to the nearest 1 mm using a metric ruler. Finally, we measured head length, width and depth to the nearest 0.1 mm using a calliper and the methods recommended by Goren & Werner (1993), as head dimensions can be correlated with Darwinian fitness in

some lizards (Hews 1990). Following dominance testing, all animals were released at the capture site.

## Field Behaviours

For a subsample of 50 males, we recorded measures of locomotor and aggressive behaviour, body and air temperatures, and characteristics of the last-used perch prior to collection. Three measures of field locomotor behaviours were obtained during 10-min observations: number of moves/min, percentage of the time spent moving (Huey & Pianka 1981; Perry 1999) and number of jumps/min (Losos 1990a; Irschick et al. 2000). We also recorded the number of assertion displays/min (Jenssen et al. 1995).

Immediately following capture, we used a Miller and Weber quick-read cloacal thermometer to measure body and ambient temperatures at the last perch to within 0.1 °C. For the last perch used by the lizard, we measured height to within 5 cm, and diameter to the nearest 0.1 mm. To avoid observer effects, a single experimenter (G.P.) collected all field behaviour data. Animals engaged in overt social interactions or thermoregulatory behaviours (e.g. shuttling in and out of sunlight) were omitted from the analysis. In anoles, assertion displays are made without reference to a specific recipient (Jenssen et al. 1995), and were thus not considered to represent an interaction.

## Locomotor Capacity

Following collection, we measured locomotor performance in captivity. General testing procedures followed those of previous studies, with multiple tests of sprint speed occurring on a single day and endurance tests conducted on separate days (Garland et al. 1990b; Robson & Miles 2000). Sprint speed, which requires a relatively brief effort, was always tested before endurance, which requires exhausting the animal. Recovery from sprint speed testing is considered to be rapid, with some researchers conducting up to eight such tests in a single day, separated by at least 1 h (e.g. Robson & Miles 2000). Consequently, we follow previous studies in expecting that a rest period greater than 12 h should minimize the impact of sprint speed testing on the results of endurance tests conducted the following day.

We measured sprint speed in 2000 using a computerized racetrack equipped with infrared photocell/receptor stations at 25-cm intervals. Because *Anolis* do not respond well to flat racetracks, we followed previous workers (Losos 1990a; Losos & Irschick 1996; Irschick & Losos 1998) in angling the racetrack and treadmill (see below) at 37°. Repeatability enhances the utility of physiological performance in evolutionary analyses (Chappell et al. 1996; Dohm 2002). To assess the repeatability of laboratory measures of speed, we conducted a preliminary study using an initial sample of 31 lizards. We followed Garland et al. (1990b) in testing sprint speed on two consecutive days. Each lizard was chased up the 2.5-m-long track. The speed measured for the fastest 0.25 m was used as an estimate of maximum sprinting speed. Each lizard was tested in two bouts, conducted on the morning and afternoon of the same day. Each bout included two runs, resulting in a total of four runs each day. Using the fastest 0.25-m interval speed from each day, speed was found to significantly decline from day to day (day 1: $1.50 \pm 0.39$ m/s; day 2: $1.40 \pm 0.47$ m/s; Wilcoxon matched-pairs signed-ranks test for repeated measures: $Z = 2.293$, $N = 31$, $P = 0.022$). Moreover, maximal values obtained on the 2 days were significantly and positively correlated (Spearman rank correlation: $r_S = 0.585$, $P = 0.001$; Pearson correlation: $r_{29} = 0.676$, $P < 0.001$). We therefore eliminated the second day of testing for sprint speed.

We measured endurance in both 1999 and 2000, using a portable electric-powered treadmill inclined at 37° as described above. Speed was controlled via a rheostat, and the moving high-traction belt (effective area 50 × 18 cm) was surrounded on three sides by Plexiglas walls to prevent escape by the lizards. To determine the appropriate speed for distinguishing performance on the treadmill, we first tested groups of six to seven lizards at speeds of 1, 0.5, 0.35, 0.25 and 0.15 km/h. No lizard was tested at more than one speed, and lizards used for this preliminary study were not included in the analyses that follow. Based on these results (Fig. 1), we conducted all further tests of endurance at 0.3 km/h. At this speed, lizards showed a relatively wide variability in endurance time, most of them between 4 and 6 min. This period is long enough to involve substantial aerobic metabolism, yet short enough to allow efficient handling of large numbers of animals. As with speed, we also tested endurance on two consecutive days, using a subsample of 26 lizards. Each lizard was tested to exhaustion, once a day, at a treadmill speed of 0.3 km/h. Endurance measures were highly repeatable ($r_S = 0.871$, $N = 26$, $r_{24} = 0.878$, $P < 0.001$ for both) and did not differ significantly between days (day 1: $240 \pm 104$ s; day 2: $237 \pm 96$ s; Wilcoxon matched-pairs signed-ranks test for repeated measures: $Z = 1.283$, $P = 0.20$). To reduce stress on study animals, we therefore also eliminated the second day of testing for endurance and only include the results of the first day of testing in the analyses that follow.

Body temperature greatly affects locomotor performance in ectotherms (e.g. Bennett 1990; Garland 1994; Autumn et al. 1999). The problem is less severe for our study organism, because the body temperature of *A. cristatellus* closely tracks that of their relatively stable environment (Huey & Webster 1976; Hertz 1992). Moreover, the optimal temperature for sprinting is close to field body temperatures (Huey 1983). We have verified this observation on Guana Island (Table 1). Nevertheless, we avoided conducting tests outside normal activity times. As an added precaution, we used a Miller and Weber cloacal thermometer to verify that body temperatures of test animals were within the normal range of field activity temperatures for Caribbean *Anolis* (28–30 °C; Irschick & Losos 1998).

## Dominance

From among the larger pool of available males, 77 pairs of *A. cristatellus* were chosen for dominance testing based

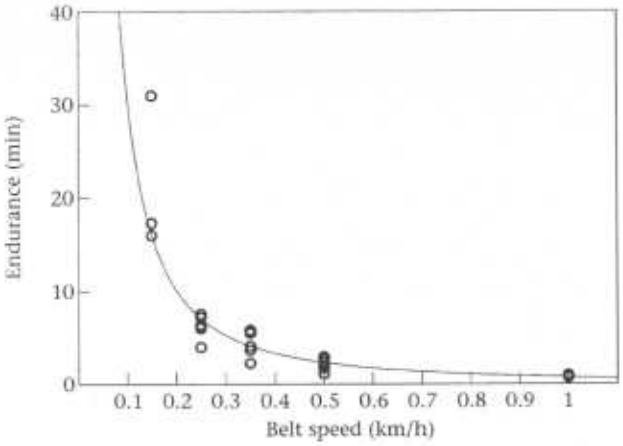


**Figure 1.** Endurance as a function of belt speed in adult male *A. cristatellus* from the British Virgin Islands. The treadmill was angled at 37°, and each symbol represents a single individual. Endurance can be predicted from belt speed using the equation: endurance = 0.761 + speed$^{-1.606}$ (Pearson correlation: $r_{26} = 0.96$, $P < 0.001$).

on matches in body size. Body size can strongly influence social dominance in lizards (Fox 1983; Tokarz 1985; Deslippe et al. 1990; Hews 1990; McMann 1993; Carpenter 1995; Molina-Borja et al. 1998). Paired animals were therefore chosen such that their SVLs were within 3 mm of one another. Because tail condition can also affect dominance status (Fox & Rostker 1982; Fox et al. 1990; Martín & Salvador 1993), we did not use as contenders any animals with recently damaged tails. Each lizard was only tested once. To control for possible effects of captivity (e.g. Navas & Gomes 2001), males were paired only with other individuals collected on the same day.

Dominance was tested under neutral conditions similar to the symmetric low-level contests of McMann (1993). Animals were not familiar with the test enclosure or with each other. Contestants were placed in a three-dimensional test enclosure (120 × 50 × 70 cm, L × W × H), containing a centre perch with a light over it (cf. Garland et al. 1990b; Robson & Miles 2000). Enclosures were made of nylon mesh and had entrances at each end. Placement of lizards at either end was simultaneous and was randomized to avoid location effects. Animals were allowed to interact until resolution was reached, a process that took 1–8 h.

At the end of each interaction, a single experimenter (G.P.) assigned a 'winner' or 'loser' status to each lizard based on posture, location within the cage, and overt signs of aggression. We also followed Fox & Rostker (1982), Garland et al. (1990b) and Robson & Miles (2000) in quantitatively scoring the behaviour of both participants during the interaction. For each interaction, a range of behaviours was recorded during at least 30 min and up to 1 h. Measurements occurred during active interactions between contestants. Because some interactions occurred slowly, longer observations were sometimes used to better represent the behavioural repertoire displayed. Aggressive behaviours, identified based on McMann (1993) and Leal & Rodríguez-Robles (1995, 1997), were assigned positive scores, whereas submissive behaviours were assigned negative ones. Pushups and dewlap extensions were assigned a weight of 0.5. Performing lateral displays (Leal & Rodríguez-Robles 1995, 1997), and chasing and biting an opponent each received a score of 1. Crouching (Leal & Rodríguez-Robles 1995) and escape merited a score of −1. Following the interaction, a single numerical score, summing all observed agonistic behaviours, was computed for each contestant.

In the statistical analyses that follow, all comparisons were made within dyads of interacting individuals. Recording was carried out, by a single observer not aware of individual endurance measurements, for both contestants simultaneously. If more than 3 h passed with no interaction between the lizards ($N = 5$), the dominance trial was terminated and the data were omitted from the

**Table 1.** Measures of behaviour and microhabitat use for a subsample ($N = 50$) of adult male *A. cristatellus* in the British Virgin Islands

| | Winners ($N = 17$) | | | | Losers ($N = 17$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD | $Z$ | $P$ |
| Activity levels | | | | | | | | | | |
| Moves/min | 0.2 | 0.0 | 0.9 | 0.31 | 0.4 | 0.0 | 1.8 | 0.53 | 0.94 | 0.35 |
| % Time moving | 0.5 | 0.0 | 2.5 | 0.79 | 0.6 | 0.0 | 2.0 | 0.77 | 0.53 | 0.59 |
| Jumps/min | 0.02 | 0.0 | 0.2 | 0.05 | 0.08 | 0.0 | 0.5 | 0.15 | 1.71 | 0.09 |
| Displays/min | 0.2 | 0.0 | 1.0 | 0.28 | 0.05 | 0.0 | 0.4 | 0.13 | 1.78 | 0.037 |
| Thermoregulation | | | | | | | | | | |
| $T_a$ (°C) | 29.7 | 26.6 | 32.6 | 1.7 | 29.6 | 26.6 | 32.6 | 1.5 | 0.19 | 0.85 |
| $T_b$ (°C) | 28.6 | 24.8 | 32.4 | 2.2 | 28.3 | 26.1 | 33.0 | 1.7 | 0.36 | 0.72 |
| Perch characteristics | | | | | | | | | | |
| Height (cm) | 141 | 35 | 600 | 246 | 85 | 25 | 135 | 37 | 1.19 | 0.23 |
| Diameter (mm) | 346 | 31 | 2000 | 546 | 231 | 43 | 2000 | 445 | 0.59 | 0.55 |

$T_b$: body temperature. $T_a$: ambient temperature. Values for winners and losers, determined in subsequent laboratory tests, were compared using the Wilcoxon matched-pairs signed-ranks test. $P$ values were two tailed except for assertion displays/min, where the prediction was for winners to have a higher value. Data for animals that participated in interactions that had no clear resolutions ($N = 16$ individuals) are not presented.

analysis. Thus, only interactions that had a clear resolution ($N = 72$) were included in the analyses that follow.

### Statistical Analyses

To avoid assumptions about normality, all statistical comparisons were performed using nonparametric statistical tests. Qualitatively similar results were obtained from parametric tests, which are reported in a few places to enhance comparability with previous work. Because lizards were tested as pairs, measures of winners and losers were nonindependent, so we used a matched-pairs test (Wilcoxon signed-ranks test) for comparing winners and losers (Garland et al. 1990b; Zamudio et al. 1995; Robson & Miles 2000). The same test was also used to compare field behaviours, observed prior to capture, between winners and losers. To fully account for differences among individuals regardless of interaction outcome, we also used a Spearman rank correlation to relate laboratory locomotor performance to field behaviours. The reported $P$ values are two tailed unless stated otherwise. Means are reported with standard deviations throughout.

## RESULTS

### Field Behaviours

Field measurements of behaviour and microhabitat usage are summarized in Table 1. Male *A. cristatellus* are sedentary foragers who maintain a body temperature near that of their immediate environment, and normally occupy a perch typical of trunk-ground species in the Williams (1983) ecotype scheme. Winners of subsequent laboratory dominance tests (see below) gave assertion displays at a significantly higher rate than losers (Table 1).

### Locomotor Capacity

We found considerable variation between males in both maximal sprint speed ($N = 112$; mean $\pm$ SD: $1.6 \pm 0.35$ m/s; range 0.6–2.6) and endurance ($N = 144$; $280 \pm 79.8$ s; range 84–502). Neither speed ($r_S = -0.115$, $N = 87$, $P = 0.291$; $r_{85} = -0.207$, $P = 0.055$) nor endurance ($r_S = 0.064$, $N = 127$, $P = 0.473$; $r_{125} = 0.050$, $P = 0.579$) were correlated with SVL within the size range examined in this study. Endurance ($r_S = 0.188$, $N = 127$, $P = 0.034$; $r_{125} = 0.167$, $P = 0.06$) but not speed ($r_S = -0.024$, $N = 87$, $P = 0.824$; $r_{85} = -0.058$, $P = 0.595$) was significantly positively correlated with body mass.

Laboratory sprint speed was not significantly correlated with any of the four measures of field behaviour taken before capture (moves/min, percentage of time moving, jumps/min and displays/min). Endurance was positively correlated with the number of displays/min recorded in the field ($r_S = 0.31$, $N = 50$, $P = 0.027$; Fig. 2), but not with any of the other three traits (NS in all cases). We obtained data on both sprint speed and dominance for 52 pairs of lizards. The distribution of sprint speeds for both winners and losers did not differ significantly from normal (Kolmogorov–Smirnov tests: $D = 0.110$ and 0.107, NS for both). Similarly, the distribution of endurance for both winners and losers did not differ significantly from normal ($D = 0.062$ and 0.077, $N = 72$, NS for both).

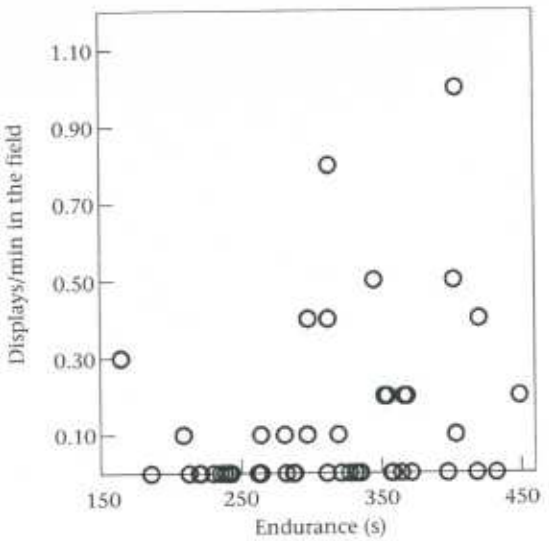


**Figure 2.** The relationship between endurance of adult male *A. cristatellus* in the laboratory and the rate at which they performed assertion displays before capture (Spearman rank correlation: $r_S = 0.31$, $N = 50$, one-tailed $P = 0.027$).

### Dominance

Lizards typically initially froze at opposing sides of the test enclosure. Once interactions began, they took a variety of forms. Some involved very subtle actions that were barely discernible, whereas others became obvious fights involving displays, chasing and biting. Escalated interactions involving biting were uncommon, compared with interactions limited primarily to displaying and posturing. The repertoire used by captive lizards was very similar to that of free-ranging animals. A dominant individual was identified, based on the observer's assessment of the interaction, in 72 of 77 staged interactions. Most interactions were prolonged, and determination of winners usually occurred more than 1 h after the interaction began. We obtained numerical dominance scores for 52 of the fights. In most cases, the numerical dominance scores agreed well with subjective assessments (Fig. 3). Winners had significantly higher scores than did losers (Wilcoxon matched-pairs signed-ranks test: $Z = 5.822$, $P < 0.001$), corroborating the qualitative assessments.

Overall, tested animals showed considerable variation in body size (Table 2). However, within a dyad, winners and losers did not differ significantly in SVL (difference between winners and losers: $1.0 \pm 1.1$ mm), reflecting our criterion for size-matched combatants. Paired animals also did not differ significantly in mass, tail length, crest length, crest height, head length or head width. Differences in these traits cannot, therefore, explain the winner or loser status of a lizard in our study. However, winners

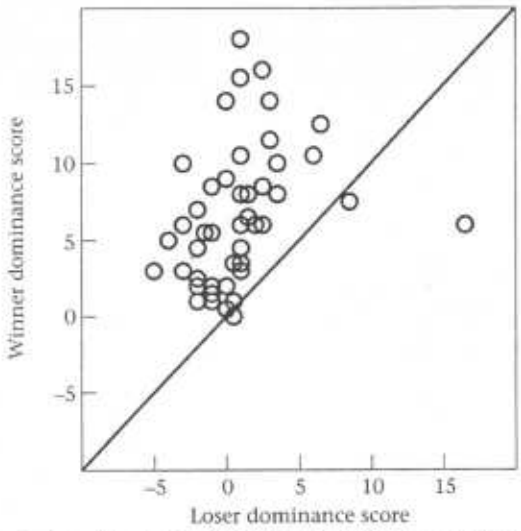


**Figure 3.** Dominance scores of winners and losers in experimentally matched pairs of male *A. cristatellus* in which an agonistic interaction occurred. The diagonal line represents equal values for the winner and loser in a pair. Winners had significantly higher dominance scores than losers (Wilcoxon matched-pairs signed-ranks test: $Z = 5.822$, $N = 52$ pairs, two-tailed $P < 0.001$).

had significantly deeper heads than did losers (Table 2), raising the possibility that relative head size helps determine the outcome of agonistic interactions between males.

As expected, winners of laboratory encounters performed more assertion displays in the field before capture than losers (winners: $0.21 \pm 0.279$ displays/min; losers: $0.05 \pm 0.133$ displays/min). However, both body and ambient temperatures were nearly identical for winners and losers prior to capture (Table 1). Winners typically used higher and wider perches than did losers, but the differences were not statistically significant. Winners and losers also did not differ significantly in the number of moves/min, the percentage of the time spent moving or the number of jumps/min.

The sprint speeds of winners ($1.61 \pm 0.39$ m/s) were not significantly different from those of losers ($1.57 \pm 0.34$ m/s; $Z = 0.537$, $N = 52$ pairs, $P = 0.591$; Fig. 4). In contrast, the endurance of winners ($300 \pm 87.7$ s) was significantly higher than that of losers ($268 \pm 69.1$ s; $Z = 2.447$, $N = 72$ pairs, $P = 0.014$; Fig. 5).

We used two methods to search for a correlation between speed and endurance. At the level of individuals, we found no significant correlations between sprint speed and endurance within either winners or losers ($P > 0.6$ in both cases). Similarly, comparing members of each dyad, we found no correlation between the difference between winners and losers in speed and the difference in endurance (Spearman rank correlation: $r_S = -0.09$, $N = 52$ pairs, $P = 0.524$).

## DISCUSSION

Our study is the first to link social dominance, locomotor performance, morphological traits and field behaviours. Experimentally assessed social dominance in *A. cristatellus* was positively and significantly related to relative head depth, laboratory-measured endurance and display frequency in the field, but was not related to most morphometric traits, microhabitat and thermoregulation measures, most field locomotor behaviours, or laboratory sprint speed. Both endurance (Garland 1994; present study) and dominance (Fox 1983; Tokarz 1985; Deslippe et al. 1990; McMann 1993; Carpenter 1995; Molina-Borja et al. 1998) often covary with body size within lizard species. However, our contestants were matched for SVL and did not differ significantly in mass, thus removing size as a possible influence on our findings.

To the extent that results obtained under artificial conditions in captivity reflect natural behaviours, social dominance in male *A. cristatellus* was positively related to locomotor performance in the present study. This result is consistent with previous work on lizards (Garland et al. 1990b; Robson & Miles 2000), suggesting that locomotor performance is a widely important correlate of social dominance in this group. Studies of social dominance in junglefowl, *Gallus gallus spadiceus*, also show a relationship between organismal performance (aerobic capacity, in this

**Table 2.** Morphometric measures taken from adult male *A. cristatellus* in the British Virgin Islands

| | Winners ($N = 17$) | | | | Losers ($N = 17$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD | $Z$ | $N$ | $P$ |
| Mass (g) | 7.8 | 4.0 | 13.5 | 1.71 | 7.6 | 3.0 | 11.5 | 1.66 | 1.08 | 71 | 0.28 |
| Tail length (mm) | 100 | 33 | 145 | 23.6 | 95 | 25 | 145 | 28.1 | 0.21 | 72 | 0.84 |
| Crest (mm) | | | | | | | | | | | |
| Length | 45 | 0 | 73 | 20.4 | 46 | 0 | 72 | 19.1 | 0.21 | 72 | 0.84 |
| Height | 7 | 0 | 17 | 3.6 | 6 | 0 | 15 | 3.5 | 1.07 | 72 | 0.28 |
| Head (mm) | | | | | | | | | | | |
| Length | 19.7 | 16.7 | 22.3 | 1.18 | 19.5 | 15.9 | 22.2 | 1.43 | 1.38 | 72 | 0.17 |
| Width | 11.9 | 9.9 | 13.9 | 0.88 | 11.9 | 8.7 | 13.7 | 0.95 | 1.54 | 72 | 0.12 |
| Depth | 9.4 | 7.8 | 11.5 | 0.85 | 9.2 | 6.9 | 10.9 | 0.91 | 2.35 | 35 | 0.019 |

Values for winners and losers were compared using the Wilcoxon matched-pairs signed-ranks test. $P$ values are two tailed. $N$ refers to the number of dyads used in each comparison.

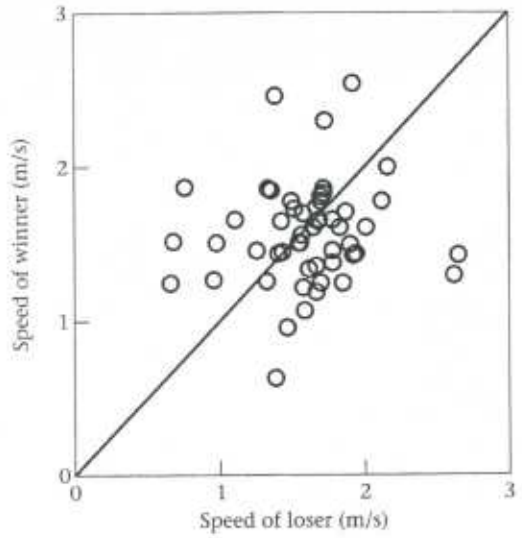


**Figure 4.** Maximum sprint speed of winners and losers in pairs of male *A. cristatellus* matched for body size. Sprint speed was measured on a 2.5-m racetrack angled at 37°. The diagonal line represents equal values for the winner and loser in a pair. Differences between winners and losers were not significantly different (Wilcoxon matched-pairs signed-ranks test: $Z = 0.537$, $N = 52$ pairs, two-tailed $P = 0.591$).

case) and the social rank of males (Hammond et al. 2000). No such relationship exists in female junglefowl (Chappell et al. 1999; Hammond et al. 2000), and it would be interesting to test for such a relationship in female *A. cristatellus*, which appear to be aggressive but not territorial (G. Perry, unpublished data).

