

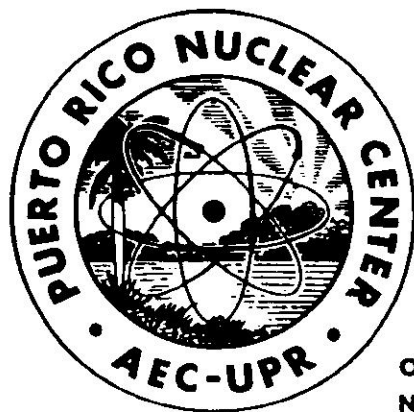
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PUERTO RICO NUCLEAR CENTER

THE RAIN FOREST PROJECT

ANNUAL REPORT

June 1970



OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT
NO. AT (40-1)-1833 FOR U. S. ATOMIC ENERGY COMMISSION

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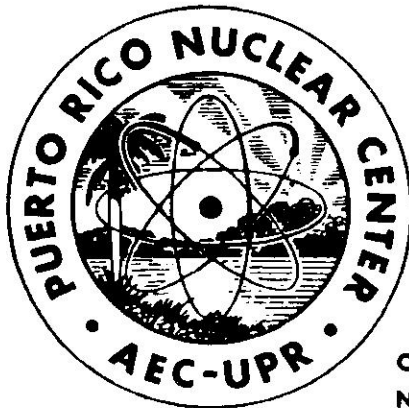
ANNUAL REPORT

Richard G. Clements, George E. Drewry

and

Robert J. Lavigne

June 1970



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TABLE OF CONTENTS

I.	Introduction	ii
II.	Forest Ecology	
	Isotope Cycling Studies	1
	A Versatile Stemflow Collar for Interception Studies	7
	Forest Recovery following Gamma Irradiation	10
III.	Animal Ecology	
	Role of Amphibians in the Ecology of the Puerto Rican Rain Forest	16
	Classification and Geographic Distribution Microhabitat	18
	Reproductive Behavior	19
	Vocal Organization	23
	Evolutionary Relationships	32
	Experiments in Niche Distributions and Energy Flow	35
	Territoriality and Population Dynamics	38
	Interspecific Territoriality	49
	 Feeding Behavior of the Frogs and Lizards in the Tropical Wet Forest	 65
	 The Ecology of Ants in the Luquillo Forest	 75
	 The Role of Insects in the Food Web of the Tropical Wet Forest	 84
IV.	Visiting Scientist Program	
	Properties of Aquatic Communities in Container Habitats	87
	Studies on the Chemistry of the Tropical Maritime Atmosphere	91
	Karyotypic Studies of Bats of the Family Phyllostomatidae	95
	Arrested Succession in Tropical, Terrestrial Ecosystems	101
	Field Studies on the Puerto Rican Tody, <u>Tody</u> <u>mexicanus</u>	109
	The Termites at El Verde - 1969 Survey	112
	Polyethism in Workers of <u>Nasutitermes costalis</u> (Holmgren).	119
	Occurrence and Distribution of Aquatic Fungi in the El Verde Forest.	129
	Radiotracer Studies and Nutrient Cycling	131

INTRODUCTION

The Terrestrial Ecology Program is now in its seventh year. Approximately the first four years were devoted to studies on the effects of gamma radiation on a tropical forest ecosystem. Beginning in 1966, emphasis was gradually shifted to the second objective; the measurement of fall-out nuclides in the rain forest system. This objective has since been modified to include the cycling of both radioactive and stable isotopes in the forest ecosystem; (1) fallout measurements; (2) tracer experiments; (3) stable element analyses; and (4) water balance measurements.

Since 1966, numerous experiments using radioisotopes as tracers have been carried out in the tropical forest. The movement of ^{134}Cs , ^{85}Sr , ^{54}Mn , ^{65}Zn in plants, animals and soil water have been studied and reported. Experimental work involving the movement of tritium in plant and soils has been successfully studied. Studies on the transfer of ^{32}P , ^{65}Zn , ^{75}Se and ^{59}Fe from litter to soil and uptake by plants were completed this past year.

Current research includes studies on insect ecology, movement of selected isotopes through the animal food web, element input via rainfall and its subsequent distribution in the forest, recovery in the irradiated area, movement and distributions of previously applied isotopes in the soil, plants and animals and Acoustical Communication and Population Ecology in *Eleutherodactylus* species.