As predicted, winners of dyadic encounters had greater endurance than did losers. However, most of the interactions we observed in the test arenas were not very intense, perhaps because males tend to match the level of aggression displayed by the other individual (McMann 1993). Endurance in our study was measured under conditions intense enough to exhaust lizards in a few minutes, whereas the dominance interactions apparently lasted much longer. Thus, although our findings could be interpreted to mean that endurance directly determines social status, maximal endurance is unlikely to have directly determined the outcome of observed fights. At least some of the processes involved in territorial acquisition in *Anolis* appear to rely more on persistence than on active aggression, and winning an interaction may not be essential to controlling a territory (Stamps 1994, 2001; Stamps & Krishnan 1995). Animals can use subtle cues to evaluate each other (McMann 1993). Such assessment of aggressive potential is predicted by evolutionary theory (Maynard Smith 1982), although the specific cues used have rarely been identified (Hofmann & Schildberger 2001). Males may be able to use the frequency or amplitude of aggressive displays as a proxy for the endurance of an opponent. That field display rates are correlated with endurance (see below) suggests that display rates may serve as honest signals of male physical prowess (sensu Zahavi 1977).

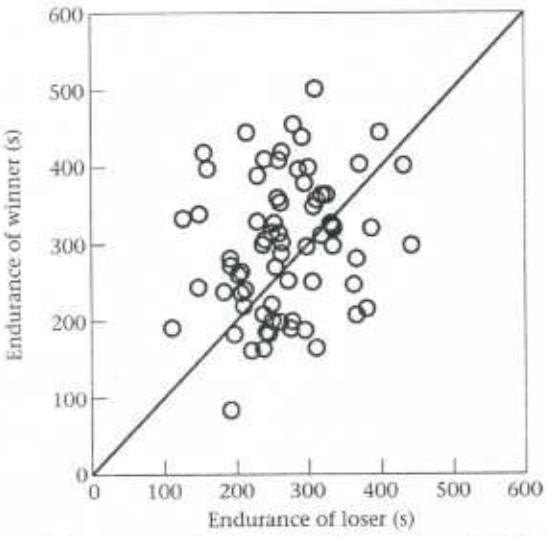


**Figure 5.** Endurance of winners and losers in pairs of male *A. cristatellus* matched for body size. The diagonal line represents equal values for the winner and loser in a pair. Winners had significantly higher endurance values than losers (Wilcoxon matched-pairs signed-ranks test: $Z = 2.447$, $N = 72$ pairs, two-tailed $P = 0.014$).

Winners in our study, matched in size to losers, had significantly deeper heads. Hews (1990) also found greater head depth in male *Uta palmeri* that won experimental dyadic interactions. She also observed a positive correlation between head depth and territory quality. Her findings that head width and length were not so correlated (Hews 1990) suggest that head length, width and depth are not necessarily part of a single evolutionary suite, but may evolve in response to separate selection pressures. Our data also support this interpretation. Two interspecific comparative studies (Carothers 1984; Kratochvíl & Frynta 2002) also reported an association between male–male aggression and sexual dimorphism in lizard head sizes, suggesting that this might be a general phenomenon associated with intrasexual selection. Head dimensions are directly related to bite force (Herrel et al. 1998, 1999). As biting is an occasional element in *A. cristatellus* agonistic interactions, head dimensions may be important for determining social dominance during male–male interactions. However, biting was rarely observed in our experimental arenas, and was not a common element during interactions we observed in the field. We therefore do not believe that head dimensions of contestants directly affected the outcome of our contests. As with endurance, head dimensions may provide information on opponent biting ability, thus providing cues that help to determine the outcome of an interaction, even in the absence of overt aggression.

An alternative interpretation of our findings would be that higher endurance and greater head size are not the direct causes of winning fights, but rather are affected in parallel by an underlying physiological trait. Testosterone levels are well known to be associated with dominance and display behaviours in lizards (Cooper et al. 1987; Moore & Marler 1987; Marler & Moore 1989; DeNardo & Sinervo 1994; Smith & John-Alder 1999; Sinervo et al.

2000). Testosterone levels are higher in males during the reproductive season (Tokarz et al. 1998). Experimentally modified testosterone levels cause changes in activity levels (Marler & Moore 1989; DeNardo & Sinervo 1994; Sinervo et al. 2000) and locomotor performance (John-Alder et al. 1996; Klukowski et al. 1998). Similarly, male *S. occidentalis* infected with malaria show lower testosterone levels, lower stamina, reduced courtship display rates, less success at territorial defence and lower social status (Schall et al. 1982; Schall & Dearing 1987; Dunlap & Schall 1995). Thus, a reasonable hypothesis would be that testosterone levels affect social dominance in male *A. cristatellus* through both increased aggression and greater locomotor performance (see also Garland et al. 1990b). Androgen levels are well known to affect changes in secondary sexual characteristics (e.g. Shine & Crews 1988; Oliveira & Almada 1998; reviewed in Thornhill & Gangestad 1999). The observed differences between winners and losers in head depth might be interpreted as another effect of increased testosterone. However, there are two potential problems with this interpretation. First, the known organizational effects of testosterone occur during development, whereas behavioural effects are the result of short-term hormone levels. Second, data on the organizational effects of hormones such as testosterone and corticosterone on lizard head dimensions are not available. Further studies of the hormonal processes involved in dominance and locomotion in *A. cristatellus* are needed before this hypothesis can be evaluated.

Both Garland et al. (1990b) and Robson & Miles (2000) found that sprint speed is positively correlated with social dominance in the species they studied. We predicted that speed would not be as important in *A. cristatellus*, because agonistic interactions in this species are rarely high-speed. We therefore did not find it surprising that maximum attainable speed was not an important predictor of social dominance in *A. cristatellus*. All *Anolis* species studied to date normally move at speeds much below their maximum levels (Irschick & Losos 1998; Irschick 2000), and all are sedentary foragers that move relatively infrequently (Perry 1999). Thus, it is also not surprising that winners and losers showed no differences in sprint speed and all field locomotor behaviours measured (moves/min, percentage of time moving and jumps/min). Nevertheless, in the distantly related *Lacerta vivipara*, Clobert et al. (2000) found that endurance (measured at birth) is associated with subsequent field activity level, growth rate and parasite load, but not survivorship. It would be of interest to conduct similar studies in the ecologically very different *A. cristatellus*.

We did not find a trade-off between speed and endurance, although it has often been predicted based on first principles (e.g. Losos 1990b; Garland & Losos 1994). Moreover, Vanhooydonck et al. (2001) found such a trade-off when comparing lacertid lizard species. However, Garland et al. (1988) did not find it among mammals, and intraspecific studies of lizards have generally failed to identify such a pattern (e.g. Garland et al. 1990b; Sorci et al. 1995; Robson & Miles 2000; this study). Perhaps the limited amount of variability available intraspecifically, compared with interspecific differences, makes identifying such correlations and trade-offs difficult.

Our second important finding was the presence of a significant positive relationship between agonistic behaviours in the field and laboratory-measured endurance and social status. Previous studies of lizards did not include measures of field behaviour (Garland et al. 1990b; Robson & Miles 2000), so the relationship between laboratory findings and aspects of fitness in the field has not been explored. That winners in dyadic encounters had both a higher display rate before capture and greater endurance greatly strengthens the likelihood that laboratory-based findings on locomotor performance and social dominance are truly instructive about natural interactions in the field. Moreover, greater signalling frequency in dominant males has also been documented in other lizard species (Deslippe et al. 1990; Molina-Borja et al. 1998).

The lack of a correlation between field locomotor activity and laboratory-measured locomotor capacity differs from what might have been expected based on interspecific comparisons conducted in the past. Garland (1999) showed that laboratory endurance capacity and field locomotor behaviour are positively correlated among lizard species, and G. Perry, K. E. Bonine & T. Garland (unpublished data) showed that endurance and home range size are positively correlated in phrynosomatid lizards. Previous work has shown that the activity levels of territory-holding male lizards might be higher (DeNardo & Sinervo 1994) or lower (Perry 1996) than those of subordinate or nonterritorial individuals. However, we found no such differences. In a similar vein, dominant individuals might be better able to monopolize preferred microhabitats than losers, resulting in differences in body temperature. However, this seems unlikely to occur in *A. cristatellus*, which is a thermoconformer (Huey & Webster 1976; Hertz 1992). Indeed, winners and losers occupied similar microhabitats and thermoregulated to a similar degree before capture (Table 1). It thus seems that the impacts of social dominance might be highly species and/or context specific. Our findings also serve to emphasize that behavioural factors can make typically realized locomotor activity differ greatly from what is physiologically feasible (Garland et al. 1990a; Garland & Losos 1994; Irschick 2000; Irschick & Garland 2001).

Reproductive success, a crucial element of fitness, is often greatly impacted by the social status of an individual. Thus, the increasing body of evidence indicating that dominance is correlated with locomotor performance abilities emphasizes the potential multilevel nature of selection. In combination with previous studies, our findings also suggest that different aspects of locomotor performance are important in different species, presumably as a function of variation in ecology and social behaviour.

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# Estimation of Phylogenetic Relationships within *Anolis* (Sauria:Iguanidae), with especial reference to *A. cristatellus wileyae*, using AFLP

By

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# Abstract

Anoles (Sauria: Iguanidae: *Anolis*) are a diverse group of neotropical lizards that have undergone an extensive adaptive radiation in the West Indies. Many interesting issues of phylogenetic relationships, at levels from within species to the earliest branchings within the genus, remain unresolved. The method of amplified fragment length polymorphisms (AFLP), a DNA fingerprinting technique, was used on seven species of anole (*Anolis cristatellus wileyae*, *A. ernestwilliamsi*, *A. extremus*, *A. grahami*, *A. leachi*, *A. pulchellus*, and *A. stratulus*) to address such questions at several taxonomic levels. Most broad was the comparison of beta (*A. grahami*) to both island (*A. extremus*) and mainland (*A. leachi*) alpha anoles, designed to see which alpha group had more similarity to the beta representative. Next was the comparison of anoles of differing series classification: *A. cristatellus wileyae*, of the cristatellus series, and *A. stratulus*, of the bimaculatus series. In addition, sympatric species, *A. cristatellus wileyae* and *A. pulchellus* with the same series were compared for similarity of banding pattern. For these three levels, AFLP was found to be an ineffective detector of similarity/dissimilarity. Phylogenetic trees constructed using 1,000 bootstrap replicates with both the Dollo parsimony and neighbor-joining methods for tree building did not contain consistent, well-supported structure for the internal branch nodes. While some terminal taxa appeared together and there was moderate tree structure observed for one of five primer pair banding patterns, it was concluded that AFLP was not useful for the levels of differentiation within the *Anolis* genus as aforementioned.

Also examined were the relationships between *A. cristatellus wileyae* individuals from different islands in the British Virgin Islands (Beef Island, Guana Island, Necker Island, Norman Island, and Tortola), as well as from six different locations on Guana Island. Also included were *A. ernestwilliamsi* individuals, a species recently (at most about 3,000 years) derived from *A. cristatellus* and found only on Carrot Rock, British Virgin Islands. Results showed very little population structuring among islands or within a single island, suggesting that adequate gene flow exists to counteract the effects of genetic drift between island locations. Interestingly, *A. ernestwilliamsi* individuals did not appear outside the *A. cristatellus wileyae* group, but rather mixed in among them on the phylogenetic trees. This result may be indicative of insufficient time for lineage sorting of genes to have occurred; the species are clearly different based on morphological features, but are at a point where they still share many of the same genetic polymorphisms.

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## I. Introduction

*General Information*

Anoles (Sauria: Iguanidae) are among the most abundant and diverse lizards of the Americas. There are approximately 300 recognized species, of which about half are West Indian, the remainder being found on the mainland from the southern United States to South America (Williams, 1992; Powell, *et al.*, 1996). As many as eleven species are found to occur syntopically, i.e. in the same habitat (Williams, 1983; Losos, 1994).

Anoles display a suite of correlated morphological, behavioral, and ecological characteristics. The correspondence between morphology and behavior on the one hand, and ecological niche on the other, is denoted by the term "ecomorph". Ecomorphs have been named primarily on the basis of perch site. The most important ecomorphic niches are the following: grass-bush, trunk-ground, trunk, twig, trunk-crown, and crown-giant. Among the morphological features correlated with the niche are coloration, number of toe pad lamellae, body size, body shape, and the lengths of the forelimb, hindlimb, and tail relative to body size (Rand & Williams, 1969; Williams, 1972; Mayer, 1989; Beuttell & Losos, 1999).

The same ecomorphic classes have arisen on each of the major Greater Antillean Islands of Cuba, Hispaniola, Jamaica, and Puerto Rico (Figure 1). Molecular evidence (Losos *et al.*, 1998) has shown the anoles of one island to be more closely related to each other than to anoles of the same ecomorph on other islands. This indicates that, on each of the Greater Antillean islands, an ancestral anole species arrived there and experienced a radiation, filling the available niches, rather than each ecomorph arising once and colonizing each island. As an adaptive radiation, anoles rival the well known examples of the Galapagos finches (Lack, 1984; Grant, 1986) and Hawaiian honeycreepers (Berger, 1981), but are even more remarkable in that the radiation has occurred independently four times on each of the Greater Antillean islands. Schluter (2000) refers to the anoles of the Greater Antilles as " a spectacular case of replicate radiations", and is perhaps the most striking example of convergent evolution of community structure known (Williams, 1972; Losos *et al.*, 1998, Mayer 1989).

*Mechanisms of Distribution*

There are three major modes by which anoles have occupied their current geographic range: vicariance, dispersal, and human introduction. As a result of vicariance (Brown &

Lomolino, 1998), organisms are found to occur on multiple landmasses because they occurred previously on a single, larger landmass that broke apart into two or more pieces. This occurs, for example, when tectonic forces break up a continental landmass into fragments, dividing the continental biota as well. Vicariance can also occur when ocean levels rise or fall, flooding or exposing low-lying ground. This has been important in the Quaternary history of the West Indies (for current island locations, see Figure 1). Shallow ocean depths separate many of the islands on the Puerto Rican island shelf. During the Pleistocene, 1.8 million to 11,000 years ago, sea-level varied between +20 and –120m, relative to the current sea-level (Fairbanks, 1989). During the most recent low sea-level stand, 11,000 years ago, Puerto Rico was joined with Caja de Muertos and all of the Virgin Islands, except St. Croix, forming an area about twice as large as Puerto Rico today. As the sea-level rose through time, the shelf was broken into many smaller islands, severing the dry land bridges between them (Heatwole & MacKenzie, 1966). The area has changed little in the last 6,000 years as the sea level has only slightly fluctuated around the current level since that time (Farrand 1962).

The dispersal ability of a species also affects its distribution. To reach new areas, species often have to cross physical or climatic barriers. In the Caribbean, the major physical barrier is the ocean. Dispersal across oceanic waters could occur during hurricanes blowing between islands, or by floating on debris across the water (Darlington, 1938; Censky *et al.*, 1998). To colonize these new areas, the organism would need to adapt to any environmental difference present between the previous habitat and the new one. Environmental differences can include vegetation, rainfall, competition, and food sources. The presence of anoles (as well as other reptiles and amphibians (Mayer & Lazell, 1988)) on low islands that did not exist during the last Quaternary high sea-level stand about 120,000 years ago shows their capacity for significant over water dispersal (Williams, 1969).

Lastly, human introduction in the past 150 years, whether accidental or intentional, has contributed to the spread anole species to many places outside their natural ranges (Losos *et al.*, 1993). Wingate (1965) discussed anole introductions to the formerly anole-free island of Bermuda. For example, *Anolis grahami* captured on Jamaica were introduced to Bermuda to aid in control of the fruit fly, *Ceratitis capitata*, in 1905. Bermuda hosts two other introduced species as well: *A. leachii* from Antigua, first noted around 1940, and *A. extremus* from Barbados, first noted in 1953.

*Speciation and the Anole Radiation*

Competition can be a strong influence on species richness in anoles, and can influence adaptation or extinction (Rand, 1969; Roughgarden, 1995). In the West Indies, anoles' competition comes primarily from other anoles, as they largely occupy the niche of diurnal, arboreal, insectivorous lizards alone (Williams, 1972). This has resulted in differential habitat use; for example, different anole species prefer microhabitats varying in perch diameter and perch height (Rand 1964; Schoener & Schoener 1971). In general, anoles from islands with three or more species, mainly in the Greater Antilles, belong in one of the ecomorphic groups mentioned above, whereas anoles on islands where two species occur, such as in the Lesser Antilles, have one large anole and one small one. Solitary anoles are of an intermediate size. Thus, on species rich islands, anoles have diverged into a variety of niches, while on less species rich have a more generalized morphology and ecology (Schoener 1969; Williams 1972; Losos, 1992a; Losos, 1992b).

Geographic isolation also encourages differentiation: gene flow is retarded, and selective pressures may differ between locations. For example, an ancestral *A. cristatellus* on the Puerto Rico bank, with time and geographic isolation, gave rise to the subspecies *A. cristatellus cristatellus* on the Puerto Rican mainland and *A. cristatellus wileyae* on the small eastern islands of the Puerto Rican Bank (Heatwole, 1976; for geographic map, see Figure 1). Later, *A. cristatellus wileyae* returned to Puerto Rico, and there now exists an intergrade region where the two interbreed (Heatwole, 1976). Within the small eastern islands, *A. cristatellus* has given rise to *A. ernestwilliamsi* on the isolated island of Carrot Rock, British Virgin Islands (Lazell, 1983; Mayer & Lazell, 2000). Through a long distance colonization event, or several shorter colonizations of intermediate islands during low ocean levels, *A. cristatellus* gave rise to *A. scriptus* of the southern Bahamas (Rand, 1962; Williams, 1969).

Based on morphological and molecular data, anoles diversified early and rapidly in their history (Jackman *et al.*, 1999). This introduces and increases difficulty in attempting to estimate the anole phylogenetic tree. The deep branching is problematic to sort out because many branchings occurred at roughly the same time. Superficial morphological features are not always helpful. Island and mainland (South/Central America) anoles have different adaptive features. Mainland anoles do not exhibit the same ecomorphic specializations as island anoles, even when some morphological features are shared (Irschick *et al.*, 1997). Additionally, molecular data

(Losos *et al.*, 1998) indicates anoles of a single island are more closely related than anoles of different islands that occupy the same ecomorphic niche. For example, a trunk-ground anole on Jamaica would be more closely related to the other anoles of Jamaica than to trunk-ground anoles of other islands. This indicates that the ecomorphic specializations observed arose multiple times in anole history (Losos *et al.*, 1998).

*Studies of Anole Phylogeny*

Linneaus (1758) included some anoles in the 10th edition of his *Systema Naturae*, the beginning of zoological nomenclature. Since that time, and with growing interest since the latter nineteenth century, people have been working to identify anole species and, subsequently, to define the relationships present within the *Anolis* genus. A variety of methods have been applied to this end.

Researchers have analyzed karyotype (Gorman & Atkins, 1969; Gorman *et al.*, 1969), allozyme (Gorman & Kim, 1976; Gorman *et al.*, 1980a), albumin immunological (Gorman *et al.*, 1980b, Shochat & Dessauer, 1981), mitochondrial DNA (mtDNA) sequence (Jackman *et.al*, 1999), and DNA:DNA hybridization data (Mayer & Kirsch, 1999). These methods, however successful with more evolutionarily recent divergences among anole species, have left some deep and intermediate branches unresolved.

Etheridge produced an estimate of anole intra-genus relationships in 1959, based on skeletal morphology. He divided the genus *Anolis* into two groups: the alpha and beta anoles. This division, which occurred early in the radiation of anoles, was based on the presence or absence of a transverse process on the anterior caudal vertebrae. Alpha anoles lack this process whereas in the beta section it is present (Etheridge, 1959). The work Etheridge did in his proposed phylogeny (1959) focused on the alpha/beta dichotomy and the organization of the species within the alpha and beta sections into series. Williams (Williams, 1976a; Williams 1976b) formalized the categories used by Etheridge (1959) and added the subseries, species group, and superspecies categories for West Indian and South American anoles. These works have been considered a baseline for the majority of subsequent studies. However, Williams himself only retained the classical classifications "in the absence of anything better" (Williams 1989).

In 1986, Guyer and Savage reanalyzed Etheridge's (1959) osteological data set. Combining this data with the karyological data of Gorman (1973) and albumin immunology data from Shochat and Dessauer (1981), they sought to bring new order to the classifications employed and escape the paraphyly of the current arrangement, caused by the beta's origin from within the alpha section. The resulting division of *Anolis* into several genera (*Anolis, Dactyloa, Semiurus, Ctenonotus, Norops*) by Guyer and Savage (1986) has not been generally accepted (see Williams, 1989; Cannatella & de Queiroz 1989).

*Differentiation on the Puerto Rican Bank*

As indicated above on page 3, *A. cristatellus* had differentiated into two subspecies (the nomite from the Puerto Rican mainland and *wileyae* on the small eastern islands) and the allospecies, *A. ernestwilliamsi*. Carrot Rock is the only location where *A. ernestwilliamsi* may be found, a unique descendent of a widespread species. This situation is shared by a fellow Carrot Rock inhabitant, *Mabuya macleani*, a recently described skink (Sauria: Scincidae) differing from the widespread species, *M. sloanii* (Mayer & Lazell, 2000). Carrot Rock is situated about 400 meters off of the coast of Peter Island, British Virgin Islands (see Figure 2); the two are separated by a shallow stretch of water, which would have been dry land approximately 3,000 years ago (Fairbanks 1989). Additionally, the ocean currents of the area are such that individuals from the Peter Island populations could disperse over to Carrot Rock; such additions to the gene pool would work to reduce the effect of genetic drift between *A. cristatellus wileyae* and *A. ernestwilliamsi*. Mayer and Lazell (2000) hypothesized that, because such unique skink and anole species are endemic to Carrot Rock, especially given the young age of the island and its proximity to Peter Island, there must be some feature or property about the island itself that promotes rapid divergence.

In addition to the morphological differentiation studies by Heatwole (1976) and Lazell (1983), physiological differentiation has also been studied. Dmi'el, Perry, and Lazell have found differences in the evaporative water loss characteristics of *A. cristatellus wileyae* collected from different islands in the British Virgin Islands (Dmi'el *et al.* 1997; Perry *et al.* 1999; Dmi'el 2001). Their work has also shown that integumentary resistance to water loss positively correlates to the aridity of the environment for eight *A. cristatellus wileyae* populations and one *A. ernestwilliamsi* population (Perry *et al.* 1999; Dmi'el 2001); it was unclear as to whether this

effect has a base in genetic factors, phenotypic plasticity, or a combination of both. Perry *et al.* (2000) collected *A. cristatellus wileyae* from eight locations on seven islands, and one *A. ernestwilliamsi* population. Work here includes six of the nine populations Perry and colleagues studied.

*AFLP Theory*

Because of the goals of the thesis included such a range of levels of differentiation (see Research Goals, page 7), ranging from the deepest divisions in the genus to differentiation at very low levels, such as species and population, a DNA fingerprinting method was chosen. Several factors were involved in the selection of a DNA fingerprinting method. RFLP (restriction fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), and AFLP (amplified fragment length polymorphism) (Vos *et al.*, 1995) were considered. RFLP analysis usually involves transfer of restriction digested DNA onto a membrane by Southern blotting. This requires a probe for a useful sequence and further time commitment for sequence identification and probe development. The AFLP technique does not require such a lengthy time investment; furthermore, no specific information about the DNA sequence of the organism is required. When comparing available fingerprinting methods, AFLP has been shown to have greater reproducibility when compared to RAPD. This is observed in both the raw banding data and when using data filtering criteria; AFLP achieved 100% polymorphic band reproducibility, while RAPD achieved only 85% (Bagley *et al.*, 2001). Mueller and Wolfenbarger (1999) reviewed AFLP, RAPD, and RFLP based on development investment, reproducibility, and other factors (Table 1). While the RFLP and RAPD techniques have names which reference the mechanism involved in the their use, the AFLP acronym should not be used in that way. AFLP detects the presence or absence of a fragment, not a difference in length (Vos *et al.*, 1995).