Future work will be in the direction of the systematic study of the movement selected radioisotopes in both the biotic and abiotic components of the forest. Increased emphasis will be placed on the physical and chemical properties of forest soils and the movement of macro and trace elements via soil water to the streams.

This report covers the studies that have been initiated or carried out this past year. Some are interim reports of on-going research while others concern short term studies that have been completed. The reports are grouped under three broad categories; (1) Forest ecology, (2) Animal ecology, and (3) Contributions from the Visiting Scientist Program.

FOREST ECOLOGY

Isotope Cycling Studies

R.G. Clements

Active research this past year has been primarily in the field of insect and amphibian ecology. Studies on radioactive and stable isotope movement in trees, litter and soil have been inactive most of the year due to completion of these studies during the first two months of 1969 and a change in staff. The results of these studies were summarized in the 1968 annual report (PRNC-131); a more detailed discussion was presented; by Jordan in the 1969 annual report of the Terrestrial Ecology Program (PRNC-129).

The contamination of terrestrial systems with radionuclides may occur either by particulate fallout or via isotopes associated with rainfall. Foliar absorption of isotopes by plants is a fact. The actual uptake of isotopes from rainfall is a function of the intensity, duration, storm size and the length of time the water is in contact with the absorbing surfaces. A review of rainfall distribution at El Verde from 1964 through May, 1966 showed that approximately 70 percent of the daily rain events were 0.5 inches or less (Table 1). Sufficient emphasis has never been placed on the importance of the small storm sizes and their role in the uptake of radionuclides.

Fassig (1916) studied the duration, frequency and intensity of tropical rains in Puerto Rico. He reported that, at the Caguas station, out of an annual frequency of 262 rain days, 160 rain days had rains between 0.01 and 0.10 inches. On the western end of the island, the highest frequency occurred between 0.20 and 0.30 inches. His data suggested that for the Luquillo area, approximately 89 percent of the rains were 0.5 inches or less. Odum, et. al. (1970) reported that light rains between 0.01 to 0.10 inches/hour accounted for 82 percent of the rains at El Verde. According to Libby (1956) cleansing of the atmosphere of particulate matter in a zone of precipitation is assumed to be fairly complete with 0.1 inch of rain.

For sometime, foliar applications have been used to correct minor element deficiencies in plants. Radioisotopes studies have confirmed that foliar applied materials can be absorbed, translocated and utilized by plants. Studies on the absorption of radioisotopes from dilute solutions have indicated that the greatest uptake occurs in the first 24 hours after application (Biddulph, 1960 and Bukovac, and Wittwer, 1960). Bukovac and Wittwer (1960) also showed that the amounts of ^{32}P absorbed vary with the moisture conditions of the leaf surface. Wittwer and Teubner (1959) have shown that ^{42}K , ^{55}Fe , ^{65}Zn ,

Table 1

Distribution of rainfall by 0.1 inch classes
for the period January 1964 - May 1966.

Storm Class	Number of Events	Percent of Total Rains	Accumulative Percentage
less than 0.1	179	27.8	27.8
0.1	96	14.9	42.7
0.2	60	9.3	52.0
0.3	61	9.5	61.5
0.4	47	7.3	68.8
0.5	26	4.0	72.8
0.6	29	4.5	77.3
0.7	16	2.5	79.8
0.8	19	3.0	82.8
0.9	9	1.4	84.2
1.0	11	1.7	85.9
1.1	12	1.9	87.8
1.2	7	1.1	88.9
1.3	9	1.4	90.3
1.4	2	0.3	90.6
1.5	4	0.6	91.2
1.6	5	0.8	92.0
1.7	3	0.5	92.5
1.8	3	0.5	93.0
1.9	6	0.9	93.9
2.0	3	0.5	94.4
2.1	5	0.8	95.2
2.2	1	0.2	95.4
2.3	.1	0.2	95.6
2.4	1	0.2	95.8
2.5	1	0.2	96.0
2.6	1	0.2	96.2
2.7	4	0.6	96.8
2.8	1	0.2	97.0
2.9	1	0.2	97.2
3.0	0	0	
3.0 +	18	2.8	100.0
Totals	643		

^{32}P and ^{35}S can be absorbed by the bark of fruit trees and contribute to the nutrition of the underlying areas. Thus the deposition and subsequent absorption of radionuclides by rainfall can be compared to foliar absorption.

Considerable work has been done on rainfall and interception in the hardwood forests of the eastern United States. Helvey and Patric (1965) summarized what was available. They found it necessary to conduct their own experiments to estimate interception, since the use of that reported in the literature was restricted by sampling and methods of measurement. However, they did find that the published data on regression equations were fairly uniform. Re-analysis of these data led to the development of generalized equations for predicting the quantity of throughfall and stemflow from gross rainfall in eastern hardwood forests.

The two equations were derived:

$$T = 0.901P - 0.031(n)$$

$$S = 0.041P - 0.005(n)$$

where T and S are the amount of throughfall and stemflow, respectively, P is gross rainfall and n is the number of storms per season.

Similar work in tropical areas is scarce. Mohr and Van Baren (1954) presented data on the amount of rainfall retained by the canopy in Surinam. They reported that with a rain of 0.04 inches, 80% was retained and this percentage decreased to 19.5% with a rain of 1.4 inches. Jordan (1968) initiated a series of studies on the water budget of the El Verde Forest. In this study stemflow was measured on 27 trees which ranged from 1.6 to 30.7 inches DBH. Throughfall was collected and measured in 12 trough type gauges located at various sites in the forest. He found stemflow to be almost a constant percentage of gross rainfall, ranging between 17.5 and 22% depending upon storm size. Throughfall was found to be approximately 70% of gross rainfall. Regression equations of the form $Y = a + bx$ were developed for each DBH class between 2 and 30 inches. It was found that intermediate size trees yielded the largest amounts of stemflow from a given storm. These equations were then combined with data on the number of trees per hectare and the DBH distribution of trees to estimate the contribution of stemflow to the water budget.

In their discussion, Helvey and Patric (1965) brought forth two important points. First, a complete interception study has never been reported and second, stemflow has not been well documented on an areal basis. Traditionally, stemflow has been measured on individual trees and the results extrapolated to an area basis as represented by the work of Jordan (1969). Hydrologic studies quite often have been too

restricted in scope and have not considered the full importance of the inter-relationships that occur on an areal basis. However, this approach has been restricted because of the lack of adequate techniques, especially in the area of stemflow measurements. Helvey and Patric (1965) pointed out that the paucity of stemflow data was no doubt due to the high cost and hard work involved in this type of study. Thus if interception studies are to be initiated on an areal basis, the question of stemflow collars and measurements must be solved. We feel that the problem has been solved and the description of the stemflow collar and techniques is presented as a separate section. The objectives, methods and procedures of the comprehensive interception study are set forth in the following sections.

Objectives: The interception study has four main objectives:

1. First, to determine the quantitative relationships between gross rainfall as measured above the canopy and the parameters of throughfall and stemflow yield, litter interception and evaporation, and soil moisture changes.
2. To determine the chemistry of each parameter and define the chemical changes that take place as rainfall is intercepted and re-distributed as throughfall and stemflow. The elements to be determined are: calcium, potassium, sodium, magnesium, manganese, iron, copper, zinc and lead.
3. To develop predictive equations through correlation-regression analysis on the relationships between gross rainfall and the associated interception parameters which will include both quantity and chemistry.
4. To utilize the information obtained for the design of future cycling studies at the watershed level using radioisotopes.

Procedure

An 80M by 80M section of forest at the El Verde Forest site in close proximity to the 72 ft. walk-up tower was selected and subdivided into 16 smaller plots of 20M by 20M. Five plots were randomly selected for the studies. A grid of 1 square meter was laid down in each of the five plots and the location of each tree species, and its DBH were recorded. Notes were made for each tree regarding its bark characteristics, leaf size, presence or absence of epiphytes, and vines, and whether the tree was erect or inclined. Each tree was classified according to its interception potential as follows:

1. Primary interceptor - Those trees occupying a canopy position and receiving direct rainfall.

2. Secondary interceptor - Those trees occupying a sub-canopy position and receiving throughfall from a primary interceptor.
3. Tertiary interceptor - Those trees occupying a sub-sub canopy position and receiving throughfall from a secondary interceptor.