Additionally, though originally tested with only bacterial, viral, plant, and human DNA (Vos *et al.* 1995), AFLP has been employed successfully for other species groups, such as fish (Albertson *et al.*, 1999; Bagley *et al.*, 2001) and insects (McMichael & Prowell, 1999; Parsons & Shaw, 2001). Giannasi, Thorpe, and Malhotra (2001) used the technique for the Asian snake *Trimeresurus albolabris* at the species and population levels with good results. Furthermore, Ogden and Thorpe (2002) have successfully used AFLP within the genus *Anolis* to assess

divergence at four graduated taxonomic levels, of which two are examined here: among series and within series.

AFLP begins with the restriction digestion of genomic DNA (see Figure 3). Two restriction enzymes are used, one 'rare cutter' (*Eco*RI, 6 base recognition sequence) and one 'frequent cutter' (*Mse*I, 4 base recognition sequence). Digestion generates three classes of fragments: in order of frequency, they are *Mse*I-*Mse*I, *Mse*I-*Eco*RI, and *Eco*RI-*Eco*RI. Adapters are ligated to the restriction sites. Adapters have two sections; one containing an enzyme-specific sequence, the other adding a primer annealing site (Figure 4). These adapters allow the AFLP process to function without prior, specific knowledge about the DNA sequence.

Next, the first PCR step occurs. This step is pre-selective amplification, in which a subset of the adapter-ligated DNA is amplified. The primers for this step consist of sequences matching the adapter (Figure 4). They also have one selective nucleotide extending 3', past the restriction site sequence, which reduces the number of fragments amplified. Primer composition and PCR strategy work to preferentially amplify the *Mse*I-*Eco*RI fragments. The *Mse*I-matching primer has a lower annealing temperature than the *Eco*RI-matching primer, resulting in much less efficient amplification of *Mse*I-*Mse*I fragments under these PCR conditions as compared to *Mse*I-*Eco*RI fragment amplification (Vos *et al.*, 1995).

The second, selective PCR step amplifies a still smaller subset of the fragments amplified in the previous step. This is accomplished by using primers with three selective nucleotides beyond the restriction site, two more than the previous step (Figure 4). For each primer pair, 50 – 100 bands are produced. More or less than this range of bands may be produced, with GC content of the selective nucleotides influencing this number; in general, higher GC content results in fewer bands amplified (Vos *et al.*, 1995).

*Research Goals*

This thesis had several goals, each dealing with a specific level of differentiation within the genus *Anolis*. They are presented as follows, starting with the broadest level:

1. To deduce whether beta anoles are more closely related to alpha anoles from the Caribbean islands or from mainland (South/Central America) alpha anoles. The beta anoles are represented by *A. grahami* and the island alpha anoles by *A. leachii*. The mainland alpha anoles are represented by the island anole *A. extremus*, which is closely related to some mainland

alphas. With this information, the colonization pattern of the beta anoles around the Caribbean can be examined. Other research to date has not been able to clearly address this question of ecological and evolutionary interest. Additionally, this compares anoles of different taxonomic sections, a branch among the deepest in the anole phylogenetic tree.

2. To compare sympatric anoles of different series. The series is the level below the alpha and beta sections of classification. *A. cristatellus wileyae* of the cristatellus series and *A. stratulus* of the bimaculatus series are representative species in the comparison. This comparison results in an estimate of the degree of genetic change present between classification series.

3. To compare sympatric species of the same series. This level of comparison, by including sympatric forms, represents "completed" speciation (Mayr, 1963), and is represented here by the comparison of *A. cristatellus wileyae* and *A. pulchellus*. This provides an estimate of the amount of genetic variation present among closely related species.

4. To compare anoles from different islands within a superspecies. This comparison includes *A. cristatellus wileyae* from several islands, and the allopatric *A. ernestwilliamsi*. *A. cristatellus wileyae* from islands both relatively near and far were collected to determine if there was a significant genetic difference between them. The populations were geographically isolated from each other, which, combined with environmental differences between the islands, could be in the process of diverging from the other populations. This results in a comparison of populations from different islands within a series, as well as a comparison of allopatric anoles of a single superspecies.

5. To compare anoles from populations within a single island. *A. cristatellus wileyae* were collected from six locations at four altitudes Guana Island, British Virgin Islands. This provides a basis for comparing individuals and populations, as well as provides information concerning the amount of genetic variation present on a single island.

To accomplish these goals, two sets of experiments, each including several AFLP primer pairs, were performed. The first set, including the species *A. cristatellus wileyae*, *A. ernestwilliamsi*, *A. extremus*, *A. grahami*, *A. leachii*, *A. pulchellus*, and *A. stratulus*, and comparing sections, series, and sympatric species, was referred to as the interspecies study. The second set, including *A. cristatellus wileyae* and *A. ernestwilliamsi*, and comparing islands and populations, was referred to as the population study.

## II. Methods & Materials

*Sample Collection*

Gregory and Nanette Mayer, and David Wingate (Department of Parks, Bermuda) collected *A. leachii, A. grahami,* and *A. extremus* on Bermuda in 1999. Arijana Barun (UW-Madison), James Lazell (Conservation Agency, Rhode Island), and Gad Perry (UW-Madison) collected *A. cristatellus wileyae* from Guana Island, Norman Island, and Necker Island, British Virgin Islands, in 2000. J. Lazell provided *A. ernestwilliamsi* individuals from Carrot Rock, British Virgin Islands, collected in 1994. With help from Arijana Barun, Kate LeVering (UW-Madison), Gad Perry, and Wenhua Lu, I collected *A. cristatellus wileyae*, *A. stratulus*, and *A. pulchellus* from Guana Island, British Virgin Islands. On Guana Island, *A. cristatellus wileyae* collection occurred at six locations (see Figure 5, Table 2). It should be noted that the distinction between the White Bay flat and plantation areas was one based largely on vegetation. White Bay flat held a sparse distribution of trees, spread in excess of about 15 meters apart and sometimes surrounded by bushes, while the plantation area as designated here was along a dirt road in a forested area, with more shade, next to the orchard.

Samples were stored either frozen at -70°C or in 95% ethanol. For ethanol-stored samples, heart, liver, muscle, and gonad tissues were dissected out for storage in 95% ethanol-filled vials. Each individual collected in the British Virgin Islands has a catalog number for identification.

*DNA Extraction*

The DNA extraction method selected employed two solvent chemicals. The first, phenol, denatures proteins effectively, while the second chemical, chloroform, acts as a solvent for RNA molecules with poly (A) tracts and removes any remaining traces of phenol from the aqueous layer (Brawerman *et al.*, 1972).

Phenol:chloroform extraction proceeded as follows: 15-20mg of tissue were homogenized and digested for 4 hours at 55°C in 500µl TNE [0.01M Tris (Fisher, Springfield, NJ, catalog #BP152-500), 0.1M NaCl, 0.01M EDTA (Fisher, catalog #BP120-500)], 50µl SDS (10% w/v) (Fisher, catalog #BP166-100), and 20µl proteinase K (10mg/ml) (Sigma, St. Louis, MO, catalog #P2308). Samples were treated with Rnase A (20mg/ml) (Sigma, catalog #R6513) and allowed to stand at room temperature (25°C) for 2 minutes. Then, an equal volume of phenol

was added and mixed for 5 minutes, followed by centrifugation at 10,000 rpm for 5 minutes (Eppendorf via Brinkmann, Westbury, NY, Centrifuge 5415c). The aqueous layer was transferred to a fresh microfuge tube. Next, a half volume each of phenol (Amresco, Solon, OH, catalog #0945) and chloroform was added and mixed for 5 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. The aqueous layer was transferred to a fresh microfuge tube. An equal volume of chloroform was added and mixed for 5 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. The aqueous layer was transferred to a fresh microfuge tube. The DNA was precipitated by the addition of 2 volumes of ice-cold 200 proof ethanol and overnight storage at -20°C. Lastly, the sample was centrifuged for 15 minutes at 14,000 rpm, the alcohol poured off, and the pellet dried using a Savant speed-vac SC110 vacuum centrifuge for 15 minutes. The pellet was rehydrated in 100µl TE [0.01M Tris, 0.001M EDTA] and stored at -20°C.

To estimate the DNA concentration of the extracted samples, 10µl of sample and 990µl TE [0.01M Tris, 0.001M EDTA] were mixed by inversion in a quartz cuvette for spectrophotometry. Optical densities (OD) were measured at 260nm and 280nm using a Beckman DU-640B (Beckman Coulter, Fullerton, CA, serial #4321101). The following calculation was used as an estimate of DNA content.

$$\frac{1000ul}{10ul} * \frac{50ug}{1ml} * OD_{260} = \frac{ugDNA}{ml} = \frac{ngDNA}{ul}$$

*Amplified Fragment Length Polymorphism (AFLP)*

AFLP System I (Invitrogen-Life Technologies, Carlsbad, CA, catalog #10544013), for use with large genomes ($5x10^8$ - $6x10^9$ bp), was selected for use. Anole genome size was calculated based on C-values, or haploid genome sizes, provided by T.R.Gregory (2001). The values listed for anoles were averaged, with an average C value of 2.12pg. Using the conversion factor 1pg = $10^9$bp, the average genome size of anoles is $2.12x10^9$bp.

AFLP proceeded according to manufacturer specifications, with some adjustments. Restriction enzyme digestion reactions contained 15.8µl $ddH_2O$, 2.0µl DNA extract (about 250ng/µl, based on estimates from spectrophotometer readings), 5.0µl 5X buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate], and 2.2µl *Eco*RI/*Mse*I enzyme mix [1.25 units/µl each in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml

BSA, 50% (v/v) glycerol, 0.1% Triton X-100], totaling 25.0µl. The reaction was mixed gently and incubated at 37°C for 4 hours. Restriction enzyme inactivation occurred at 70°C for 15 minutes.

Ligation of adapters followed digestion. To each digestion reaction, 24.0µl adapter/ligation solution [EcoR I/ MseI adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate] and 1.0µl T4 DNA ligase [1 unit/µl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% glycerol (v/v)] were added, mixed gently, and incubated for 2 hours at 20°C in a Perkin-Elmer Cetus thermalcycler. Diluting 10µl into 90µl TE created a 1:10 dilution. Both the diluted and undiluted samples were stored at -20°C.

Pre-selective amplification took place as follows: 40.0µl pre-amp primer mix, 5.0µl diluted DNA template, 6.0µl 25mM $MgCl_2$, 5.7µl 10X PCR buffer [500mM KCl, 100mM Tris-HCl (pH 9.0), 1.0% Triton X-100], and 1.0µl *Taq* polymerase were mixed gently and subjected to 30 PCR cycles as follows: 94°C for 30s, 56°C for 60s, and 72°C for 60s. The Perkin-Elmer Cetus thermalcycler was used, requiring a mineral oil overlay on all PCR reactions. Diluting 3.0µl PCR product into 147µl TE formed a 1:50 dilution. Both the diluted and undiluted samples were stored at -20°C.

Selective amplification was the final AFLP PCR step. Each reaction contained 5.0µl pre-selectively amplified, diluted DNA template, 0.18µl *Eco*RI primer (27.8ng/µl), 4.5µl *Mse*I primer (6.7ng/µl, contains dNTPs), 2.0µl 10X PCR buffer [500mM KCl, 100mM Tris-HCl (pH 9.0), 1.0% Triton X-100], 8.22µl $ddH_2O$, and 0.1µl *Taq* polymerase. In this step, each of the two primers was selected from a set of eight. The selections for the *Eco*RI primers were as follows, with 'E' representing core and restriction site-matching sequences: E-AAC, E-AAG, E-ACA, E-ACT, E-ACC, E-ACG, E-AGC, and E-AGG. The selections for the *Mse*I primers were as follows, with 'M' representing core and restriction site-matching sequences: M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, and M-CTT. Each pair of primers was assigned a number for ease of reference (See Appendix A). The Perkin-Elmer Cetus thermalcycler was used, with the required mineral oil overlay. The first two cycles denatured at 94°C for 30s, then annealed at 65°C for 30s, then elongated at 72°C for 60s. For the next nine cycles, the annealing temperature decreased by one degree each cycle. When the final annealing temperature of 56°C was achieved, 30 cycles were completed at that temperature. The product was then stored at

-20°C.

The AFLP products were then electrophoresed in a 6.0% denaturing PAGE gel (19:1 acrylamide:bis-acrylamide) at 50°C and 45W with 1X TBE running buffer for 3 to 4 hours (see Table 3 for specific times). Samples were prepared for loading by placing 3.0µl of the selective PCR reaction in a 0.65ml microfuge tube with 3.0µl of AFLP dye (98% Formamide [Fisher, catalog #BP227-500], 0.05% bromophenol blue [Sigma, catalog #B-5525], 0.05% xylene cyanol [Acros, Morris Plains, NJ, catalog #422690050]). The marker used was a 10bp DNA ladder (Invitrogen – Life Technologies, catalog #10821015). Marker was prepared like the samples, except using 2.5µl of AFLP dye and 2.5µl of a 0.04µg/µl stock dilution (0.10µg/lane). All sample and marker microfuge tubes were heated in a hot water bath at 90-95°C for 5 minutes, then quench cooled on ice for a minimum of 10 minutes. Each lane was loaded with 4µl. Data for each primer pair's analysis was collected from a single gel. In this way, between-gel error and inconsistencies were avoided, as well as resulted in more consistent band scoring.

*Research Design*

For the interspecies study, a minimum of two individuals from each of the seven species available were included, as well as markers and an outgroup sample (*Iguana iguana*). The resultant banding patterns of five primer pairs were examined: 15, 21, 29, 37, and 43 (see Appendix A for numerical designations and associated primers).

For the population study, each gel contained a negative control sample, two *A. ernestwilliamsi*, one outgroup (*A. stratulus*), three *A. cristatellus wileyae* from each of ten different locations (five different islands, six localities on the same island), except for Beef Island and White Bay Flat, which were each represented by two individuals, and two to four marker lanes. Again, banding patterns for five primer pairs were examined: primer pairs 22, 25, 34, 47, and 64 (see Appendix A).

*Visualization: Silver Staining*

There are several methods available for visualizing DNA in polyacrylamide gels, including radiography, colorimetry, fluorescence, and silver staining. Silver staining was selected for use for two reasons: first, there are no radioactive chemicals or substances involved; second, solutions can be produced in the lab economically. The protocol employed here was modified

from Bassam *et al.* (1991) by Pham (2001), and has demonstrated detection sensitivity down to 1pg/mm$^2$ (Bassam *et al.* 1991). The required DNA quantity for visualization with this protocol increases to 5-10pg/mm$^2$ for fragments smaller than 310bp (Bassam *et.al* 1991).

The staining protocol began by waiting for the glass plate and bound gel to cool to <40°C, or about 10 minutes. Each step, fixing through staining and the final rinse, occurred with rocking on a Belly Dancer (Stovall Life Science, Inc., Greensboro, NC). The plate, with the gel firmly affixed, was then submerged in 1L of fixing solution [95% dd$H_2O$, 5% reagent grade acetic acid (Fisher, A38S-212)] for 2.5 hours in a dedicated plastic pan. This fixing solution was reserved for later use. Then, the gel was rinsed in 1L dd$H_2O$ 3 times for 5 minutes each in a dedicated plastic pan, followed by submersion in 1L staining solution [99.9% dd$H_2O$, 0.1% (m/v) silver nitrate (Fisher, S181-100), and 0.056% formaldehyde (Fisher, BP531-500)] for 30 minutes in a dedicated plastic pan. Formaldehyde was added immediately before use. The plate and gel were then briefly but thoroughly rinsed with dd$H_2O$ to remove excess silver nitrate particles. Ahead of time, the metal developing pan was chilled in the -20°C freezer, and the first 2 components of the 1L developing solution [97% dd$H_2O$, 3% (m/v) sodium carbonate (Fisher, BP357-1), 0.056% formaldehyde, and 0.2% 10 mg/ml sodium thiosulfate pentahydrate] were mixed and chilled to 4°C in the refrigerator. The formaldehyde and sodium thiosulfate were added immediately before use. The developing solution was used in two equal aliquots, and a swirling motion, not the automatic rocking used in previous steps, was maintained for the duration of developing to prevent silver particles from settling on the gel surface and producing background. The first aliquot was used and drained off when it contained an amount of reduced silver particles to moderately darken the solution. The remaining solution was added to the pan and developing proceeded for an additional four to six minutes, or until the desired amount of band development occurred. Then, the reserved fixing solution was added to stop the developing process and allowed to rest for five minutes. Lastly, the plate and gel were rinsed in one liter dd$H_2O$, with rocking on a Fotodyne Bellydancer, for ten minutes, three times. To dry, the gel was covered with several sheets of filter paper, a thick layer of paper towels, and then a board with weights on top; this worked to wick away the liquid from the gel surface. Twenty minutes later, the gel was sufficiently dry enough to scan.

*Data Collection & Storage*

Gels were scanned in black and white at 400dpi using a flatbed scanner (Plustek, Taiwan, Optic Pro 9630P) to preserve them for analysis and review after the physical gel was destroyed. Adobe Photoshop v5.5 (Adobe Systems Inc., San Jose, CA) was used to adjust brightness and contrast. The DNA ladder has bands from 30 bp to 330bp, with bands located every 10bp. Bands were detected and scored manually with the results encoded into an Excel 2000 (Microsoft Corp.) spreadsheet matrix using a binary scoring scheme, where 0 represents the lack of a band and 1 represents the presence of a band. These were the primary data sets upon which all subsequent analyses were based.

*Data Analysis*

Two methods of analysis were used. First, Dollo parsimony was used to construct trees from character data by finding the tree for which the least number of changes occur. It assumes that it is more difficult to gain a complex feature, such as a restriction site or recognition sequence, than it is to lose that feature. Thus, in this method, a specific sequence of characters can be gained only once, but can be lost several times. A more general method, such as Wagner parsimony, does not have this limitation (Swofford *et al.*, 1996).

Neighbor-joining constructs trees from dissimilarity, or distance, data by finding the shortest, or minimum evolution, tree for a data set. It accomplishes this by starting with a star tree: one in which all taxa, or individuals, connect to the same node. Pairs are examined to find the two with the least distance between them. Those two taxa are then considered one, and compared to all remaining taxa to again find the one giving the least distance, and so on.

Bootstrapping can be used to obtain approximate standard errors and confidence limits for some statistics, but is not a statistical test in and of itself. It has been shown that the means and standard deviations of estimated statistics obtained utilizing bootstrapping approximate those of the population. Introduced by Felsenstein (1985), the practice is applied in phylogenetic research to aid in the evaluation of clades in trees. It involves taking one data set and creating many pseudoreplicate data sets by sampling from the original set with replacement; in this way, some values are sampled multiple times while others are not sampled at all. The additional data sets are referred to as 'bootstrap replicates'. Bootstrapping data 100 times results in 100 replicate data sets. These sets are processed identically by some method, i.e. neighbor-joining, and then a

consensus tree is generated. This tree depicts the nodes/relationships that occur most often in the bootstrap replicates, and labels that node with the percentage of times it occurs. Thus, bootstrapping provides a way to look at the data and have an idea of how robust the relationships/nodes depicted are. For example, a bootstrap value of 95% would denote a well-supported clade, whereas 25% would not be well-supported (Sokal & Rohlf, 1995).

The Excel matrices containing binary banding data were converted to text files for use in Phylip v3.5 (Felsenstein, 1993) using the PERL script excel_2_seqbt.pl, written by Peter Ladvienka. For character data analysis, the data were bootstrapped using Phylip's seqbt.exe module (data type: discrete morphology, replicates: 1,000). Each matrix was bootstrapped to form 1,000 psuedoreplicate matrices, except the data for primer pair 34, which resulted in a downstream file size too large for processing. In that case, the original matrix was bootstrapped into 2 files containing 500 psuedoreplicates each, which were processed as the other files were, replacing 1,000 with 500 in the program settings where appropriate, with a manual consensus at the end. After bootstrapping, Dollo parsimony was conducted using Phylip's dollop.exe module (jumble: 10 times, multiple data sets: 1,000). Finally, the multiple trees yielded from parsimony analysis were made into a single consensus tree using Phylip's consense.exe module (specify outgroup). Images of the resulting trees were viewed and manipulated using Treeview v1.6.6 (Page, 1996)

For comparison, general or Wagner parsimony was performed in addition the Dollo parsimony presented. Wagner parsimony, as opposed to Dollo, allows the gain of a feature more than once. The Wagner trees showed the same structure as the Dollo trees; however, the bootstrap support values were not as clear-cut as for Dollo parsimony (trees not shown). Well supported clades had lower bootstrap values in the Wagner trees than the Dollo trees, while the ill-supported clades had higher support in the Wagner trees as compared to Dollo. Clearly, the use of Dollo parsimony, as recommended, was a better choice (Swofford *et al.*, 1996) .

The consistency index (Kluge & Farris, 1969; CI) was computed for each of the ten parsimony trees. It is a measure of the amount of homoplasy present in a tree and the consistency of the data. Homoplasy occurs when a character is present in multiple individuals, but due to mutation and reversion, it is not the same, unchanged character. The CI may be calculated for each character separately, or for all the characters at once; it is then called the ensemble CI, and is the calculation employed here. Computation proceeds as follows:

$$CI = \frac{M}{S}$$

where M = the minimum number of steps required to fit the data, or the number of bands scored, and S = the actual number of steps in the most parsimonious tree(s). An increase in the number of steps results in a lower CI number and indicates homoplasy exists. Data sets rarely attain 100% consistency, and the index can become infinitely small but will never reach 0%. The interpretation of CI values is similar to that of the bootstrap analysis in that a higher CI value corresponds to more consistent data and less homoplasy in a linear fashion. However, when considering CI values, one must keep in mind two facts. First, the inclusion of uninformative characters can serve to inflate the CI value. Second, with increasing numbers of taxa, the observed CI shows decreasing values. Therefore, the CI values for data sets with differing amounts of parsimony uninformative characters cannot be directly compared, as cannot data sets with differing taxa numbers (Forey *et al.*, 1998).

For distance analysis, the bootstrapped data files containing 1,000 psuedoreplicate data sets from the parsimony analysis were used. For this portion, primer pair 34 psuedoreplicates did not need to be divided as before, but primer pairs 21 and 29 did require such division. The bootstrapped matrices were converted to Excel format using the PERL script seqbt_2_excel.pl, written by Peter Ladvienka. The data files were then converted into the format employed by NTSYSpc v2.11n (Exeter Software, New York). Binary character data was made into similarity distance data using the Jaccard coefficient (Jaccard, 1908) by the SIMQUAL module (coefficient=J). The Jaccard coefficient is defined as follows:

$$\frac{a}{a+b+c}$$

where a = number of shared bands, b = number of bands in individuals *i* but not individual *j*, and c = number of bands in individuals *j* but not individual *i*. Conversion to similarity data was followed by transformation into dissimilarity values using TRANSF module [MUL(1)+ ADD(1)]. Neighbor-joining occurred with the NJOIN module (root with outgroup, specify outgroup OTU). Lastly, the resultant trees were consensed into a single tree using 50% majority rule. The coefficient accompanying the neighbor-joining trees is a dissimilarity index calculated using the Jaccard coefficient. Colless' index ($CI_c$; Colless, 1995), or the consensus fork index,

was calculated. The value refers to how much the 1,000 individual trees agree, or the robustness of the result. It is calculated as follows:

$$CIc = \frac{Nset}{n - 2}$$

where $N_{set}$ = number of subsets in the consensus tree, excluding the total set and subsets containing only one taxon, and n = number of taxonomic units.

## III. Results

*Evaluation of the AFLP Method*

In both studies, bands appear in some of the negative control lanes (Figures 6, 17, 19, 21, and 23). Not all of the gels contained a negative control lane; some controls were on optimization gels conducted previous to the ones used for scoring, were clear of any banding, and were omitted to make room for additional marker lanes. However, once bands did appear in the negative control lanes, they consistently appeared. Efforts were made to eliminate the issue, including the use of new, freshly autoclaved aerosol barrier pipette tips, PCR reaction tubes, and distilled water. The water, PCR buffer, $MgCl_2$, and mineral oil aliquots were UV treated for a 20-minute duration. Primers that had either not been used, or were used little, were tested. Samples were loaded in alternate lanes to eliminate the possibility of well leakage issues. Latex gloves were changed midway through the PCR preparation. Despite these efforts, the bands still appeared.

Three to four bands were visible; usually two single bands and one doublet, regardless of the primer pair used. In addition, the bands were located at roughly the same base pair (bp) regions; for example, the doublet occurred between about 130bp and 150bp. Lastly, a band corresponding to the negative control band *does not* always appear in sample lanes, as would be expected if contamination had occurred (see Figure 21; very little corresponds to the negative control band at about 132bp). For these reasons, it is believed that the bands present do not represent contamination of the samples, and likely represent some kind of artifact, such as a primer dimer. In fact, Vos *et al.* (1995) note that when <1pg DNA template *or no DNA at all* was added to a reaction, they still observed template-independent bands. As a conservative measure,

any bands corresponding to any found in the negative control lane were excluded from scoring and inclusion in any analyses.

The number of bands scored for this thesis were not very different in number than that reported by Ogden and Thorpe (2002) for anoles. Using 3 primer pairs, they scored a range of approximately 180bp for each, yielding over 300 bands total. This results in about 0.56 bands per base pair. This thesis research scored 474 total bands over about 1300 base pairs, resulting in about 0.36 bands per base pair. This is comparable to the amount scored by Alberson *et al.* (1999), who viewed about 0.37 bands per base pair. Given that the number of bands amplified varies between primer pairs, there is no evidence that the amount or quality of banding present here differs from other AFLP studies.

*Interspecies Study*

This interspecies study sought to compare anoles of three taxonomic categories. First, a comparison between anoles from each of the two sections of the genus: the alpha and beta sections. A single beta representive, *A. grahami*, was compared to each of two alpha anoles, one from the mainland, *A. extremus*, and one from the islands, *A. leachii*. In this way, the origin locality of the beta anoles may be theorized, based on which of the alpha anoles it more closely relates to. This constitutes the broadest level examined. Next, within the alpha section, two comparisons were made. Two anoles of differing series, *A. cristatellus wileyae* and *A. stratulus*, were compared, as were two sympatric anoles of the same series, *A. cristatellus wileyae* and *A. pulchellus*. These provided comparisons of levels between the alpha/beta comparison and the population study.

For the interspecies study, a total of five primer pairs were used to generate the scored banding patterns (summary, Table 3), Dollo parsimony trees (summary, Table 4), and neighbor-joining trees (summary, Table 5).

The banding patterns produced by primer pair 21 (Figure 6) resulted in no structure in the 50% majority rule Dollo parsimony tree (Figure 7b), except to separate an *A. pulchellus* sample (AP3) from the anole group with 53.1% support in a tree with a CI of 0.92, though the CI value was inflated by the inclusion of 21 bands possessed by either all anole samples or just the outgroup (Table 3). In the neighbor-joining tree (Figure 7c, $CI_c$ 0.21429), sample AL1 (*A. leachi*) was separated from the others. These two samples seemed to amplify differently than the others,

and that is reflected in the fact they are separated out from the rest of the anole group for this and some following primer pairs. The samples AP3 and AL1 contained bands the others did not, and lacked other, common bands (for data, see Appendix C). The data for this primer set was not helpful with respect to the research goals pertaining to between section, between series, and within series comparisons.

Primer pair 29 produced a banding pattern (Figure 6) which, when scored (Appendix D), produced 50% majority Dollo parsimony trees which only showed strong support for one relationship: the pairing of the *A. stratulus* samples (89.9%, Figure 8b). The CI for the tree was 0.76, inflated by the inclusion of 18 bands possessed by either all anole samples or just the outgroup (Table 3). Neighbor joining analysis required the use of two files containing 500 bootstrap replicates, instead of a single file with 1,000 replicates, due to computational limitations; however, both of the resultant trees were identical excepting an unresolved point with less support than the tree shown here in Figure 8c. The neighbor-joining tree (Figure 8c, $CI_c$ 0.42857) placed both AL1 and AP3 outside the other anoles in about 72% of cases. Other branchings occurred, but were supported in less than 50% of the bootstrap replicate trees. With respect to the research goals, this primer pair was not informative for the three comparisons above the species level.

Data for primer pair 15 (Appendix E) based on its banding pattern (Figure 9) were analyzed. A Dollo parsimony tree utilizing the 50% majority rule again placed the AL1 sample outside the other anoles and apart from the 2 other *A. leachii* samples (AL2 and AL3), but inside relative to the outgroup, 52.0% of the time. The paired *A. stratulus* sampled were again well-supported (78.1%) in the tree, which had a CI of 0.58, inflated by the inclusion of 6 ingroup parsimony0uninformative bands (Table 3). The data also resulted in a neighbor joining tree distinguishing only the outgroup from the other taxa (Figure 10c, $CI_c$ 0.14286). This primer pair did not produce data useful in attaining the relevant research goals.

Primer pair 37 banding patterns (Figure 11) contained data (Appendix F) demonstrating slightly more structure than the previous primers. The beta anoles (*A. grahami*) individuals were paired together in the 50% majority Dollo parsimony tree (66.8%, CI 0.92, Figure 12b), as were the *A. stratulus* and *A. cristatellus wileyae* individuals (68.1% and 81.0%, respectively). The CI value was inflated slightly by the inclusion of 2 ingroup parsimony-uninformative bands (Table 3). Unfortunately, neither the island nor mainland alpha anoles (*A. extremus* and *A. leachi*,

respectively) were also together, nor were any of the internal nodes supported in the bootstrap analysis to allow comparison to see which, island or mainland alpha, the beta anole *A. grahami* was most closely related to. The same lack of internal node structure prevented the between-series analysis of *A. stratulus* and *A. cristatellus wileyae*. Neighbor-joining analysis resulted in a very similar tree (Figure 12c, $CI_c$ 0.28571). Though an improvement over the previous primer pairs, this pair also did not contribute to the research goals.

Primer pair 43 was used to generate a banding pattern (Figure 13, data in Appendix G). While the neighbor-joining tree (Figure 14c, $CI_c$ 0.42857) showed many of the same relationships at the deeper nodes, the 50% majority Dollo parsimony tree showed the greatest amount of structure attained in the interspecies study (Figure 12b, CI 0.61). Four ingroup parsimony-uninformative bands inflated the CI value slightly (Table 3). Sample AP3 was again placed outside the anole group with 72.2% bootstrap support. Four species samples paired as expected: *A. leachi* (56.2%), *A. cristatellus wileyae* (69.9%), *A. stratulus* (71.6%), and *A. ernestwilliamsi* (76.5%). The two species of differing series classification, *A. cristatellus wileyae* and *A. stratulus*, were placed in separate groups with an intermediate bootstrap support of 50.8%. *A. stratulus* was placed closer to the outgroup, as it also was in the neighbor-joining tree. This support is not strong enough to make assertions about between-series relationships. This primer pair did not aid the alpha/beta analysis; none of the three anoles involved showed unambiguous placement or pairing. It is worth noting that the *A. cristatellus wileyae* and *A. ernestwilliamsi* samples both showed with-species pairing, as aforementioned, but also showed a supported separation from each other (58.1%).

Pooling the data from all primer pairs for analysis was considered. As seen in the heterogeneity of the CI, which ranged from 0.58 to 0.92, the most parsimonious tree for each of the primer pairs expressed a varying degree of goodness of fit. This great range supported an analysis in which data for each primer pair was examined independently of the others, rather than an analysis in which data for all primer pairs was pooled together. In summary, of the five primer pairs examined for the interspecies study, none displayed a sufficient amount of resolution to make assessments concerning the research goals pertaining to comparisons between sympatric species of the same series, allopatric species of differing series, or species of differing sections. Futhermore, two samples (AL1 and AP3) were declared outliers based on clear amplification differences as compared to other samples both visually and in terms of scored bands.

*Population Study*

This portion of the thesis sought to find genetic differences among islands for a single species, *A. cristatellus wileyae*, as well as assess genetic diversity or distinguish populations within a single island. Additionally, a genetic comparison between *A. cristatellus wileyae* and a descendant species, *A. ernestwilliamsi* was sought. A total of five primer pairs were used in the population study to generate the scored banding patterns (summary, Table 3), Dollo parsimony trees (summary, Table 4), and neighbor-joining trees presented (summary, Table 5). The average number of bands scored per base pair of scoring range for the interspecies and population studies overlapped ($0.454 \pm 0.182$, versus $0.384 \pm 0.102$, respectively); similar amounts of bands were scored for both studies, though in the population study the bands tended to be present or absent in either most or few samples as compared to the interspecies study where there appeared to be a more continuous distribution of samples containing or lacking a particular band.

Primer pair 34 resulted in a banding pattern (Figure 15), which was subsequently scored (Appendix H). Neighbor-joining analysis (Figure 16c, $CI_c$ 0.03448) showed only that the outgroup was well-supported. Dollo parsimony analysis proceeded with 2 parallel analyses, of 500 bootstrap replicates each, instead of the single analysis used for the other primer pairs because of computational limitations; a single file was unable to be consensed by Phylip's CONSENSE module. The most parsimonious tree for the two files differed by five steps (80 and 85 steps, see Table 4). The fully bifurcating tree with the least steps appears as Figure 16a (CI 0.43). All characters included were polymorphic. The 85-step tree (CI 0.40) differed slightly in topology (tree not shown). Both trees collapse identically, showing only the outgroup apart from the other taxa (Figure 16b).

The banding pattern for primer pair 22 (Figure 17) was converted to binary data (Appendix I). Both the collapsed, 50% majority rule Dollo parsimony tree (Figure 18b, CI 0.35) and the neighbor-joining tree (Figure 18c, $CI_c$ 0.06897) showed the same relationships: the outgroup was placed outside the other samples with high support, and individuals AC24 and AC29 were paired together (66.7% support in the parsimony tree). Individuals AC24 and AC29 were collected on Guana Island at the plantation location and Norman Island, respectively; an interesting result, given that the two islands are separated by Tortola and Peter Island (Figure 2). The CI value was inflated by the inclusion of four ingroup parsimony-uninformative characters and two characters shared by all samples including the outgroup.

Data (Appendix J) extracted from the banding patterns produced by primer pair 64 (Figure 19) were analyzed. Again, both the tree building methods resulted in the same tree after collapse: separation of the outgroup and a single supported pair. That pair was AC18 and AC21, from White Bay Flat and Longman's Point on Guana Island, present in the neighbor-joining tree (Figure 20c, $CI_c$ 0.06897) and with 73.6% bootstrap support in the parsimony tree (Figure 20b, CI 0.49). These two locations are about 1,400m apart, though one would estimate the home range of *A. cristatellus wileyae* to be $10^1$ to $10^2 m^2$, based on diet and snout-vent length (Perry & Garland, 2002), and mark/recapture data (Mayr, pers. comm.). It was interesting to note that those two locations should have a link. Seven ingroup parsimony-uninformative characters and six characters shared by all samples, including the outgroup, inflated the CI value.

Primer pair 47 data (Appendix K) was collected from the banding patterns produced (Figure 21). Neither the 50% majority Dollo parsimony tree (Figure 22b, CI 0.63) nor the neighbor-joining tree (Figure 22c, $CI_c$ 0.13793) showed supported internal structure. Parsimony analysis resulted in the support of two individual pairs: AC24 and AC29, as for primer pair 22, with a bootstrap support of 86.9%, and AC7 and AC30, both from Norman Island, with 58.9% support. These two relationships were also supported in the neighbor-joining analysis, with the addition of an AC15 (Hotel), AC19 (White Bay Flat) pairing, localities about 425m apart. This pair appeared in the fully bifurcating parsimony tree (Figure 22a), but with only 44.6% support, it did not survive the collapse to majority rule. Ten parsimony-uninformative bands and two bands shared by all samples, including the outgroup, inflated the CI value.

Finally, data from the banding patterns produced by primer pair 25 (Figure 23) were collected (Appendix L). Dollo parsimony (CI 0.69), collapsed to majority rule (Figure 24b), displayed support for three pairs, one of which was the same AC24, AC29 pair (95.2%) shown in two data sets above, indicating 3/5, or 60% of primer pairs tested, support a Guana-Norman Island connection. Collected at locations about 710m apart, individuals AC5 (Muskmelon Bay) and AC14 (hotel) paired with 57.8% bootstrap support. Lastly, an AC6 (Necker Island), AC15 (hotel) pair appeared (65.4% support). Virgin Gorda, The Dogs, and Great Camanoe lie between Guana and Necker Islands (Figure 2). The neighbor-joining tree also displayed these three relationships (Figure 24c, $CI_c$ 0.13793). The CI value was inflated by eleven parsimony-uninformative bands and ten bands shared by all samples, including the outgroup.

## IV. Discussion

*Interspecies Study*

The interspecies study, including fifteen anole individuals of seven species, examined genetic differences of three major types: between anoles of differing sections (alpha or beta), between sympatric anoles of differing species, and among allopatric anoles of the same series.

Some samples presented difficulties. *A. extremus* samples AEX1 and AEX2 did not consistently pair with each other, or any other sample, in any of the five 50% majority rule parsimony consensus trees. AEX1 was never placed, with support, outside the other anoles. Sample AEX2 was placed outside all other anoles once, with a bootstrap value of 531, or 53.1% (Figure 7b). Sample AP3 was placed, with support, outside all other anoles in three of the five majority rule consensus trees with percentage bootstrap values of 52.0%, 53.1%, and 72.2% for primer pairs 15, 21, and 43, respectively (Figures 10b, 7b, 14b). Sample AL1 was also supported in a position outside all other anoles for primer pair 15 (52.0% bootstrap support, Figure 10b). This indicated that samples AL1 and AP3 contained bands that no other anoles or the outgroup had, and lacked bands that all other anoles had, and in quantities such that their placement outside the anole group received more support than some relationships between anoles of the same species. While the source of the phenomenon was unclear, and not noted in publication (see all AFLP papers cited here), these particular samples amplified differently than the others, and can be considered outliers.

The CI values (Table 4) for the primer pairs employed in the interspecies study were not consistently high; only primer pairs 21 and 37 exceeded a CI of 0.9, and that of primer pair 21 was inflated. The next highest CI, 0.76 for primer pair 29, also demonstrated inflation; if there were no inflation, all three would be considered good values. However, considering the numbers of uninformative bands included (Table 3), only the CI for primer pair 37 would remain near 0.9. This indicates that, for some primer pairs, the data do not point to a single, fully resolved tree. In the 50% majority rule trees, the two *A. stratulus* individuals appear together with a minimum bootstrap support of 68.1%, except for primer pair 21, which showed no fine structure. For primer pair 29, the only structure present among anoles was the *A. stratulus* pairing. *A. grahami*, *A. ernestwilliamsi*, and *A. leachi* individuals each exhibited within-species pairing, with 50% or greater bootstrap values, only once in five primer pairs. *A. cristatellus wileyae* pairs appeared twice (Figures 12b and 14b).

The greatest amount of structure evident was for primer pair 43 (Figure 14). The M-CTC, E-ACA primer combination seemed to result in more informative bands than the other primer pairs surveyed. The anoles of differing series, *A. cristatellus wileyae* and *A. stratulus*, were placed apart with 50.8% bootstrap support; however, this value was not high, less than 1% above the 50% majority rule cut-off value. The support was weak and not robust or definitive. This tree did not allow the comparison of beta anoles in relation to the two alpha anole taxa, since the branching was unresolved in the collapsed tree. The mainland anole, *A. extremus*, was ambiguously placed as the two individuals of the species did not pair and were in two groups. The interesting outcomes to note in this tree were the following: first, note that both the *A. cristatellus wileyae* and *A. ernestwilliamsi* samples paired with each other, and with good bootstrap support (69.9% and 76.5%, respectively). Secondly, note that the two species pairs were placed in different groups with 58.1% bootstrap support. This result gains interest after reviewing the results for the population study presented below. The bootstrap support placing the two species apart was not high. This may be due to a sample size effect, for in the population study, *A. ernestwilliamsi* samples appear intermixed within the *A. cristatellus wileyae* group, never pairing with each other with support.

Neighbor-joining trees displayed structures quite similar to the 50% majority rule parsimony trees. Similar tree topologies arrived at via differing analysis methods reinforced the idea that the data were represented in a consistent, reproducible manner. Based on the rather limited amount of supported structure in any of the trees resultant of the five primer pairs explored, it was concluded that AFLP may not have the needed power to resolve the deep and intermediate areas of the anole phylogenetic tree that have plagued systematists for so long. As a result, some of the goals of this research will remain unfulfilled and open to future endeavours: the goals dealing with how the beta anoles are related to alpha anoles, including the beta colonization of the Caribbean and South American mainland, as well as the goal to apply AFLP to compare anoles of differing series and sympatric anoles at a molecular level. The method applied to these issues would need to use genetic markers or loci that experience a much slower mutation rate. A single answer is not clear; sequence data from 5 mitochondrial transfer RNA genes and the mitochondrial NADH dehydrogenase subunit 2 gene also were unable to resolved the deepest branchings (Jackman *et al*, 1999). AFLP surveys the entire genome in areas both within and outside genes or other functional regions. While the functional DNA regions change

more slowly because of the functional constraints present, the other, 'nonfunctional' DNA regions do not experience such selective constraint (Graur & Li, 2000). Thus, these areas can change much more frequently, increasing the occurrence of homoplasy when comparing taxa that radiated long ago. AFLP has most frequently been applied in studies at the population or closely related species levels (Mueller & Wolfenbarger, 1999; Albertson *et al.*, 1999; McMichael & Prowell, 1999; Giannasi *et al.*, 2001; Parsons & Shaw, 2001); results here agree that that is where the method yields most information.

*Population Study*

*A. cristatellus wileyae* from five islands, and six localities on a single islands, were compared to assess relationships among islands and within an island. Included as well were *A. ernestwilliamsi* samples, a species which only recently diverged from the *A. cristatellus* superspecies group and is endemic to the island of Carrot Rock, British Virgin Islands.

The CI values for these five Dollo parsimony trees were lower than those for the interspecies study; however, this was not unexpected, and the CI values from both studies cannot be directly compared based on the taxa number consideration discussed above. Because the population study included such a large number of individuals (31, versus 16 in the interspecies study), the CI was expected to display a lower value (Forey *et al.*, 1998). As in the interspecies study, the neighbor-joining trees displayed very similar, if not identical, topology as the 50% majority rule parsimony trees. Concerning the neighbor-joining consensus trees, four of five primer pairs (22, 25, 47, and 64) show a coefficient of 0.50 at the node where most individuals join because the consensus method used was a 50% majority rule method; the taxa have to have a minimum of 50% similarity, or a maximum of 50% dissimilarity. The coefficient for the neighbor-joining consensus trees is a dissimilarity index, so a lower coefficient corresponds to a greater degree of similarity.

In the population study, even in the two parsimony trees with the highest CI values (0.63, Figure 22; 0.69, Figure 24), the bootstrap values of most internal nodes were exceptionally low. This results in trees displaying supported relation only between pairs of individuals, and only once between individuals from the same location (for a listing of individuals by location, see Table 1). Two individuals from Norman Island, AC7 and AC30, paired at 58.9% in data from primer pair 47 (Figure 22b). Pairing most frequently, AC29 and AC24, from Norman Island and

the plantation area on Guana Island, respectively, were present in 3 of 5 majority rule parsimony trees, and in the other 2 fully bifurcating parsimony trees (see figures 18b, 22b, 24b, 16a, 20a). The pair displayed bootstrap support of 66.7%, 86.9%, and 95.2% for primer pairs 22, 47, and 25, respectively (see Figures 18b, 22b, and 24b). It is notable that these two individuals come from such geographically separate islands as Norman and Guana.

Another result of exceptionally low internal node bootstrap values was the collapse of all internal nodes for all five primer pairs, all the way back to the outgroup, *A. stratulus*. This result was not the expected one; based on the fact that others have found phenotypic difference between *A. cristatellus wileyae* living in differing environments and islands (Heatwole, 1976; D'miel *et al.*, 1997; Perry *et al.*, 1999, Perry *et al.*, 2000), and the fact that five separate islands were represented in the study, it was expected that there would be at least some geographic structuring. This thesis was not interested in differences based on a single gene, rather accumulated changes based on geographic isolation and differing selective pressures between environments. AFLP markers are randomly scattered throughout the genome, and are probably not subject to forces like functional or selective constraint. Because of this, most of the differentiation revealed is neutral. Thus, this may not have been unexpected, as Lewontin (1984) showed that neutral genetic characters differentiate at a slower rate than do phenotypic characters subject to selection.

The complete lack of supported geographic structuring suggests that there exists adequate gene flow or movement of individuals between islands to maintain a single large, multi-island population. Heatwole (1976) proposed great gene flow among islands as well, based on morphological features such as dewlap coloration, with movement east to west using water currents. Such flow prevents any one group from differentiating from the rest, though it is unclear how much or often this individual movement could occur. Introduction, both accidental and intentional, would increase gene flow among islands; for example, an anole could board and travel on a boat, getting off on a new island. Additionally, anoles are very small, light lizards, and could be moved on debris during hurricanes, as has been recorded for the larger *Iguana* lizards that colonized the island of Anguilla (Censky *et al.*, 1998). The results also lend more support to phenotypic plasticity as the reason for observed interlocational differences, rather than a basis in significant genetic change. However, there may exist genetic differences undetectable using the AFLP technique.

As aforementioned, Ogden and Thorpe (2002) conducted an evaluation of AFLP over graduated taxonomic levels. The analysis performed using principal coordinate analysis (PCOA; Sokal & Rohlf, 1995), a method in which one locates the data axis describing the greatest amount of variation among an infinite number of axes. When they examined sample localities for a species differing only morphologically, with no previously demonstrated genetic variation, only 13.2% of variation was described by the first two axes, as compared to 26.3% along the first two axes for populations of a different species *with* demonstrated genetic variation. Given these low values and the occurrence of gene flow among islands, it is perhaps not so surprising that the population study for this thesis, examining localities varying morphologically without demonstrated genetic variation, was not able to distinguish clear population groups.

*Concerning A. ernestwilliamsi*

Any information obtained about the relationship between *A. cristatellus wileyae* and *A. ernestwilliamsi* has interest because *A. ernestwilliamsi* is a recent descendent of the *A. cristatellus* superspecies group. As further discussed later, *A. ernestwilliamsi* is endemic to Carrot Rock, British Virgin Islands, an island not long above sea level. The clear morphological differences between *A. ernestwilliamsi* and *A. cristatellus wileyae* also developed in a relatively short timespan.

In the interspecies study, *A. cristatellus wileyae* and *A. ernestwilliamsi* did not group together with support; indeed, they were once placed in separate clades, with a weak support of 58.1%, in a parsimony majority rule tree (see primer pair 43, Figure 14b). Yet, in the population study comparisons, the two *A. ernestwilliamsi* individuals did not group with one another, even when considering the fully bifurcating parsimony trees.

There exist multiple possible explanations for this observation. A sample size effect may be present, making AEW1 and AEW2 appear within the *A. cristatellus wileyae* group when they should be placed together and outside; an increased number of *A. ernestwilliamsi* samples may resolve the tree. Another consideration could lie in tissue preservation. All sample tissues for the population study were immersed in ethanol for one year or less, while the *A. ernestwilliamsi* tissues were stored in ethanol for a much longer time; an additional 6-7 years as compared to the *A. cristatellus wileyae* samples. Though unlikely, degradation of the DNA in those two samples may have occurred, preventing adequate amplification of some species-specific fragments, but at

a rate which allowed the two to pair in the interspecies study above. However, the number and quality of the bands amplified was not noticeably different from the other samples; the bands were neither more nor less numerous, nor was their distribution or intensity different, unlike the outlier samples AL1 and AP3. If the DNA had degraded, one would have expected a noticeably different size distribution and fewer bands, as many of the bands that *would* have been included under normal cirsumstances would have been missing an adapter site. This would prevent amplification.