One plot, from the five set aside, was randomly selected to initiate the interception studies. All trees in the plot having DBH's of 3 inches or larger, 45 in total, were fitted with a stem flow collar (see section on collars).

To measure throughfall, six troughs 8 inches wide by 24 feet long were fabricated from galvanized metal. Each trough was lined with polyethylene sheeting. The troughs were given a 10 percent slope to facilitate rapid drainage of water. Throughfall will be collected in covered 20 gallon plastic containers and quantities determined by measuring with a calibrated stick. The effective collecting area of the troughs is 13,696 cm². Theoretically one inch of throughfall would yield 34,789 ml. of water thus allowing the measurement of small quantities of throughfall. The troughs were selected over other means of gaging because of the interest in the chemistry of the water. In this way the troughs integrate the throughfall and give a more representative sample of the area than would spot sampling.

Gross rainfall measurements are measured from a one square meter stainless steel collector located on top of a 72 ft. tower. With an effective collecting surface of 10,000 cm², a one inch rain would yield 25,400 ml. thus giving a sensitivity of 0.001 inch.

Measurements will be made the morning following each rain and the data transferred to IBM cards for subsequent correlation-regression analysis. Samples will be collected from each rain for chemical analysis which will be done by atomic absorption spectrophotometry.

As the study proceeds, litter interception and evaporation loss studies will be incorporated into the program. Likewise, soil moisture blocks will be installed to monitor soil moisture conditions. Pressure membrane techniques will be used to determine the water holding capacity and drying cycles for soil layers down to a depth of 36 inches.

REFERENCES

- Bidduph, O. 1960. Radioisotopes in plants: Foliar entry and distribution. Radioisotopes in the Biosphere. Ed., R.E. Caldecott and L.A. Snyder. Printing Department, University of Minnesota. pp. 73-85.
- Bukovac, M.J. and S.H. Wittwer, 1960. Absorption and distribution of foliar applied mineral nutrients as determined with radioisotopes. Plant Analysis and Fertilizer Problems. Ed., W. Reuther. Lord Baltimore Press, pp. 215-230.
- Fassig, Oliver L. 1916. Tropical rains - their duration, frequency and intensity. Monthly Weather Review, pp. 329-337.
- Jordan, C.F., 1968. The Rain Forest Annual Report. PRNC-119.
- Helvey, J.O. and J.P. Patric, 1965. Canopy and Litter Interception of Rainfall by Hardwoods of Eastern United States. Water Resources Research, Vol. 1, No. 2. pp. 193-206.
- Libby, W.F. 1956. Radioactive fallout and radioactive strontium. Sci. 123: 657-660.
- Mohr, E.C.J. and F.A. Van Baren, 1954. Tropical Soils. Interscience Publishers, Inc., New York, pp. 17-99.
- Odum, H.T., G.E. Drewry and J.R. Kline, 1970. Climate at El Verde. A Tropical Rain Forest. H.T. Odum, Ed. pp. B-347-418.
- Wittwer, S.H. and F.C. Teubner, 1959. Foliar absorption of mineral nutrients. Ann. Review of Plant Physiology 10: 13-32.

A Versatile Stemflow Collar for Interception Studies

R.G. Clements and José A. Colón

One of the limiting factors in the design and initiation of large scale rainfall interception studies in forests has been the lack of an inexpensive, practical and rapidly assembled stemflow collar. Helvey and Patric (1965) have suggested that the relative paucity of stemflow data was no doubt due to the high cost and hard work involved. This may explain the great diversity of collars used in previous studies and the reason why many investigators have dealt with individual trees rather than attempting studies on an areal basis.

While the individualistic approach may provide an approximation of stemflow on an areal basis it has two basic faults which are inter-related. First, and by far the most important, it excludes the interdependence or the influence of one tree on its nearest neighbor from consideration. Yet today, the ecosystem approach and systems analysis stress these inter-relationships. To ignore these relationships leads to the second fault which is that considerable error may be introduced as the results from individual studies are extrapolated to an areal basis. Thus there is a need to revise the approach and begin studies on an areal basis as suggested by Helvey and Patric (1965).

The problem at the El Verde site centers around the cycling of both stable and radioactive isotopes in a forest ecosystem. In planning the interception studies on an areal basis, it was necessary to solve the problem of high cost and ease of installation of stemflow collars. This was further complicated by the requirement that the collars had to be made of relatively inert material so as not to effect the chemistry of the water collected.