The pairing and placement of *A. cristatellus wileyae* and *A. ernestwilliamsi* in the 50% parsimony majority rule tree could result from a sample size effect. Comparing 2 *A. ernestwilliamsi* to 2 *A. cristatellus wileyae* maybe quite different than with 29 *A. cristatellus wileyae*. A last explanation, that *A. ernestwilliamsi* is not truly a separate, allopatric species, but rather an extreme phenotypic variation of *A. cristatellus wileyae*, was not supported by the interspecies study above.

The most interesting possibility, of course, would be if the data are accurate and representative of the genetic similarity. Given the pronounced phenotypic differences noted by Lazell (1983), including size, scalation, and color pattern, this may be another instantiation of the phenomenon of phenotypic change not being paralleled by neutral genetic changed (Lewontin, 1984). Phenotypic differences do not imply neutral genetic differences. The most exciting possibility for these results is that *A. ernestwilliamsi*, as a very recent descendent of *A. cristatellus*, has had insufficient time for the ancestral polymorphisms to be sorted between the species, or for detectable genetic change to occur.

Lineage sorting is the process by which the phylogenetic relationships between genes become the same as the phylogenetic relationships between populations or individuals. When one species becomes two, they share many of the same polymorphisms, and an allele found in one species has closer relation to an allele in another species, rather than another allele within the species; the genes are polyphyletic. Eventually, some allele lineages die out as happens during periods of genetic drift, and genetic monophyly is achieved. This process of lineage sorting is called coalescence. The time to coalescence ($T_c$) requires a number of generations equal to about 4 times the effective population size ($N_e$) to occur (Futuyma, 1998). Using independent estimates of time and population size, $T_c$ and $N_e$ may be calculated. First, the time to coalescence may be

calculated using the *A. ernestwilliamsi* census population estimate of 2,000 to 3,000 individuals, provided by Lazell (1983):

$$Tc = 4Ne$$

$$Tc = 4(2{,}000) \quad Tc = 4(3{,}000)$$

$$Tc = 8{,}000 \quad Tc = 12{,}000$$

The time to coalescence required is 8,000 to 12,000 generations, or about 8,000 to 12,000 years, using 1 year as the generation time (Andrews, 1976). Next, the effective population size, $N_e$, may be estimated based on the time the island of Carrot Rock has been exposed, an upper limit of 3,000 years (Fairbanks, 1989). Assuming that the time now ($T_n$) is less than the time required for coalescence ($T_c$), and substituting into the equation $T_c = 4N_e$:

$$Tn < 4Ne$$

$$3{,}000 < 4Ne$$

$$750 < Ne$$

$$Ne > 750$$

The effective population size of the *A. ernestwilliamsi* population on Carrot Rock is greater than 750, which agrees with the estimate of 2,000 to 3,000 for the population size. It was noteworthy that the effective population size calculated was a lower bound; usually, the available values are upper bounds obtained from census surveys. An effective population size like this, of several hundred individuals, would take many thousands of years to achieve lineage sorting by genetic drift. Thus, both estimates support the theory that *A. ernestwilliamsi* has not yet completed coalescence.

## V. Conclusions

The AFLP technique does not appear to be sufficiently powerful enough to resolve the deep and intermediate branches of the anole phylogenetic tree. It would appear that there exists too great an amount of diversity between distantly related Caribbean anoles. For the population study, the level at which AFLP has been applied most frequently, that lack of structured, well-supported results was itself significant, indicating that gene flow is effective and/or isolation times insufficient for geographical structuring to evolve.

In regards to future work, several recommendations may be made. More primer pairs could be explored, only 7.8% (5 in 64 available primer pairs) having been scored here for the

population study. This may uncover a set of selective nucleotides that produce the set of bands that could resolve the trees; however, based on the current findings, I hesitate encourage further use of AFLP on this project. Another improvement to the subsequent methods would be to use more recently preserved *A. ernestwilliamsi* tissues. These modifications would serve to both verify if *A. ernestwilliamsi* should be placed with support among *A. cristatellus wileyae*, or in a monophyletic position. In further studies, the following additional *A. cristatellus wileyae* locations should be included: Peter Island, Virgin Gorda, Anegada, and the Sage Mountain location of Tortola, British Virgin Islands (BVI). Peter Island is directly north of Carrot Rock, the only location at which *A. ernestwilliamsi* occurs. Virgin Gorda lies near Necker Island, while Anegada is about 13 miles north of Virgin Gorda and the most "remote" BVI island. Sage Mountain on Tortola has an environment significantly more wet than that found on Guana or many of the other islands (Beard, 1949); it is extreme, and as such warrants inclusion. A greater number of locations across the *A. cristatellus wileyae* species range would give a broader perspective, and may uncover a location with geographical population structuring. The additional inclusion of two skinks: *Mabuya macleani* and *Mabuya sloanii*, may also produce interesting results. As aforementioned, these two skinks display the same phenomenon observed with *A. ernestwilliamsi* and *A. cristatellus wileyae*; a uniquely occurring species surrounded by a more widespread close relative. By including the skink, one could compare similar situations in different species.

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**Table 2**. Sample Collection Summary.

| Species | Island Group | Island | Locality | Altitude | Study Individuals |
|---|---|---|---|---|---|
| *Anolis cristatellus wileyae* | BVI* | Norman Island | N/a | N/a | AC7, AC29, AC30 |
| *Anolis cristatellus wileyae* | BVI* | Necker Island | N/a | N/a | AC6, AC16, AC17 |
| *Anolis cristatellus wileyae* | BVI* | Tortola | University Grounds | N/a | AC26, AC27, AC28 |
| *Anolis cristatellus wileyae* | BVI* | Beef Island | N/a | N/a | AC3, AC10 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Hotel Area | 100m | AC2, AC14, AC15 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Whitebay Flat | 0m | AC18, AC19 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Plantation area | 0m | AC23, AC24, AC25 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Red & blue trail confluence | 150m | AC11, AC12, AC13 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Muskmelon Bay | 0m | AC5, AC31, AC32 |
| *Anolis cristatellus wileyae* | BVI* | Guana Island | Longman's Point | 90m | AC8, AC21, AC22 |
| *Anolis stratulus* | BVI* | Guana Island | Hotel | N/a | AS1, AS3 |
| *Anolis pulchellus* | BVI* | Guana Island | Hotel | N/a | AP3 |
| *Anolis pulchellus* | BVI* | Tortola | University Grounds | N/a | AP1 |
| *Anolis leachii* | Bermuda | Bermuda | Barnes' Corners | N/a | AL1 |
| *Anolis leachii* | Bermuda | Somerset Island | Mangrove Bay | N/a | AL3 |
| *Anolis leachii* | Bermuda | Bermuda | Sea Swept Farm | N/a | AL2 |
| *Anolis grahami* | Bermuda | Nonsuch Island | N/a | N/a | AG2 |
| *Anolis grahami* | Bermuda | Bermuda | Barnes' Corners | N/a | AG1 |
| *Anolis extremus* | Bermuda | Somerset Island | Mangrove Bay | N/a | AEX1 |
| *Anolis extremus* | Bermuda | Ireland Island North | Naval Dockyard | N/a | AEX2 |
| *Anolis ernestwilliamsi* | BVI* | Carrot Rock | N/a | n/a | AEW1, AEW1 |

N/a: Not applicable
BVI*: British Virgin Island

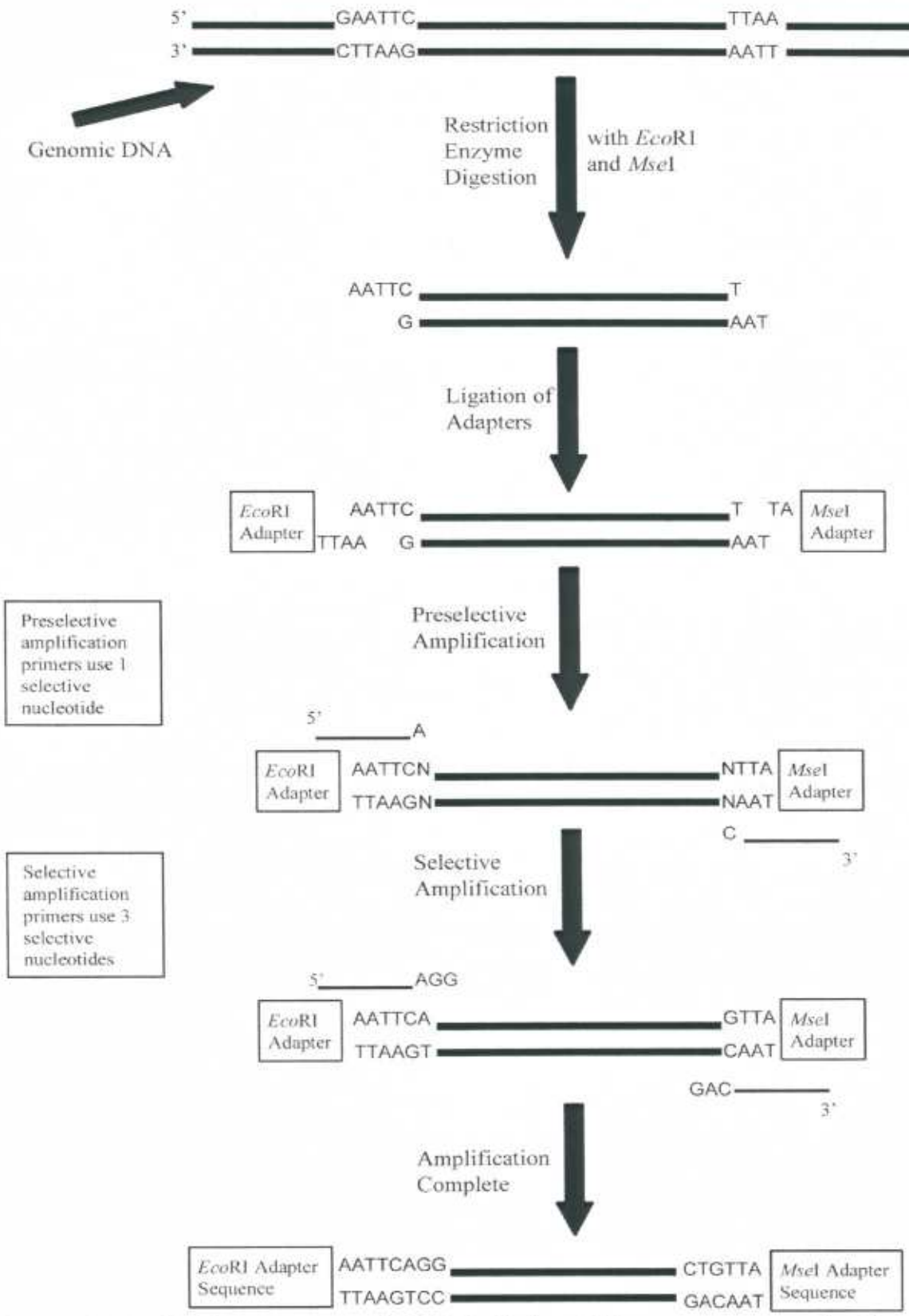


**Figure 3**. Graphic Representation of AFLP Technique. "N" represents any nucleotide.

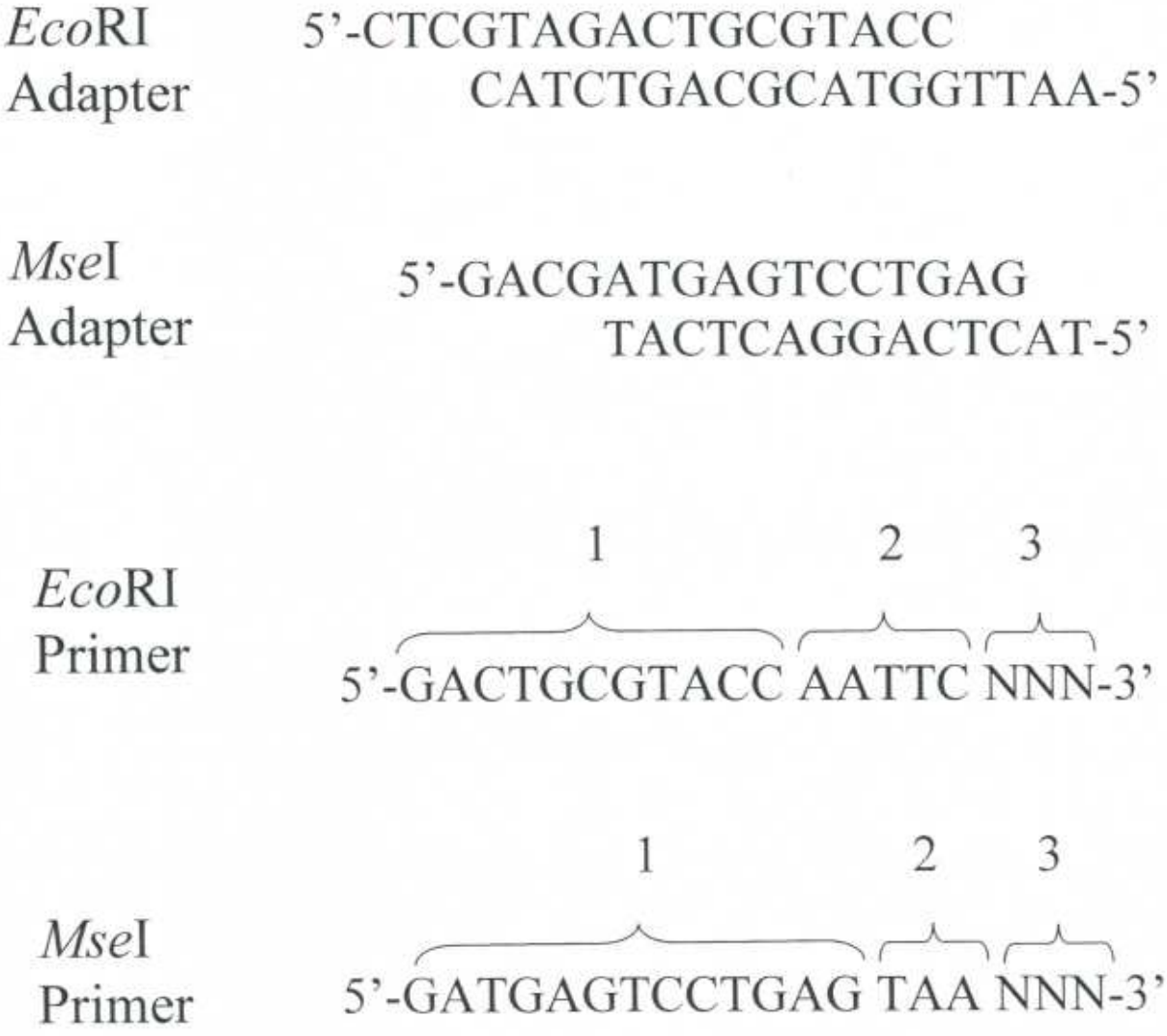


**Figure 4.** Adapter and primer structures for AFLP.
1: The core sequence complementary to the adapter
2: The sequence complementary to the enzyme recognition sequence
3: The selective nucleotide(s). "N" represents a nucleotide. Preselective amplification primers have 1 selective nucleotide, while selective amplification primers have 3 selective nucleotides

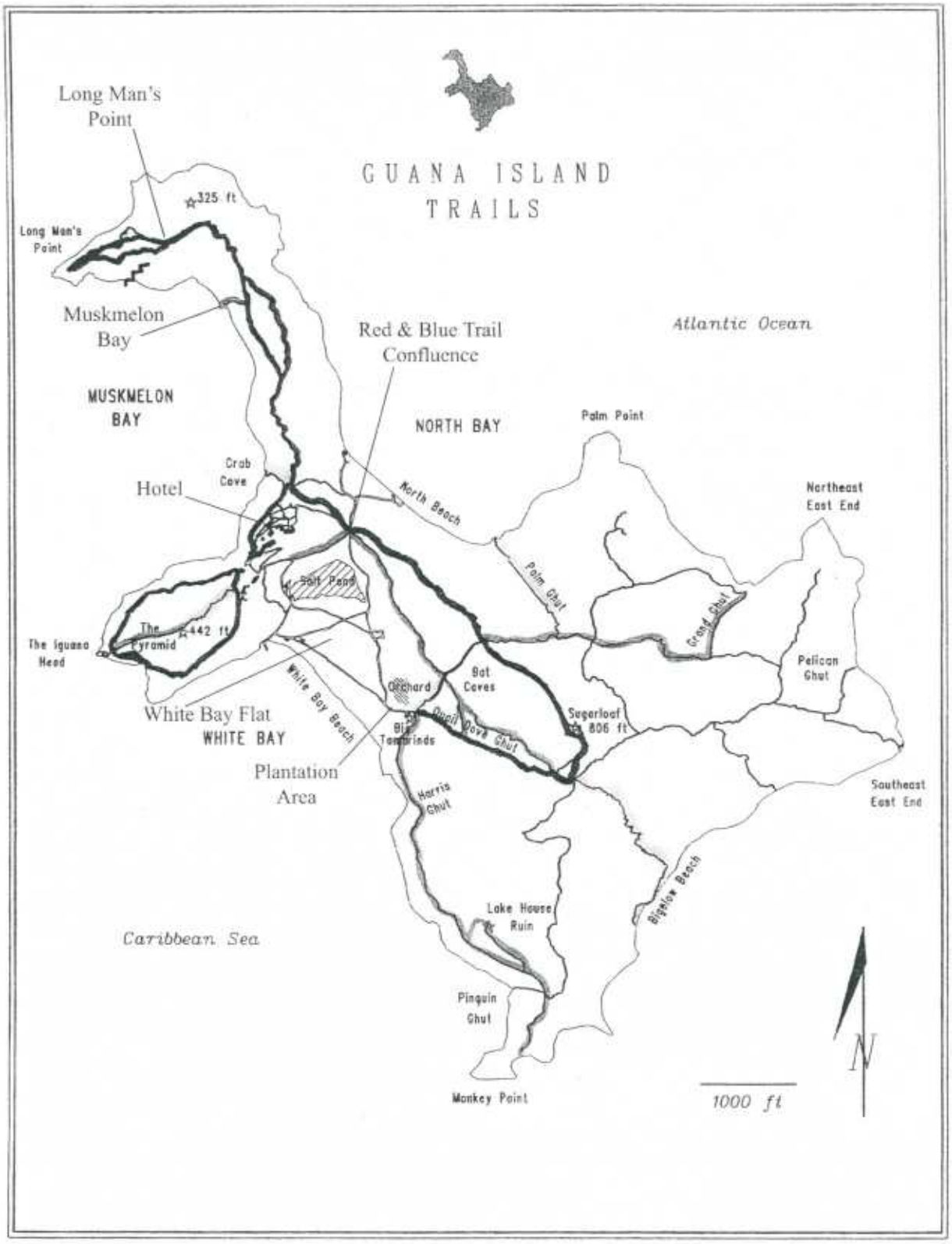


**Figure 5**. Locations of *Anolis cristaellus wileyae* collection sites on Guana Island. See Table 2 for site altitudes. Map provided by Guana Island Resort, Guana Island, British Virgin Islands.

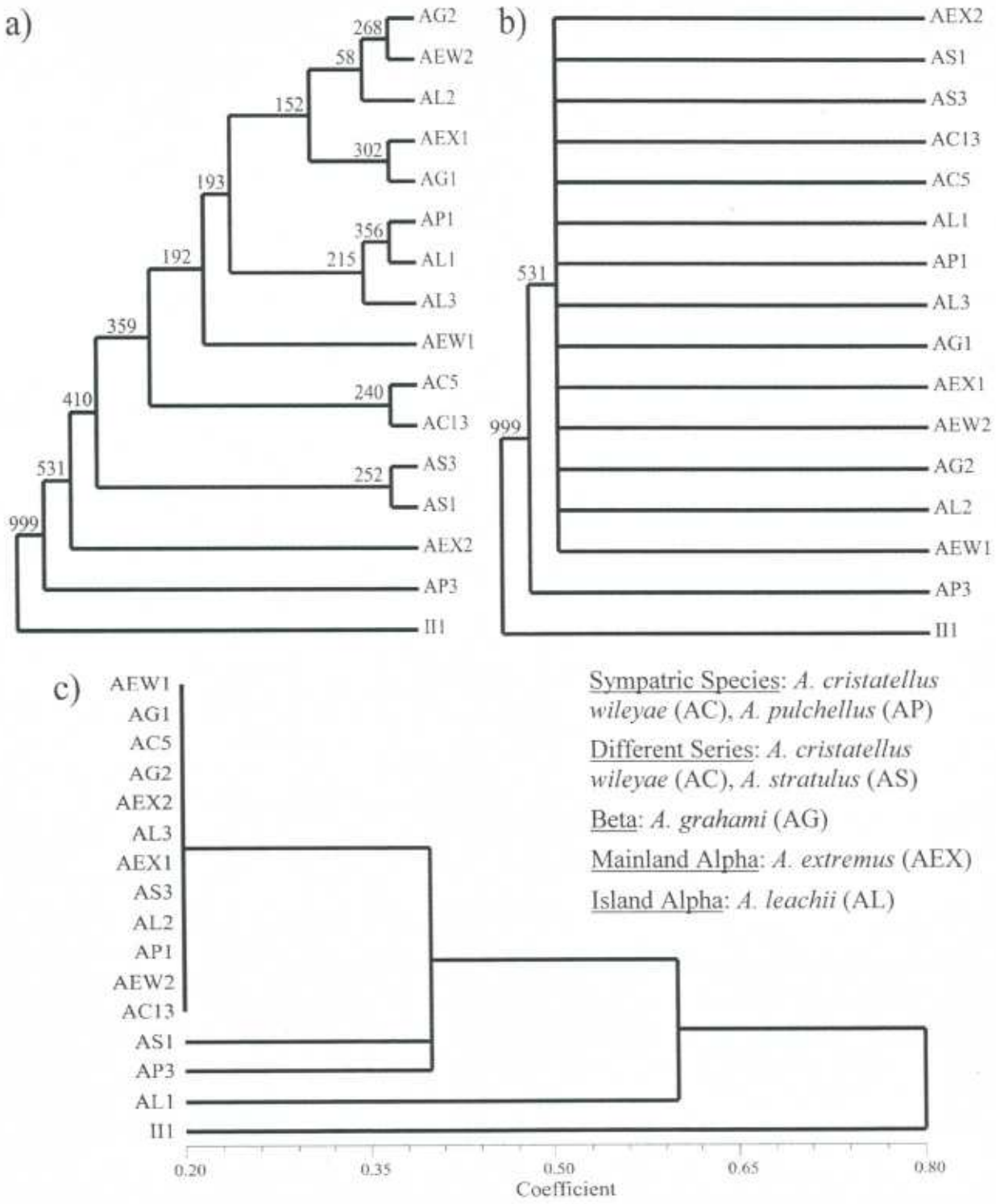


**Figure 7.** Phylogenetic trees based on data from primer pair 21 in the interspecies study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.92. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.21429. Coefficient is a dissimilarity index.

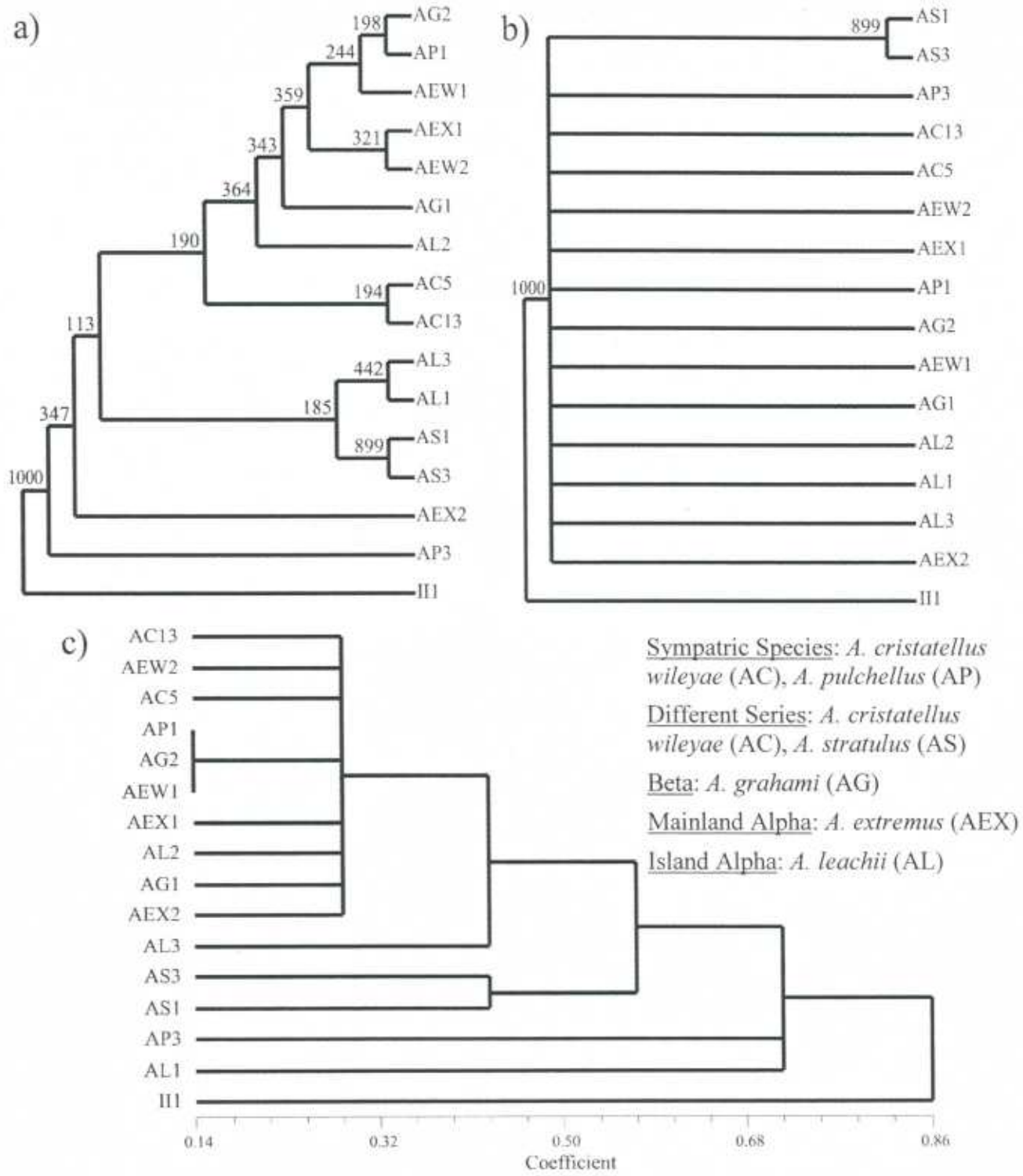


**Figure 8.** Phylogenetic trees based on data from primer pair 29 in the interspecies study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.76. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree with 500 bootstrap replicates. Colless' consensus index ($CI_c$) = 0.42857. Coefficient is a dissimilarity index.

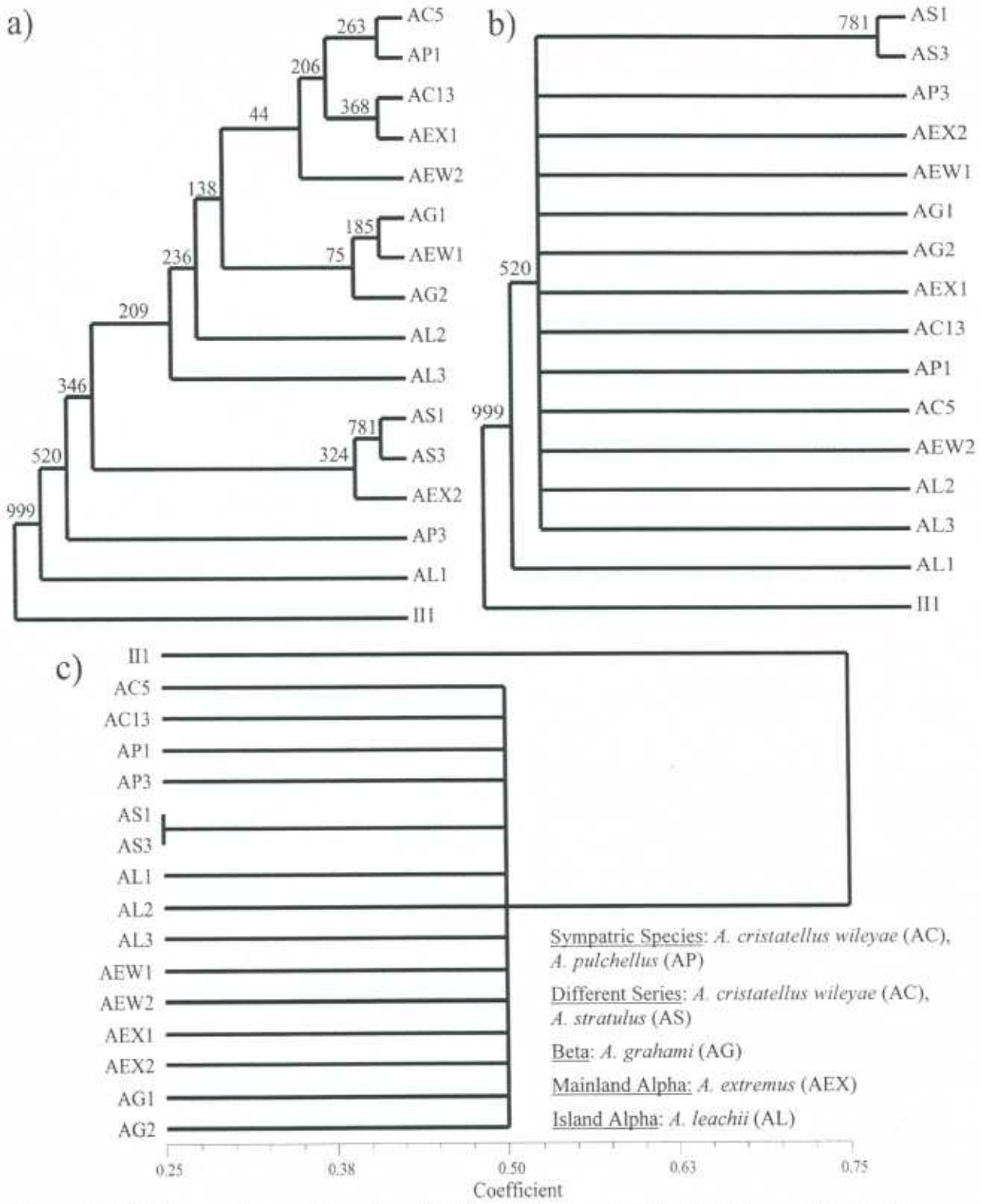


**Figure 10.** Phylogenetic trees based on data from primer pair 15 in the interspecies study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.58. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.14286. Coefficient is a dissimilarity index.

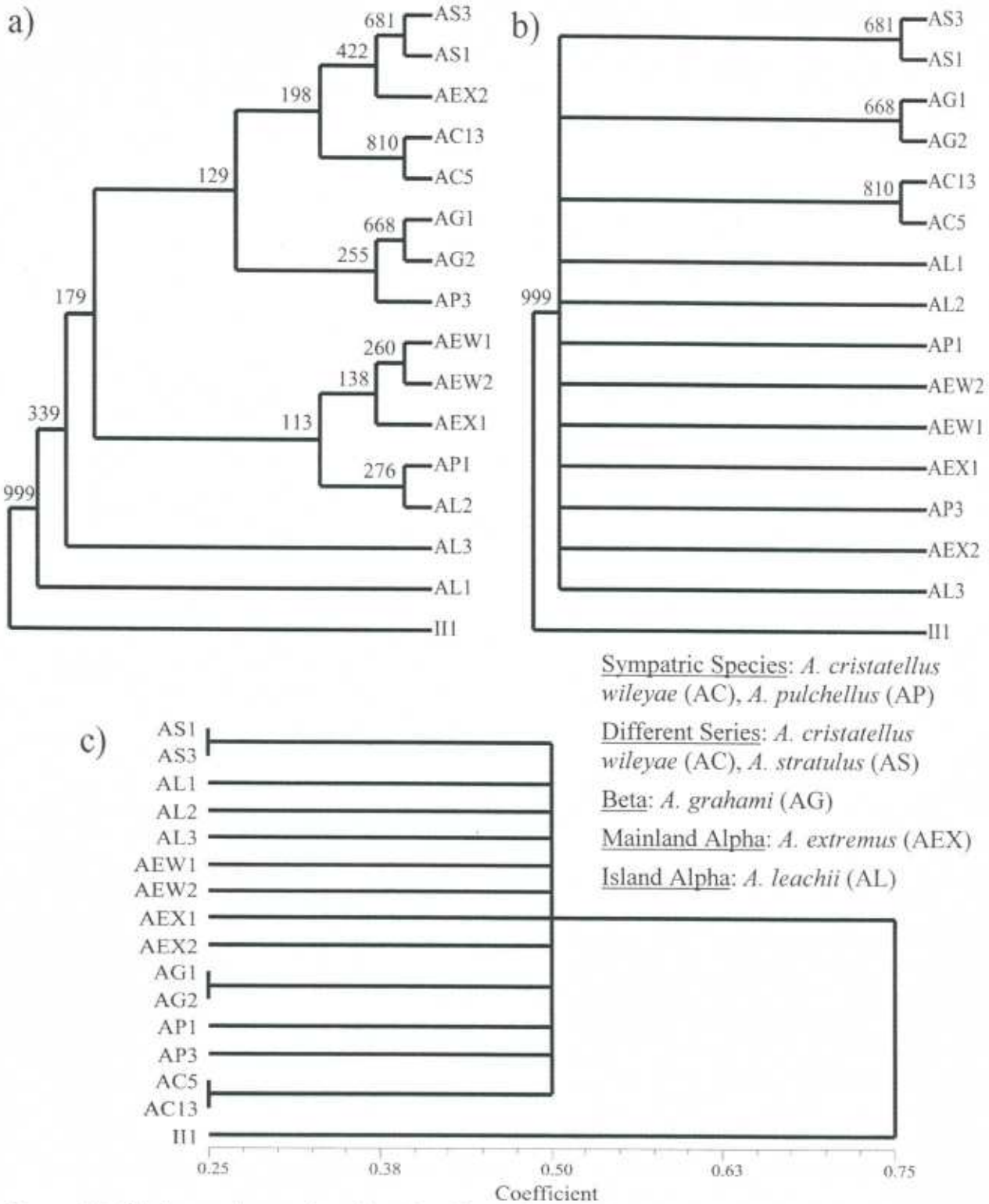


**Figure 12.** Phylogenetic trees based on data from primer pair 37 in the interspecies study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.92. b) Collapsed Dollo parsimony tree showing only the 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.28571. Coefficient is a dissimilarity index.

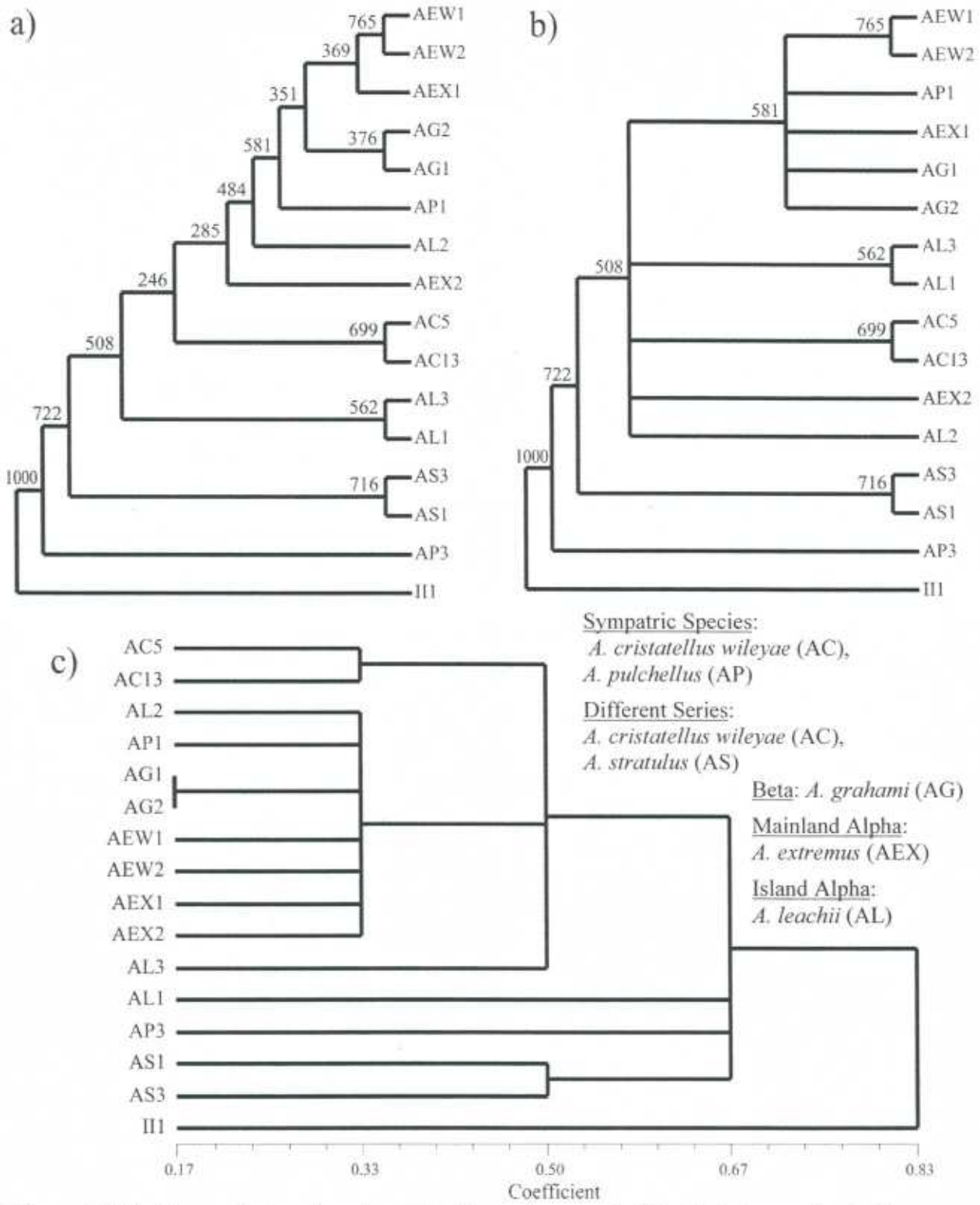


**Figure 14.** Phylogenetic trees based on data from primer pair 43 in the interspecies study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.61. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.42857. Coefficient is a dissimilarity index.

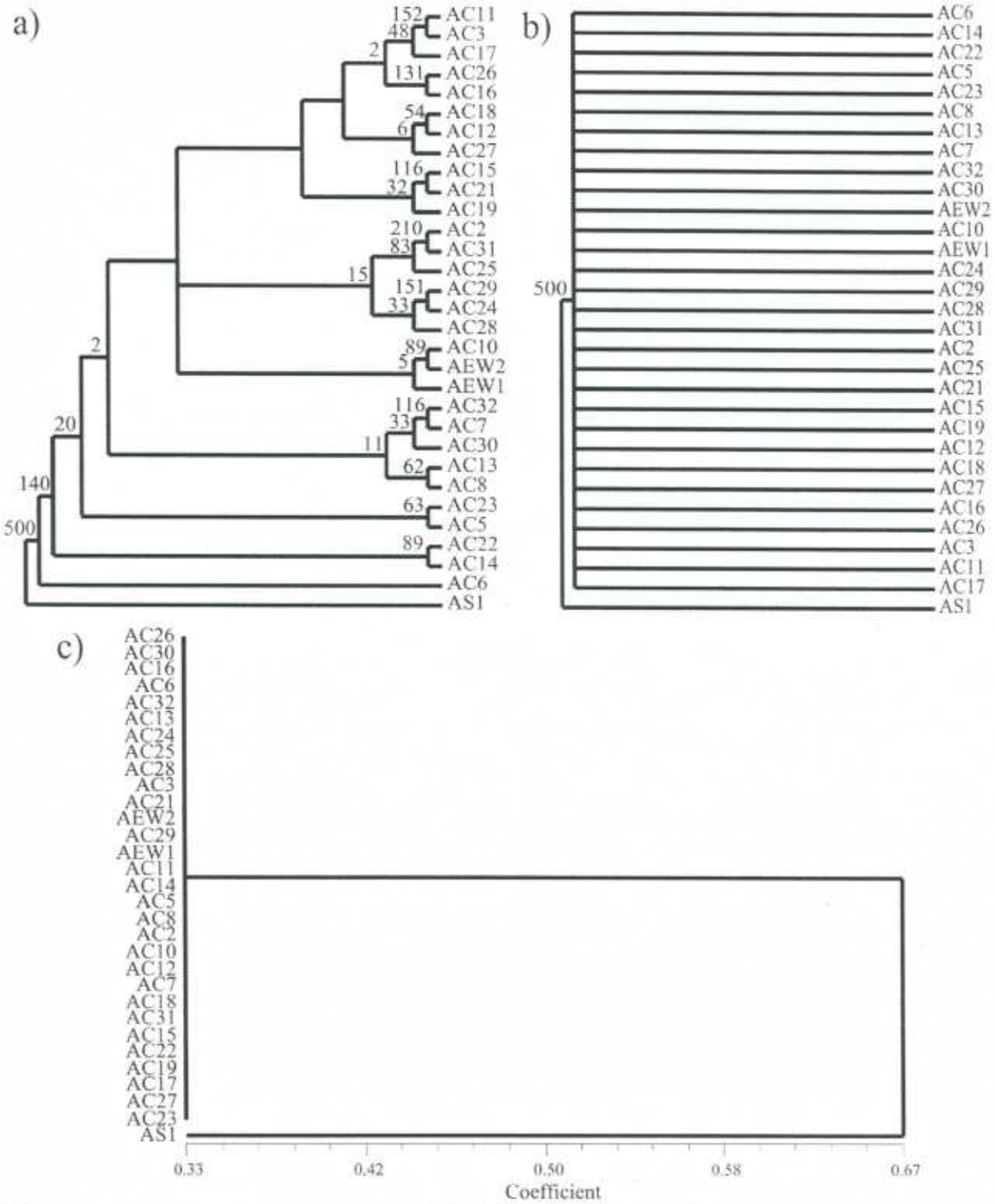


**Figure 16.** Phylogenetic trees based on data from primer pair 34 in the population study. Numbers at the nodes in a) and b) indicate the number of times in 500 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.43. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree using 1,000 bootstrap replicates. Colless' consensus index ($CI_c$) = 0.03448. Coefficient is a dissimilarity index.

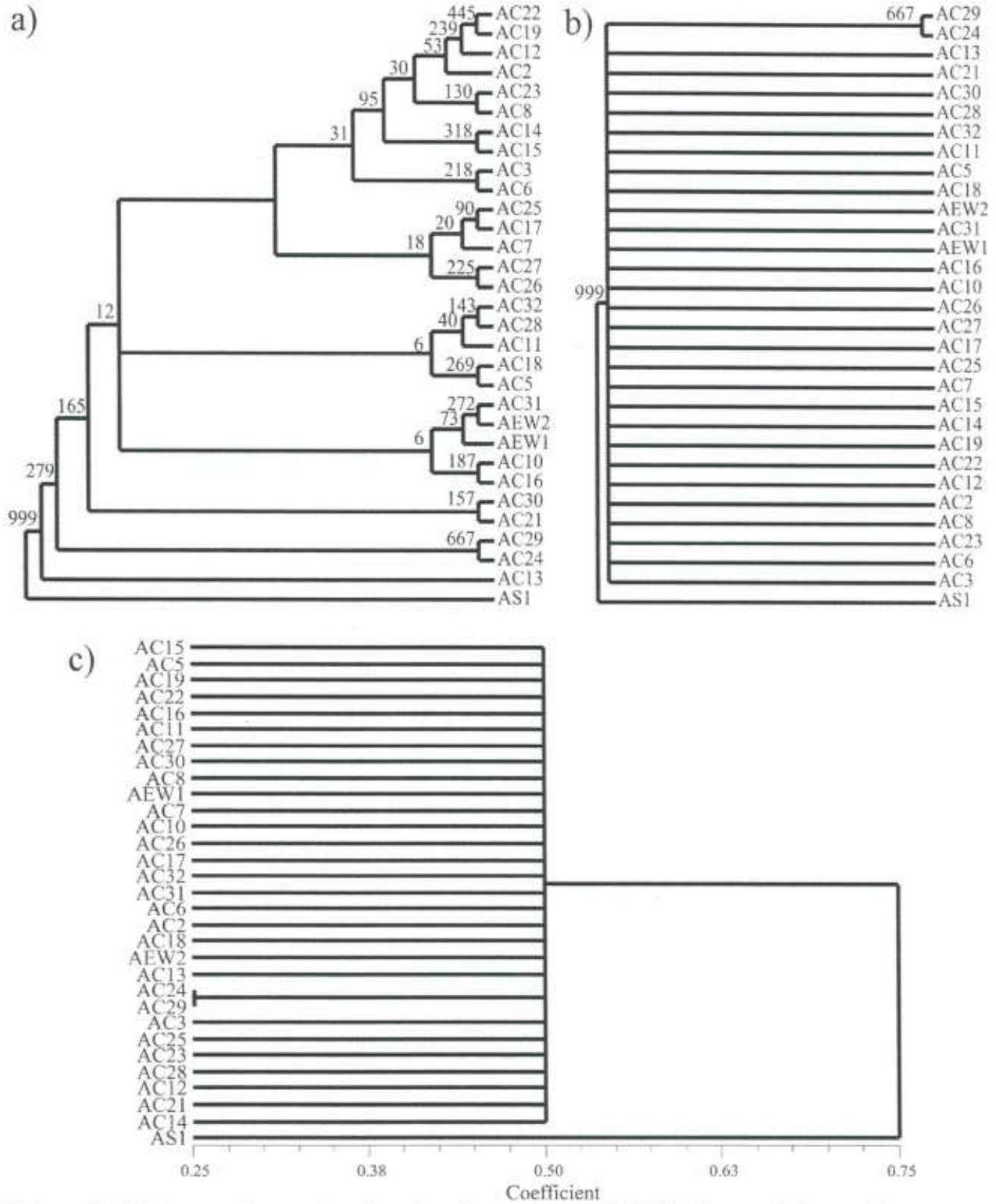


**Figure 18.** Phylogenetic trees based on data from primer pair 22 in the population study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.35. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.06897. Coefficient is a dissimilarity index.