A literature search provided little if any help. After rejecting many concepts and suggestions because they did not meet the three criteria mentioned, a solution was found.

The materials consist of foam rubber sheeting one inch thick, 4 mil polyethylene plastic, rigid polyethylene tubing, contact cement and refined parafin. The foam rubber is cut into strips one inch wide, and the length depends on the circumference of the tree. The two ends are coated with contact cement and allowed to dry. The foam rubber strip is stretched around the tree and the two ends joined (Figure 1A).

With a standard cork borer, a hole is cut on the drainage side of the collar. A rigid polyethylene tube is cut to the desired length and inserted into the collar (Figure 1B). This tubing may be heated and bent slightly and secured to the tree as shown in Figure 1C.



1a



1b



1e



1c



1d

Figure 1 Pictorial sequence of stemflow collar installation at El Verde

Two inch strips are cut from 4 mil polyethylene sheeting. Contact cement is applied to the outside edge of the collar and one side of the polyethylene strip. When the contact cement has dried, the polyethylene strip is applied to the outside edge of the collar to form the trough (Figure 1C).

Refined parafin is melted in a glass beaker on a portable burner. The area surrounding the polyethylene tube is saturated with melted parafin and when cooled provides additional strength to the foam rubber-tube joint. The upper surface of the collar and the area between the collar and the tree receive several coats of parafin to seal the surfaces (Figure 1D).

The completed collar may then be connected to the collection container by the appropriate tubing (Figure 1E). The plastic container shown has a 5 gallon capacity. We have found it necessary on some trees with high stemflow potential to insert a Y tube at the outlet of the collar and pass the stemflow water to two 5 gallon containers. The collars have been in field use for many months and show no sign of deterioration under the high rainfall and humidity conditions of the El Verde Forest. Repairs if needed, are simple and rapid with melted parafin.

The actual cost of the collars is approximately \$0.25 per tree exclusive of the collecting containers. The materials are inert and cause no problem with the chemistry. Installation is rapid. We estimate that 40 to 50 collars can be installed in a single day.

References

- Helvey, J.O. and J.P. Patric, 1965. Canopy and Litter Interception of Rainfall by Hardwoods of Eastern United States. Water Resources Research, Vol. 1, No. 2. pp. 193-206.

FOREST RECOVERY FOLLOWING GAMMA IRRADIATION

George E. Drewry and Alejo Estrada

A fourth annual census was made of all plants growing in a 476 m² sample plot inside the area of damage from a 1965 ¹³⁷Cs gamma irradiation. The census was completed during the months of August and September 1969, making results comparable to those of previous years. Mr. Jack Ewel provided valuable advice and assistance on field procedures.

Data was processed according to general procedures outlined in the FY-1969 Rain Forest Annual Report (PRNC 129). Vegetation was separated by species within established categories listed in Table 1. As mentioned in previous reports, an arbitrary method of separating individual grasses was used, each species occurring within a square meter quadrat being scored as one individual. Extensive rootstocks in some species cause this method to overestimate the number of rootstocks actually present, with a consequent reduction in apparent diversity.

Three separate diversity indices were calculated for each category, as presented in Table 2, species per thousand individuals by the maximum likelihood method, average slope of the composite ratio curve, and Brillouin's H. Composite ratio curves were constructed according to the methods described in PRNC 129. Additional experience with these indices has increased our understanding of their advantages and shortcomings. Brillouin's H, derived from information theory, is receiving wider usage among ecologists, although some persist in using the inappropriate (for biological diversity) Shannon formula. The principle shortcomings of the H parameter are its relative insensitivity to interrelationships among the rarer species of a sample and its dependence on sample size. Species per thousand, obtained by any method, is complementary in that it is insensitive to gross differences in relative ratios of common species. In fact, counting the number of species in a random sample of 1000 individuals ignores all ratios. The maximum likelihood method of extrapolation ties this index to the ratios of the rare species taken as a group. It normally errs slightly in predicting the exact number of species in a thousand, but errors appear random in either direction, and agreement in a large series of indices now calculated has been uniformly good.

The composite ratio curve, which is the reciprocal of the relative abundance curve, can exhibit a variety of shapes, but its average slope in semilogarithmic plot achieves a compromise between the other two indices. It is less sensitive to sample size than

Table 1. Plant composition of post-irradiation sample area
(476 m²) in summer 1969.

<u>Category</u>	<u>Individuals</u>	<u>Species</u>
Radiation survivors	482	40
New plants	8,129	122
Total plants	8,611	126
Total trees	6,353	73
Total new trees	5,871	68
Total non-trees	2,258	53
Tree seedlings	5,064	67
Tree saplings	807	28
Sprouts	442	36
Old trees	40	14
Vines	983	17
Grasses	775	9
Other Herbs	500	27

Table 2. Diversities within selected plant categories by
three different indices, summer 1969.

<u>Category</u>	<u>Species per 1000</u> <u>(extrapolated)</u>	<u>Average CR slope</u> <u>(species per tenfold)</u>	<u>Brillouin's H</u> <u>(bits per ind.)</u>
Tree seedlings	52.2	26.2	4.15
Tree saplings	27.2	10.5	3.21
Sprouts	44.2	18.5	3.41
Old trees	35.8	10.6	2.59
Vines	16.7	5.8	2.55
Grasses	9.0	2.5	1.73
Other Herbs	30.2	11.2	3.06
Total New Vegetation	76.6	43.7	4.95

Brillouin's H and reflects relationships in both rare and common species, with some emphasis on the rare.

Breaks in the composite ratio curve are specific to certain vegetation types and have received special study. They are sharpest, and set off fewest common species, in habitats that are most divergent from the area norm. Within a given successional stand they seem to intensify with competition until numbers stabilize. Probably they indicate a second order diversity in degree of specialization, and would be minimized if either all or none of the species present were highly specialized for that habitat.

Figure 1, taken in conjunction with Tables 1 and 2, illustrates some of the properties of the diversity indices. The H parameter for both the vine category and the old trees is nearly the same, while species per thousand (a considerable extrapolation in the case of old trees) differ by a factor of more than 2. The smallness of the old tree sample probably affected H, for in two years since 1967, H declined by 26% while the average composite ratio slope declined by only 18%. At any rate the diversity among the rare tree species is much greater than among the rare vine species, a phenomenon apparent in the composite ratio slope but not in the information theory index.

The composite ratio of vines does not exhibit a break, which leads to two hypotheses. One is that vines are not strongly different from one another in specialization, partly supported by the fact that none show the sharp soil drainage preferences that some tree species exhibit. A second hypothesis is that vines do not constitute any coherent community by themselves, but are instead members of the tree or herbaceous communities, depending on their climbing habits. They all have a clear cut specialization in common, relatively rapid growth without necessity for extensive lignification, which gives them a distinct niche yet makes them dependent on other plants.

In overall diversity structure, the 1969 data is very similar to the 1967 data. Figure 2 presents composite ratio curves for 1966, 1967 and 1969. Discrepancies involving the mis-identification of grasses were discovered in the data from the 1968 census. Pending recalculation, the 1968 curve has been omitted and will be reported later. The similarity in diversity and composite ratio between 1967 and 1969 conceals some very real changes in relative abundance between individual species. In particular the seedlings of some shade-adapted species such as Dacryodes excelsa moved into the abundant side of the composite ratio, displacing sun-adapted successional species. The only noticeable difference in the profiles is a slight increase in the diversity of the most common species at the left of the curves. There was an increase of species per thousand from 74.9 to 76.7, and in H from 4.77 to 4.95.