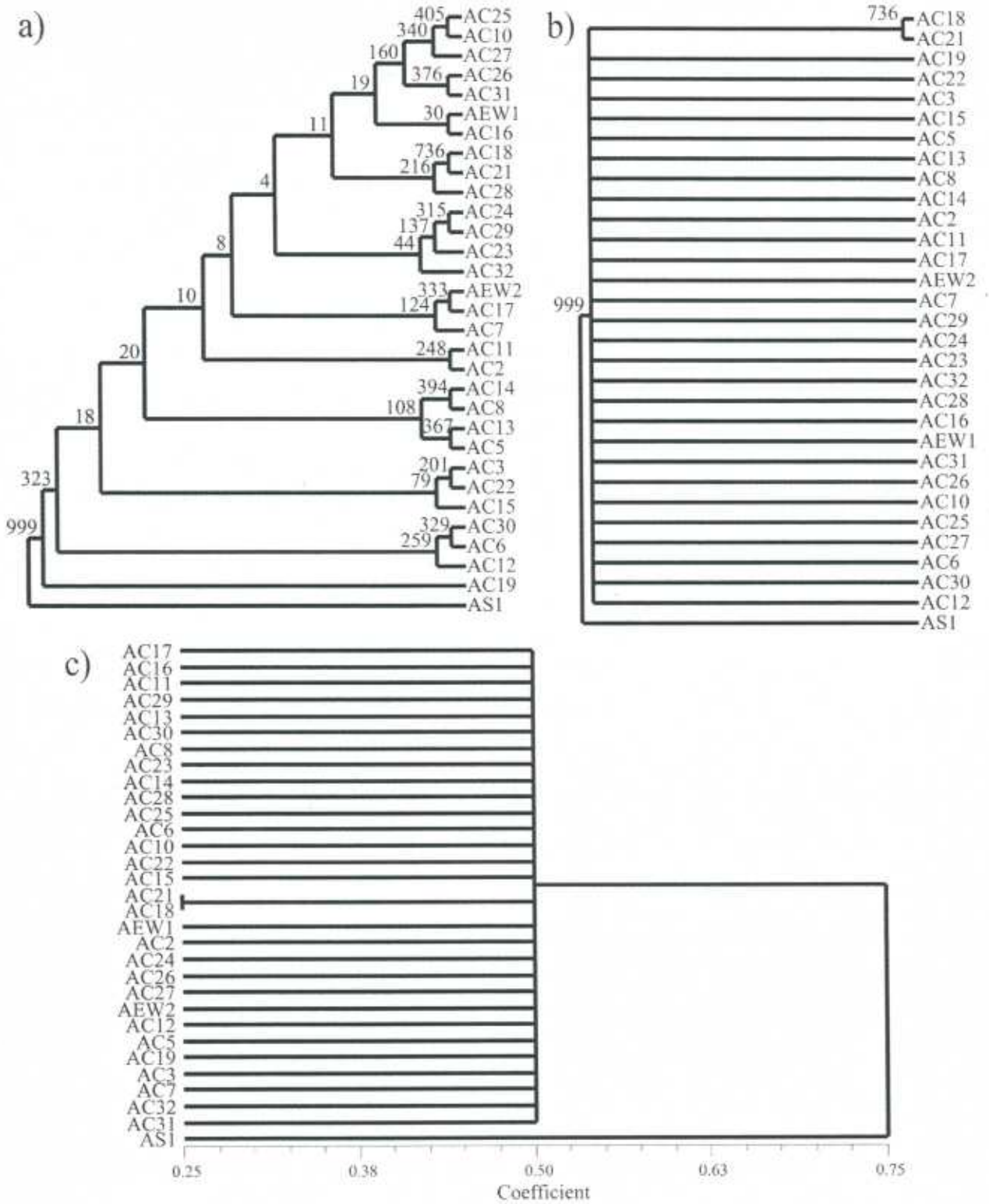


**Figure 20.** Phylogenetic trees based on data from primer pair 64 in the population study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.49. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.06897. Coefficient is a dissimilarity index.

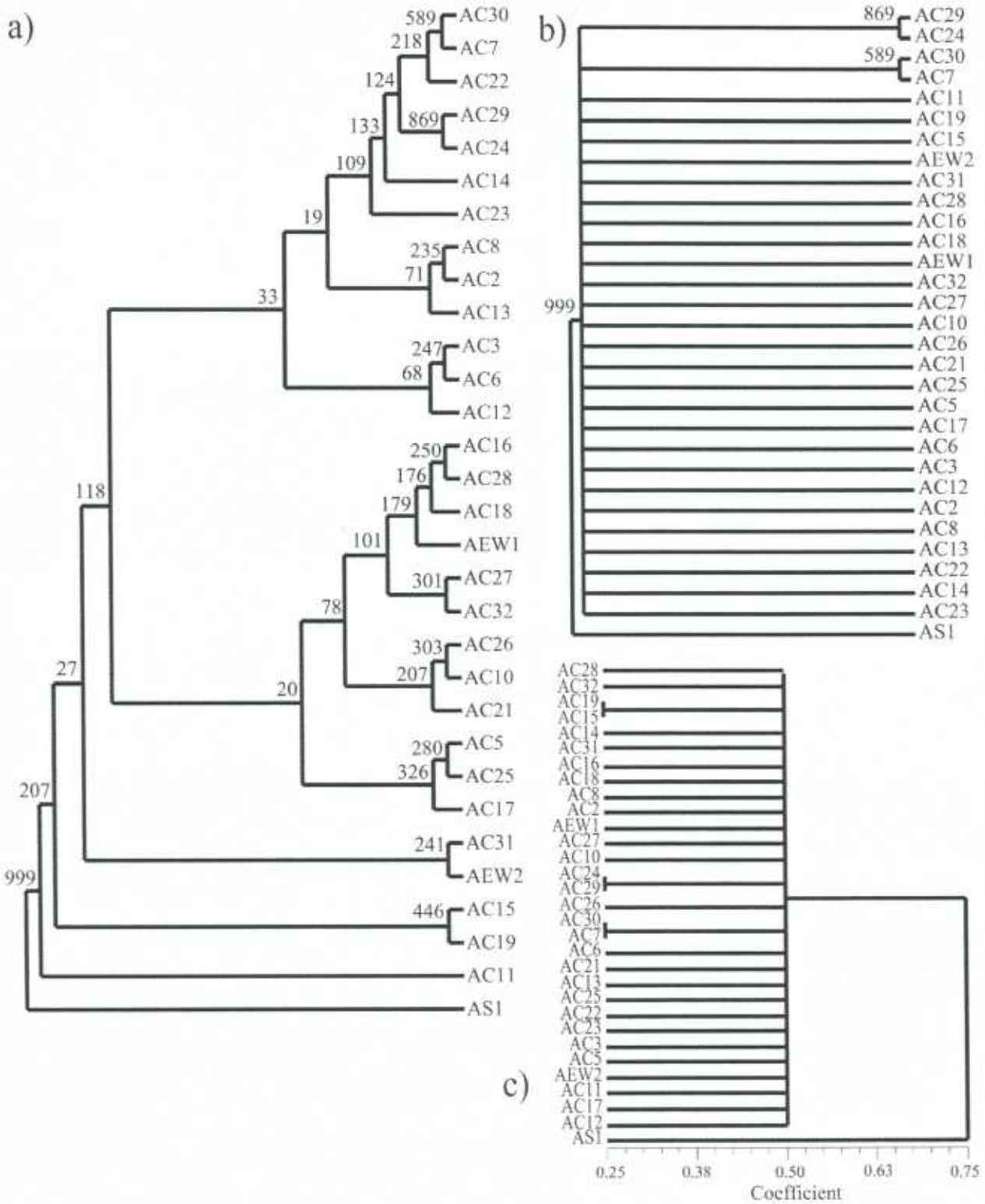


**Figure 22.** Phylogenetic trees based on data from primer pair 47 in the population study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.63. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.13793. Coefficient is a dissimilarity index.

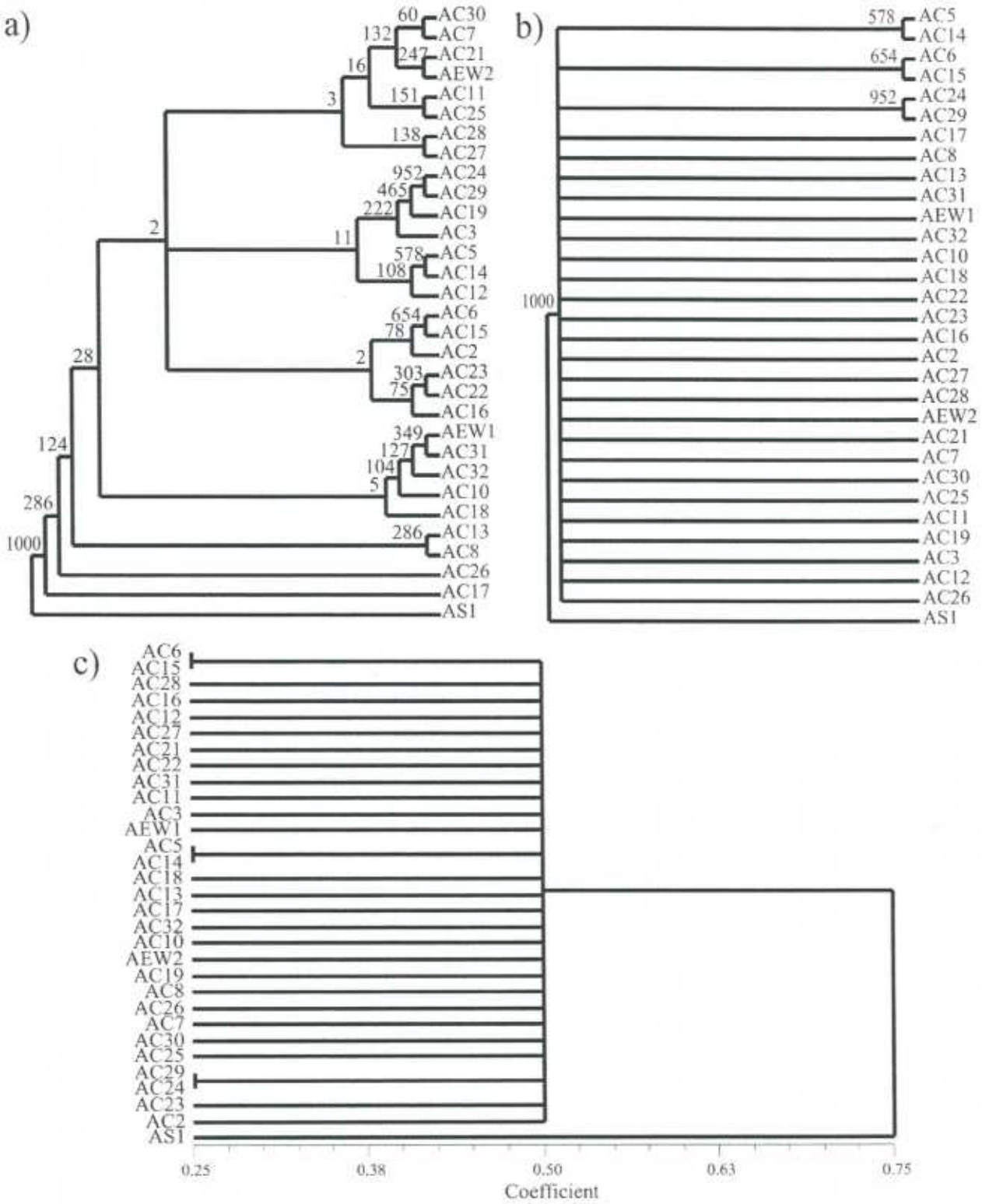


**Figure 24.** Phylogenetic trees based on data from primer pair 25 in the population study. Numbers at the nodes in a) and b) indicate the number of times in 1,000 that arrangement occurred. a) Fully bifurcating Dollo parsimony tree. Consistency Index (CI) = 0.69. b) Collapsed Dollo parsimony tree showing 50% majority rule consensus. c) Neighbor-joining consensus tree. Colless' consensus index ($CI_c$) = 0.13793. Coefficient is a dissimilarity index.

# Ground Lizards of Guana

Ground Lizards in the genus *Ameiva* are widely distributed in the West Indies. About 20 species occur in the Bahamas and in the Antilles from Cuba to Grenada. The species on Guana, *Ameiva exsul*, is found in Puerto Rican lowlands and the Virgin Islands. This is a large species; adult male head-body lengths may exceed 20 cm, although such large individuals have not been observed on Guana.

Ground Lizards are active foragers. Except for short, intermittent periods during which they bask in patches of sunlight, these lizards appear to be constantly on the move. They scratch in the leaf litter or root under surface debris looking for food. They may consume fruit, flowers, or even garbage, but their diets include mostly insects, spiders, other small invertebrates, and smaller lizards. Because Dwarf Geckos, *Sphaerodactylus macrolepis*, are so phenomenally abundant on Guana (densities of up to 67,000 per hectare have been recorded), some Ground Lizards appear to "specialize" in finding and eating these little geckos.

Ground Lizards are most active when conditions are very warm and may be active when surface temperatures hover around 40°C. Conversely, they are quite intolerant of cool conditions and may retreat to self-excavated burrows when temperatures drop or clouds cover the sun. On cool days, they may not emerge at all. Consequently, they frequently exhibit relatively short activity periods, emerging by mid-morning and retreating again by mid-afternoon, sometimes to reappear for short periods early in the evening.

Because Ground Lizards are widespread and often quite abundant in the West Indies, they are important components of island food webs. Not only do they consume a variety of prey, they in turn are eaten by a number of predatory birds and many snakes. As a matter of fact, a large percentage of snakes in the region feed largely or exclusively on lizards.

Despite their near ubiquity in the West Indies, little is known about most species and assumptions about their physiology and ecological relationships are based on relatively few studies of only a few species. Because fundamental biological attributes may vary even among populations of the same species occupying slightly different habitats only a short distance apart, additional investigations into the activity, reproduction, and ecology of these important animals are necessary to better understand West Indian ecosystems.

In October 2003, we initiated a study of Guana's Ground Lizards. Using trails or borders of beachside vegetation as transects through different habitats, we recorded times and frequencies of encounters with individual lizards in an effort to establish rough estimates of population sizes and densities, relative proportions of different size classes (large adult males, smaller adults, juveniles, and hatchlings), associations with specific habitats, and periods of activity.

To date, we have determined that Ground Lizards are found in nearly all habitats on Guana. They are absent only in the cleared flats and the area immediately around the salt pond. Unlike most other West Indian populations, Guana's lizards are not concentrated in patches of "ideal habitat," but are rather sparsely distributed across all acceptable habitats — although encounter rates appeared to be slightly higher in somewhat more open situations where penetration of sunlight through the canopy was more abundant. The highest calculated densities ranged from 33.8 lizards/ha in forested areas to 52.2/ha along White Bay Beach. Using estimates of the relative extent of suitable habitats and extrapolating to the entire island, these data result in an estimated population size of over 8100 animals on Guana. Because all of the individuals in a given area are not active at the same times, these estimates are all undoubtedly very conservative.

Adult:juvenile ratio was 1.24:1, with the large number of young individuals reflecting the recruitment of hatchlings during the current reproductive season. Because mortality of juveniles is probably much higher than that of adults, sampling during different times of the year probably would result in other ratios and different density and population size estimates.

We encountered by far the most Ground Lizards from 0930–1400 h, although one lizard was active at 0708 h on an east-facing slope and a few were foraging as late as 1630 h. Because we did not mark each animal and only a few were sufficiently distinctive to allow recognition, we could not determine the activity periods of individuals.

Now that we have a feel for where Guana's Ground Lizards live and what they do, we anticipate that the next stages in our investigation will involve individual mark and resighting data and examinations of activity and numbers during different times of the year. The latter also would allow us to evaluate seasonal reproductive activity and possible dietary shifts as lizards respond to the varying abundance of prey species or of fruits and flowers.

**Robert Powell**, Avila University (Kansas City, Missouri) and **Robert W. Henderson**, Milwaukee Public Museum, examining lizard-eating snakes (*Alsophis portoricensis*) on Guana.

***AMPHISBAENA FENESTRATA*** (Virgin Islands Amphisbaena). **PREDATION**. Relatively little is known about the biology of many fossorial reptiles and amphibians. For example, almost nothing is known about the predators of any species of *Amphisbaena*. Here we report, for the first time, two cases of predation on *A. fenestrata* observed on Guana Island, British Virgin Islands. In both cases, predation was by the locally abundant colubrid snake *Alsophis portoricensis*.

On 7 October 2001 at 1000 h (air temp. 27.2°C), we captured a male *Alsophis portoricensis* (520 mm SVL, 285 mm TL, mass 52.3 g). It was encountered at an elevation of 160 m near a forest trail on a substrate of dry leaves and small pebbles. The snake was placed in a cloth bag and ca. 30 min later regurgitated a freshly eaten adult *A. fenestrata* (210 mm SVL, 15 mm TL). The specimen was in good condition and was deposited at the Yale Peabody Museum (accession number YPM 12060).

A second predation event was recorded on videotape by Troy Peliwan on 20 October 2001. Around 1800 h, he encountered an *A. portoricensis* attempting to capture and subdue an adult *A. fenestrata*. The snake was first observed on a concrete floor. When disturbed, it moved into the nearby bushes, dragging the *Amphisbaena* with it. Swallowing continued until the snake disappeared, with the prey still in its mouth ca. 10 min later. Both predator and prey were identified from the videotape by GP.

*Alsophis portoricensis* is known to prey primarily on lizards, though other small vertebrates are also commonly taken (Henderson and Sajdak 1999. *In* Powell and Henderson [eds.], Contributions to West Indian Herpetology: a Tribute to Albert Schwartz, pp. 327–338. Soc. Study Amphib. Rept., Ithaca, New York, 457 pp.). However this is the first record of amphisbaenians in its diet.

We thank J. Lazell and R. Henderson for discussions of these events and the staff of Guana Island for technical assistance. Financial support was provided by The Conservation Agency through a grant from the Falconwood Foundation.

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# Guana Island Racers

Two species of snakes are known to occur on Guana Island. The larger and most common of the two is in the genus *Alsophis*. Eleven species of these snakes, often called "Racers," occur on about 100 islands in the West Indies, ranging from the Bahamas southward to Dominica in the Lesser Antilles. Only Hispaniola harbors two species of *Alsophis*; all others islands support a single species.

The species that occurs on Guana is *Alsophis portoricensis*; it is found on about 40 islands on the Puerto Rico Bank (= Puerto Rico, the U.S. Virgin Islands, and the British Virgin Islands), more islands than any other *Alsophis*. Considering the number of species, and how geographically widespread these snakes are in the West Indies, we know surprisingly little about their natural history.

On those West Indian islands (e.g., Antigua, St. Martin) where mongooses were introduced in the 19th century (usually in order to control rats that were damaging sugarcane crops), Racer populations have been eliminated or severely reduced. Today, some species of *Alsophis* are considered to be among the world's most endangered snakes. Fortunately, the mongoose is not present on Guana, and the Racer population appears to be very healthy.

On Guana, Racers occur just about everywhere, including walkways around the resort buildings, the dining area, and the beach. Activity may start as early as 0700 h, and it usually tapers off before dusk. Occasionally, however, these diurnal snakes are observed active after dark as they hunt diurnal lizards that are attracted to the insects that are, in turn, attracted to the ground-level lights along the paved walkways. Guana is the first place where documentation of a secondary predator (the snake) successfully exploiting the so-called "night-light niche" has occurred.

These slender, fast-moving, largely ground-dwelling snakes feed on a wide range of vertebrate prey, mostly Whistling Frogs (*Eleutherodactylus*) and lizards. Dwarf Geckos (*Sphaerodactylus*), Anoles (*Anolis*), and Ground Lizards (*Ameiva*) are consumed most frequently, but large Racers also may eat an occasional baby Iguana (*Cyclura*). Largely active foragers, the snakes move slowly and silently through Sea Grape (*Coccoloba uvifera*) litter near the beach or leaf litter along the Iguana Trail, around rocks and boulders in Quail Dove Ghut, or among decorative plants around the resort. When a lizard is captured, the snake usually works it quickly to the back of its mouth where enlarged teeth in the upper jaws puncture the lizard's skin and allow a weak venom to enter the victim's blood. Once subdued, the prey is swallowed whole (snakes are unable to bite off and chew small pieces) and usually head first.

Guana's Racers are essentially harmless to humans. They generally flee when encountered and bite only when handled. However, some individuals may act ferocious and spread their "hoods" like a cobra when threatened.

During October 2003, we explored the potential for conducting long-term research on the ecology of Racers on Guana. We were pleased to find the snakes ecologically widespread and common on the island, where we averaged about eight snake encounters per day. We sexed, weighed, and measured 38 snakes before placing a microchip (about the size of a long grain of rice) under the skin and releasing them at the exact site of capture. The microchips provide a means of identifying individual snakes, and, based on recaptures, we will accumulate information on growth, movements, habitat use, longevity in the wild, and survivorship. Perhaps we will even learn why, of the 38 snakes we captured, 31 (81.6%) had incomplete tails. Are the hermit crabs pinching them off? Several snakes had mere stubs of tails, and a few had healed scars resulting from what appeared to be quite severe injuries on their bodies.

During the very short period we spent on Guana, we encountered only a few of our marked snakes. Most had moved only very small distances during that short time period, but one moved well over 300 m in just a few days.

Visitors to Guana should take time to admire and enjoy the subtle beauty of the island's Racers, and appreciate the grace and speed with which these limbless creatures flow, seemingly without effort, across the ground, over a rock wall, or up a tree. Respect the healthy snake populations on Guana and do whatever is necessary to keep them that way.

**Robert W. Henderson**, Milwaukee Public Museum, and **Robert Powell**, Avila University (Kansas City, Missouri) examining Racers (*Alsophis portoricensis*) on Guana.

# Snaking around

◆ Eva Baskin holds a racer snake after a lecture on snakes and lizards at the H. Lavity Stoutt Community College on Wednesday. The racer came from Guana Island, where U.S.-based researchers have implanted microchips into 29 snakes. The microchips will allow researchers to identify the captured snakes so they can better assess how the snakes grow and change over time. "They're like bar codes for snakes," Bob Henderson, a researcher with the Milwaukee Public Museum, explained. Henderson and Bob Powell, of Avila University, Missouri, were in the territory last week performing research on Guana Island. Powell studies lizards. The HLSCC Lecture Series continues on Friday with the annual Guana Island Science Symposium, where researchers and students who spent part of the summer conducting research on Guana will deliver presentations on what they learned. Topics to be discussed this year include raptors of the BVI, invader plants, and lizards of the BVI and distress calling. The symposium begins at 5 p.m. Refreshments will be served. (Photo by SUSANNA HENIGHAN)

# GEOGRAPHIC DISTRIBUTION

***ELAPHE GUTTATA GUTTATA*** (Corn Snake). USA: US VIRGIN ISLANDS: St. Thomas: Crown Bay Cargo Port Area (18°20.29'N, 64°56.84'W). 3 October 1999 and two undated specimens from the 1990s. Judy Pierce and Donna Griffin. Both specimens verified by Jose Rosado. The dated specimen, an adult female, 66 cm SVL, 80 cm TL, was found at the industrial park near Victor's Hideout restaurant, ca. 600 m W of the Crown Bay cargo port area. It is preserved in the collection of the US Virgin Islands Division of Fish and Wildlife (address below). The two undated specimens are at the Museum of Comparative Zoology. MCZ 183544 is an adult male, SVL 83 cm, TL 103 cm; MCZ 183545 is a juvenile, SVL 31 cm, TL 37 cm.

Circumstantial evidence suggests these specimens might have arrived in cargo containers originating in Florida, a pattern which is consistent with other reports of introduced reptiles and amphibians in the Caribbean (e.g., Powell 2002. Herpetol. Rev. 33:321). Repeated sightings, as well as the capture of juveniles at the site, suggest a nascent population might be in the process of emerging. This is a first documented record for this part of the Caribbean. Previous published records include Antigua (Powell and Henderson 2003. Herpetol. Rev., *in press*), Anguilla (Hodge et al. 2003. The Reptiles and Amphibians of Anguilla, British West Indies. Anguilla National Trust, The Valley), and St. Barts (Breuil 2002. Patrimones Naturels 54:1–339). In addition, although no specimens are available, corn snakes have also been reported from Curaçao and Bonaire. These too might have arrived from Florida, and juveniles have been reported on Curaçao as well (Gerard van Buurt, unpubl. obs.). If the presence of juveniles indicates local reproduction, this is a source of conservation concern because, similar to the invasive brown treesnake (*Boiga irregularis*; Rodda et al. 1999. Problem Snake Management: the Habu and the Brown Treesnake. Cornell University Press, Ithaca, New York. 534 pp.), *E. g. guttata* has a generalized vertebrate diet. What effect the presence of introduced Indian mongooses in St. Thomas (Horst et al. 2001. *In* Woods and Sergile [eds.], Biogeography of the West Indies: Patterns and Perspectives, pp. 409–424. CRC Press, Boca Raton, Florida) might have on the future and impact of the species remains to be seen.