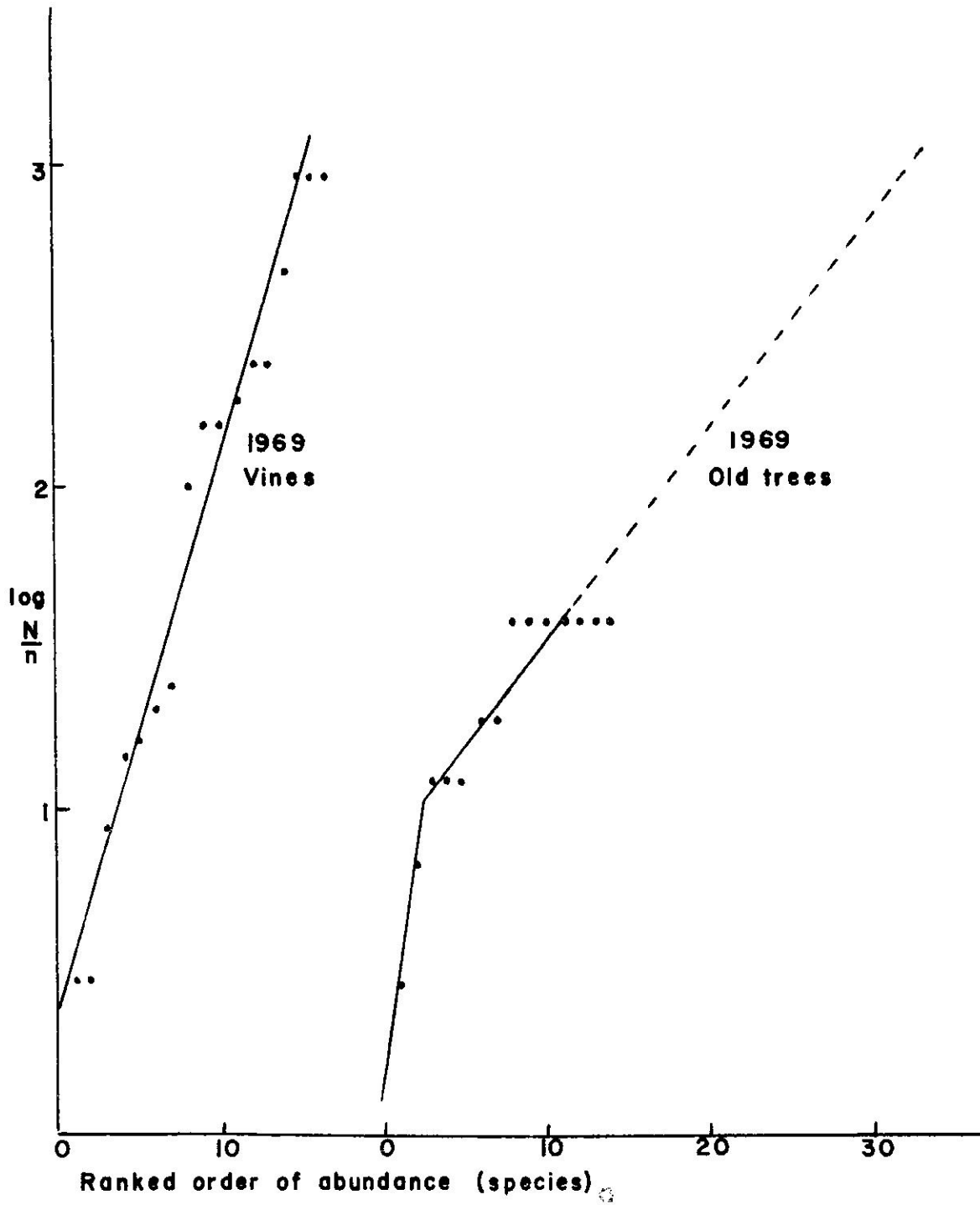


Figure 1. Composite ratios of vines and old trees in the irradiated area, 1969 census.