We thank Kate LeVering and Robert Henderson for critically reading the manuscript. Financial support was provided by The Conservation Agency through a grant from the Falconwood Foundation. This is manuscript T–9–973 of the College of Agricultural Sciences and Natural Resources, Texas Tech University.

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**Male and female *E. antillensis* after oviposition in leaf litter**

***ELEUTHERODACTYLUS ANTILLENSIS*** (Coquí Churí). **REPRODUCTION**. *Eleutherodactylus antillensis* is a widespread and abundant species in Puerto Rico and adjacent islands on the Puerto Rico Bank, but no published records of eggs and nests exist from within the species' natural range. Here we report on 8 clutches found in nature, 7 on Guana Island, British Virgin Islands (23 Oct 1993, 17 and 20 Oct 1994, 12 Oct 1997, 14 and 23 Oct 1999, 23 Oct 2001; observed by KO and J. Caldbeck) and 1 in Puerto Rico (on 10 Aug 2002, observed by ARE). Like other species of *Eleutherodactylus*, this species has direct development and eggs are laid on land.

On Guana Island, the egg masses were on the ground in a shrub forest: 6 were under a thin (ca. 2 cm deep) layer of leaf litter, and 1 was under a grass mat. The clutch size of 6 newly laid egg masses (located by following courting pairs) ranged from 25 to 42 eggs (mean = 31, SD = 5.8); the remaining clutch (found by raking of leaves) contained 9 eggs. Newly laid eggs were round, opaque white, and were laid in a globular cluster. Individual eggs measured ca. 4–4.5 mm in diameter. The development of one clutch, followed from oviposition to hatching, took 15 days at temperatures of ca. 25–30C° at the natural location. No frog was in attendance at any of these clutches during several day- and night-time inspections.

In Puerto Rico, a clutch of *E. antillensis* with 24 eggs was found near Tetas de Cayey, Barrio Cuyón, Salinas (825 m elev). Individual eggs measured ca. 3.2–4.0 mm in diameter. The eggs were in an advanced stage of development (Stage 7 or later; Townsend and Stewart 1985. Copeia 1985:423–436), based on strong, rapid movements of the embryos. The clutch was under a small wood block (20 x 10 x 5 cm) over reddish soil, clean of vegetation, and near a house in construction. An adult male *E. antillensis* was adjacent to and in physical contact with the egg mass. The clutch and frog were held captive in a small plastic terrarium, with soil taken from the site as substrate, and were maintained at 28–32°C with relative humidity of 75–87%. The male frog sheltered under the same piece of bark where the clutch was placed but did not crouch on top of the eggs, as do males of *E. coqui* when attending eggs. Nine to 11 days after collection, the eggs hatched. The froglets measured 4–5 mm SVL. All froglets had a bicuspid egg tooth on the median margin of the upper lip; no vestigial tail was present at hatching.

Our observations indicate two features of particular interest: oviposition on the ground, rather than in vegetation or arboreal locations, and apparent absence of parental care on Guana Island. In contrast, male parental care of eggs occurs in *E. coqui* and several other Puerto Rican species of *Eleutherodactylus* (Townsend 1996. *In* Powell and Henderson [eds], Contributions to West Indian Herpetology: a Tribute to Albert Schwartz, pp. 229–239. Society for the Study of Amphibians and Reptiles. Ithaca, New York). Our observations also suggest a clutch size that is somewhat larger than reported for *E. antillensis* in captivity (11–32 eggs; Michael 1997. Herpetol. Rev. 28:141–143; Joglar 1998. Los Coquís de Puerto Rico. Su Historia Natural y Conservación. Editorial De La Universidad De Puerto Rico, San Juan, Puerto Rico) and for an introduced population within residential gardens in Panama City (11–28 eggs; mean = 19 eggs; Castillo and Mayorga 1984. Distribucion, habitos ecologicos, reproduccion y embriologia externa de *Eleutherodactylus antillensis* [Anura]. Unpubl. Thesis. Universidad de Panama [Facultad de Ciencias Naturales y Farmacia], Panama City, Panama). Whether the presence of a male frog in the vicinity of the clutch from Puerto Rico represented parental care remains enigmatic.

Support for studies on Guana Island came from the Conservation Agency through grants from the Falconwood Foundation. We thank Skip Lazell for invaluable support and Jeannine Caldbeck for assistance in the field. ARE was supported by NSF/MIE Project at Universidad Metropolitana, San Juan, Puerto Rico.

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**Wenhua Lu**

**From:** "Caitlin O'Connell-Rodwell" <ceoconnell@stanford.edu>
**To:** "Wenhua Lu" <wenhua@etal.uri.edu>
**Sent:** Tuesday, April 06, 2004 1:44 AM
**Attach:** mating1.mpg
**Subject:** the incriminating evidence

Hi Skip,

Just thought I'd send along the incriminating video clip of #22 jumping off of #9 postcopulation. The study went extremely well, whether it was the right season or several years exposure to the decoys or both, the birds displayed significantly more after decoy setup than before as was hoped, but way more than they did in July 2002, culminating in a copulation event during the last watch. Don't know if an egg will get laid, but that would be really exciting. It's going to be fun to write this one up! We also made it over to Anegada with Raymond Walker to check out the situation there and got to see the beginnings of 17 nests being built and saw the one surviving juvenile from the previous season. We gave them a data form that would provide us with much better information about the population. Hopefully it won't be too difficult to get this information. Raymond was very positive and interested in improving their monitoring, so we'll see.

Thanks so much for the check. Hope all is well there and look forward to catching up one of these days soon.

All the best to you and Wenhua,

Caitlin

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U.S. GEOLOGICAL SURVEY
BIOLOGICAL RESOURCES DIVISION

7 January 2004

Dr. Skip Lazell
The Conservation Agency
P.O. Box 86
Lane, OK 74555

Dear Skip:

Enclosed are three hard copies of the project report for "Ornithological Monitoring and Research on Guana Island, British Virgin Islands". I have added a few images of birds. I think the hooded warbler and yellow-throated vireo images are nice but, unfortunately, most of my pictures were not well lit. I will have to be more exacting in getting good photos next year. Also, I am emailing a pdf file of this report as well. I will send it to the email Wenhua gave me.

On another note, I emailed an excel spreadsheet to Wenhua that had the weather data she requested. If it did not get there, please let me know and I will mail a floppy disk with it.

Happy New Year

Clint Boal
Assistant Unit Leader - Wildlife

# ORNITHOLOGICAL MONITORING AND RESEARCH ON GUANA ISLAND, BRITISH VIRGIN ISLANDS

## PROJECT REPORT FROM THE OCTOBER 2003 FIELD SESSION

**Dr. Clint W. Boal**
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**Texas Tech University, Lubbock, TX 79409-2120**

6 January 2004

**Worm-eating Warbler (*Helmitheros vermivora*)**

# ORNITHOLOGICAL MONITORING AND RESEARCH ON GUANA ISLAND, BRITISH VIRGIN ISLANDS: PROJECT REPORT FROM THE OCTOBER 2003 FIELD SESSION

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

## INTRODUCTION

In March 2003 I was invited to join the team of scientists conducting research on Guana Island. I had conducted a limited project in the Luquillo Experimental Forest, Puerto Rico, in January 1998 (Boal et al. 2003), and had been looking for an opportunity to develop a research program in the Caribbean. Thus, the invitation was a welcome opportunity for me. I approached the invitation with the objectives of 1) gaining familiarity with the geography, vegetative communities, and logistical constraints of working on the island, 2) continuing the long-term mist-netting operations to monitor neotropical migrant bird use of the island as a stopover location during autumnal migrations, and 3) identifying research opportunities and needs for which I could develop subsequent studies.

## RESULTS

### *Island Familiarization*

We arrived on Guana Island, British Virgin Islands at mid-day of 10 October 2003 and departed on the morning of 22 October 2003. We spent much of the first two days familiarizing ourselves with the geography and vegetative characteristics of the

islands, and identification of locations to set up mist nets. Fred Sibley was a great help in this period of familiarization.

We continued familiarization of the island and conducted cursory surveys for avian species during the mid-day hours when mist-nets were closed. These surveys included the Pyramid Trail, the trail to Long Man's Point, Guanaberry Trail, Liao Wei Ping Trail, the lower reaches of Quail Dove Ghut, the North Beach area, and repeated routes through the Salt Pond and adjacent flats to and including the Orchard. During these surveys we gained an appreciation for the ruggedness and difficulty of getting around in many parts of the island. We were struck by the variety in physiographic characteristics, with obvious microclimate differences related to elevation, slope orientation, and landscape type (i.e., drainages, hillsides, flats). Also, we noticed the variability in understory development, apparently due to presence or absence of sheep grazing. This variability in vegetation communities, microclimates, understory development, etc., leads to some pertinent research questions discussed below.

***Bird Monitoring Operations***

We initiated bird-banding efforts on 12 October and completed banding efforts on the afternoon of 20 October. We conducted passive mist-netting for songbirds over an eight-day period. We also conducted targeted mist-netting for shorebirds at the Salt Pond one afternoon. Our mist netting efforts totaled approximately 184.5 net hours (Table 1). Coincidentally, during our mist-netting efforts we made 185 captures of birds, for an average of 1 bird per net hour (Table 1). The majority of these were new captures that we banded (120) or did not band (26). The majority of the birds that were captured but

not banded were hummingbirds (17) for which we did not have bands. The 146 individual birds that were new captures represented 25 species (Table 2).

We recaptured 39 individual birds representing seven species. The majority of these were Bananaquits (*Coereba flaveola*; 27) and Black-faced Grassquits (*Tiaris bicolor*; 5) (Table 3). Two of these recaptures were of Caribbean Elaenias (*Elaenia martinica*), one of which was banded seven years ago (F. Sibley, pers. comm.). We are attempting to determine if this is an age record for the species. One recaptured Black-faced Grassquit was five years old (F. Sibley, pers. comm.).

In general, mist-netting and surveys indicated 2003 was a good year for migrants on Guana Island. In addition to the more commonly observed and banded migrants such as the Blackpoll Warbler (*Dendroica striata*) and Black-and-white Warbler (*Mniotilta varia*), we found several uncommon birds and one which we believe is a new record for Guana Island (Table 4, Appendix 1). We mist-netted and banded one Swainson's Thrush (*Catharus ustulatus*) and visually observed a second, unbanded, Swainson's Thrush. This is interesting because Raffaele (1989) does not list the species as occurring in Puerto Rico or the Virgin Islands. However, this was not the first time a Swainson's Thrush has been found on Guana Island. We plan to examine autumn and winter reports and Christmas Bird Count data from Tortola to elucidate meaningfulness of the occurrence of Swainson's Thrushes on Guana Island.

Other species of note included our capture and banding of a Yellow-throated Vireo (*Vireo flavifrons*), a Red-eyed Vireo (*Vireo olivaceus*), a Hooded Warbler (*Wilsonia citrina*), a Magnolia Warbler (*Dendroica magnolia*), a Kentucky Warbler (*Oporornis formosus*), an Indigo Bunting (*Passerina cyanea*), and a Rose-breasted

Grosbeak (*Pheucticus ludovicianus*) (Table 4). Other Magnolia Warblers, Kentucky Warblers, Indigo Buntings, and a Rose-breasted Grosbeak were also seen in other areas of the island and an individual Black-throated Blue Warbler (*Dendroica caerulescens*) and Blackburnian Warbler (*Dendroica fusca*) were also observed (Table 4).

Raffaele (1989) indicates the Worm-eating Warbler (*Helmitheros vermivorus*) can be found throughout the Greater Antilles but is an uncommon visitor to Puerto Rico and the larger Virgin Islands and is typically found in heavy forests of interior mountains. Thus, it was a surprise when we captured (and re-captured two days later) a Worm-eating Warbler on Guana Island. To our knowledge, this is a new species record for the island, although Rowan Roy (pers. comm.) reports rare winter and spring sightings of the species have been made elsewhere in the British Virgin Islands.

Because I am interested in the ecology of American Kestrels (*Falco sparverius*) in the Caribbean, I brought materials to construct a bal-chatri trap. Construction of the trap took two afternoons, but I deemed it easier to build one on site than try to bring one with me. Only one hour was needed to trap the pair of kestrels residing on the flat adjacent to the Salt Pond. Two other kestrels where trapped in mist-nets, and a fifth, un-banded, female kestrel showed up on our last day but we were unable to set a trap for her. One of the kestrels captured in the mist-nets was a male that had been banded the previous year (F. Sibley, pers. comm.).

***Identification of Research Opportunities***

Conducting research on Guana Island involves the logistical constraint of studies being conducted in October. This seriously hampers any investigation focused on breeding ecology or productivity for most avian species. However, I believe there are

abundant research opportunities and information needs that could be addressed under these constraints.

Some possible research projects I am interested in pursuing are:

1. Population structure and dynamics of the Bridled Quail Dove (*Geotrygon mystacea*).
2. Pearly-eyed Thrasher (*Margarops fuscatus*) food habits and impact as a predator of native birds, reptiles, and fruit crops.
3. Genetic relationships of American Kestrels throughout the Caribbean.
4. Island vegetation and physiographic influences on distribution and sympatry of Antillean Crested Hummingbirds (*Orthorhyncus cristatus*) and Green-throated Caribs (*Eulampis holosericeus*).
5. Island vegetation, physiography and microclimate influences on distribution of island species.

At this time I anticipate developing a study design and proposal for project 1. I believe it is one of the more important questions regarding native species on the island and one which, with a little creativity and persistence, could be addressed. The project would require capture and color banding of individual doves and at least 4 continuous years of data collection to draw any meaningful conclusions. I would like to initiate this project in October 2004. I am currently exploring funding possibilities for financial assistance with this project. Considerable equipment, such as special mist nets and walk in traps will be needed.

Project 2 addresses a very important issue with respect to bird and lizard ecology and fruit production on the island. This project would be easy to conduct but would

require lethal collection of individual thrashers. The legalities and permits to do this would need to be worked out. I am currently exploring the feasibility of doing this study with Dr. Gad Perry at Texas Tech University, who would be a collaborator.

Project 3 is underway in that I am attempting to gain feather samples for DNA extraction from researchers on other islands throughout the Caribbean. Funding will need to be raised to conduct genetic analysis or it may be developed as a student project.

Projects 4 and 5 are important questions for the avifauna of Guana Island. Guana is possibly the least impacted island in the BVI. Developing an understanding of resource and space use and partitioning among native species under natural conditions would be very beneficial in maintaining those species on the island but also in recovery or habitat restoration efforts on other islands. These projects would require collaboration with scientists with different specialties, notably the botanist Rudy O'Reilly.

**ACKNOWLEDGMENTS**

Tracy Boal, Fred Sibley, and Ann Sutton all assisted with mist-netting and banding. Fred Sibley also helped with orientation to the island. I thank the USGS Cooperative Research Units for facilitating this study and The Conservation Agency for inviting me to participate in research on Guana Island and providing local support.

Table 1. Mist netting effort and capture rates at Guana Island, BVI, 12 – 20 October 2003.

| Date | Net Hours | New Band | Recaptures | Not Banded[a] | Total Captures | Captures /net hour |
|---|---|---|---|---|---|---|
| 12-Oct | 20.0 | 5 | 2 | 6 | 13 | 0.65 |
| 13-Oct | 35.8 | 10 | 3 | 4 | 17 | 0.48 |
| 14-Oct | 32.3 | 6 | 5 | 7 | 18 | 0.56 |
| 15-Oct | 23.0 | 16 | 3 | 4 | 23 | 1.00 |
| 16-Oct | 17.5 | 17 | 3 | 0 | 20 | 1.14 |
| 17-Oct | 21.0 | 15 | 3 | 2 | 20 | 0.95 |
| 18-Oct | 22.0 | 21 | 5 | 2 | 28 | 1.27 |
| 20-Oct[b] | 13.0 | 30 | 15 | 1 | 46 | 3.54 |
| Total | 184.5 | 120 | 39 | 26 | 185 | 1.00 |

[a] These were birds for which the appropriate size bands were not available or were not banded for other reasons.

[b] Trapping efforts focused on Bananaquits at the nets erected at the kitchen area and on shorebirds at the salt pond.

Table 2. Species banded by day of the week on Guana Island, BVI, 12 – 20 October 2003.

| | Date in October | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Species | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 20 | TOTAL |
| Semipalmated Plover | | | | | | | | 1 | 1 |
| Ruddy Turnstone | | | | | | | | 3 | 3 |
| White-rumped Sandpiper | | | | | | | | 2 | 2 |
| American Kestrel | | | | | 2 | | 1 | | 3 |
| Common Ground-Dove | | | | 1 | | | | | 1 |
| Caribbean Elaenia | 1 | 1 | | | | 1 | | 1 | 4 |
| Swainson's Thrush | | | | | 1 | | | | 1 |
| Pearly-eyed Thrasher | | 2 | 1 | 3 | 1 | 2 | 2 | 1 | 12 |
| Yellow-throated Vireo | | | | 1 | | | | | 1 |
| Red-eyed Vireo | | | | | | 1 | | | 1 |
| Black-and-white Warbler | | | | 2 | | | | | 2 |
| Magnolia Warbler | 1 | | | | | | | | 1 |
| Blackpoll Warbler | | | | 5 | 6 | 5 | 3 | | 19 |
| Kentucky Warbler | | | | | | | | 1 | 1 |
| Hooded Warbler | | | | | | 1 | | | 1 |
| Worm-eating Warbler | | | | | | | 1 | | 1 |
| Ovenbird | | | | | 1 | | | 1 | 2 |
| Rose-breasted Grosbeak | | | | | | 1 | | | 1 |
| Indigo Bunting | | | | | 1 | | | | 1 |
| Black-faced Grassquit | 1 | 1 | | | 2 | 2 | 2 | | 8 |
| Bananaquit | 2 | 6 | 5 | 4 | 3 | 2 | 12 | 20 | 54 |
| TOTAL BANDED | 5 | 10 | 6 | 16 | 17 | 15 | 21 | 30 | 120 |

Table 3. Total birds captured and banded, recaptured, and captured but not banded, and percent of total during mist-net and bal-chatri trapping efforts, Guana Island, BVI, 12 – 20 October 2003.

| Species | Banded | Recapture | No Band | Total | Percent |
|---|---|---|---|---|---|
| Bananaquit | 54 | 27 | 0 | 81 | 43.8 |
| Pearly-eyed Thrasher | 12 | 2 | 7 | 21 | 11.4 |
| Blackpoll Warbler | 19 | 0 | 0 | 19 | 10.3 |
| Green-throated Carib | 0 | 0 | 17 | 17 | 9.2 |
| Black-faced Grassquit | 8 | 5 | 0 | 13 | 7.0 |
| Caribbean Elaenia | 4 | 2 | 0 | 6 | 3.2 |
| American Kestrel | 3 | 1 | 0 | 4 | 2.2 |
| Ruddy Turnstone | 3 | 0 | 0 | 3 | 1.6 |
| Black-and-white Warbler | 2 | 0 | 0 | 2 | 1.1 |
| Worm-eating Warbler | 1 | 1 | 0 | 2 | 1.1 |
| White-rumped Sandpiper | 2 | 0 | 0 | 2 | 1.1 |
| Ovenbird | 2 | 0 | 0 | 2 | 1.1 |
| Indigo Bunting | 1 | 0 | 0 | 1 | 0.5 |
| Hooded Warbler | 1 | 0 | 0 | 1 | 0.5 |
| Magnolia Warbler | 1 | 0 | 0 | 1 | 0.5 |
| Yellow-throated Vireo | 1 | 0 | 0 | 1 | 0.5 |
| Red-eyed Vireo | 1 | 0 | 0 | 1 | 0.5 |
| Swainson's Thrush | 1 | 0 | 0 | 1 | 0.5 |
| Semipalmated Plover | 1 | 0 | 0 | 1 | 0.5 |
| Common Ground-Dove | 1 | 0 | 0 | 1 | 0.5 |
| Kentucky Warbler | 1 | 0 | 0 | 1 | 0.5 |
| Rose-breasted Grosbeak | 1 | 0 | 0 | 1 | 0.5 |
| Zenaida Dove | 0 | 1 | 0 | 1 | 0.5 |
| Bridled Quail Dove | 0 | 0 | 1 | 1 | 0.5 |
| Black-necked Stilt | 0 | 0 | 1 | 1 | 0.5 |
| TOTAL | 120 | 39 | 26 | 185 | 99.57 |

Table 4. Full list of species seen, captured, or both by personnel on Guana Island, BVI, 12-20 October 2003.

| SPECIES | Seen | Captured | Both |
|---|---|---|---|
| Magnificient Frigatebird | X | | |
| Red-billed Tropicbird | X | | |
| Brown Pelican | X | | |
| Brown Booby | X | | |
| Cattle Egret[a] | X | | |
| Yellow-crowned Night-heron | X | | |
| Little Blue Heron | X | | |
| Green Heron[a] | X | | |
| Greater Flamingo | X | | |
| American Oystercatcher | X | | |
| Black-necked Stilt | | | X |
| Wilson's Plover | X | | |
| Semipalmated Plover | | | X |
| Lesser Yellowlegs | X | | |
| Solitary Sandpiper | X | | |
| Ruddy Turnstone | | | X |
| Semipalmated Sandpiper | X | | |
| Spotted Sandpiper | X | | |
| Least Sandpiper | X | | |
| White-rumped Sandpiper | | | X |
| Royal Tern | X | | |
| Red-tailed Hawk | X | | |
| American Kestrel | | | X |
| Zenaida Dove | | | X |
| Scaley Napped Pigeon | X | | |
| Common Ground-Dove | | | X |
| Bridled Quail Dove | | | X |
| Mangrove Cuckoo | X | | |
| Yellow-billed Cuckoo | X | | |
| Antillean Crested Hummingbird | X | | |
| Green-throated Carib | | | X |
| Belted Kingfisher | X | | |
| Gray Kingbird | X | | |
| Caribbean Elaenia | | | X |
| Caribbean Martin[c] | X | | |
| Barn Swallow | X | | |
| Swainson's Thrush | | | X |
| Pearly-eyed Thrasher | | | X |
| Yellow-throated Vireo | | X | |
| Red-eyed Vireo | | X | |
| Black-and-white Warbler | | | X |

| | | | |
|---|---|---|---|
| Black-throated Blue Warbler[d] | X | | |
| Blackburnian Warbler[e] | X | | |
| Magnolia Warbler | | | X |
| Blackpoll Warbler | | | X |
| Kentucky Warbler | | | X |
| Hooded Warbler | | X | |
| Worm-eating Warbler | | X | |
| Ovenbird | | X | |
| Rose-breasted Grosbeak | | | X |
| Indigo Bunting | | | X |
| Black-faced Grassquit | | | X |
| Bobolink[b] | X | | |
| Bananaquit | | | X |

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[a] Reported by members of the reptile team.
[b] Observed by Fred Sibley
[c] Observed by Clint Boal
[d] Observed by Tracy Boal
[e] Observed by Adrian Andre

Appendix 1. Images of some species captured and banded on Guana Island, British Virgin Islands, October 2003.

Female American Kestrel (*Falco sparverius*)

Yellow-throated Vireo (*Vireo flavifrons*)

Male Hooded Warbler (*Wilsonia citrina*)

Green-throated Carib (*Eulampis holosericeus*)