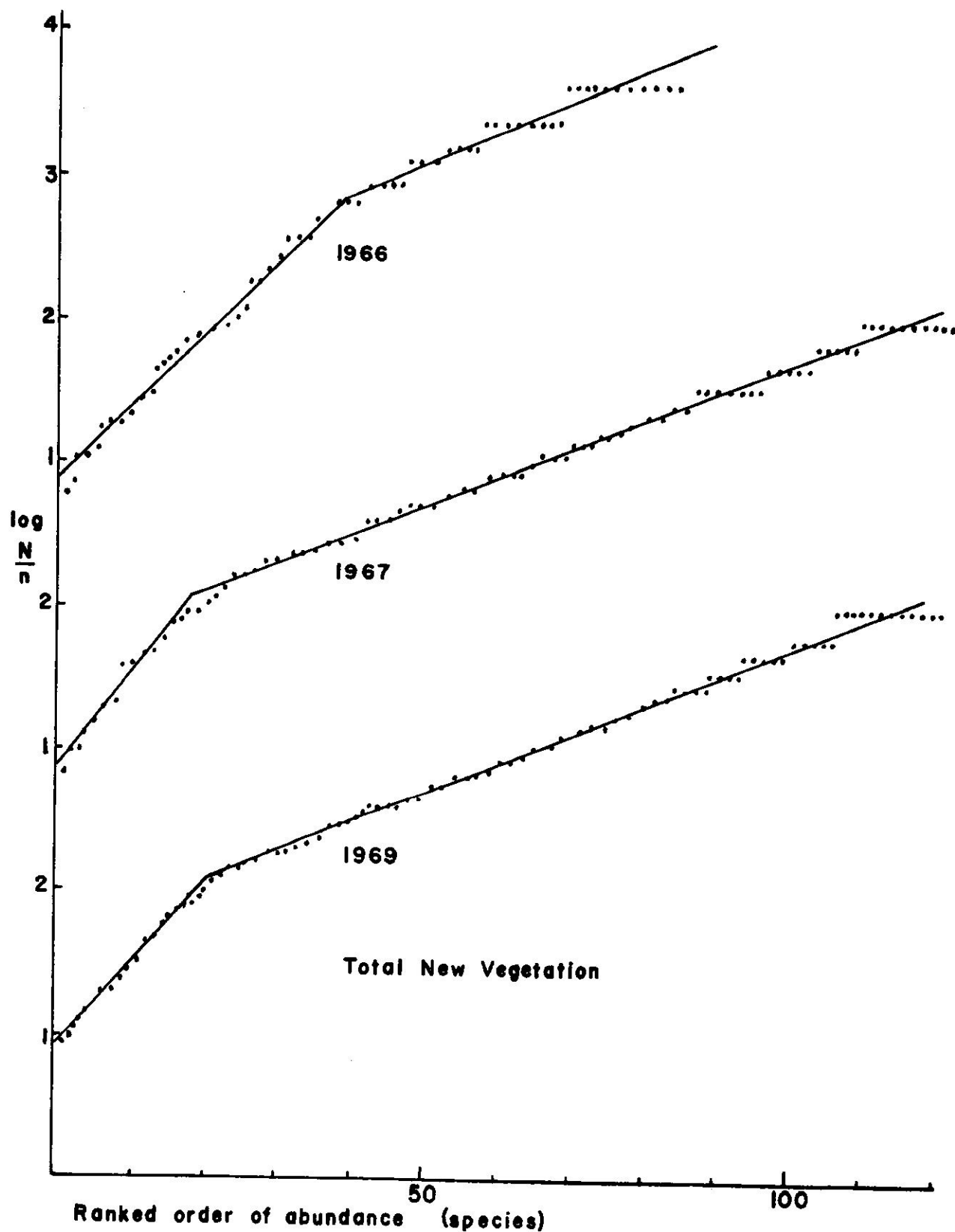


Figure 2. Composite ratios of new vegetation in the irradiated area for 1966, 1967 and 1969.

Deaths among the old trees surviving the irradiation accelerated somewhat during the fourth year, only 40 individuals representing 14 species remaining, compared with 386 of 42 species in 1967. Many of the post-irradiation sprouts, however, became established as part of the regrowth, and have become somewhat difficult to distinguish from the sapling category. One species, Sloanea berteriana, poses no difficulty, as it has not yet established seedlings in the area, but is represented by a vigorous sprout population.

Vines have followed the tree species in increasing numbers of individuals, species and diversity, the increase since 1967 being about 8% in each parameter. Herbs, on the other hand, have decreased by about the same amount. Within the herb category, the ferns have all increased, serving to balance an even greater decline in most other herbaceous species.

The irradiated area has continued to lag behind the mechanically defoliated control center in its recovery, at least partly because of shade cast by damaged but still foliated old trees. Perhaps the most significant aspect of the radiation damage is that unshielded plant tissues have been unable to recover, even by sprouting. Many trees that appeared to be on the way to recovery by sprouting have died, and the only individuals that have recovered in this way originated sprouts from below ground level or in rock shadows, etc. At present rates of removal, just a few more years should suffice to eliminate all of the above ground tissue exposed to radiation damage. The percentage of regrowth plant tissue showing apparent genetic damage from irradiation has remained almost negligible.

ANIMAL ECOLOGY

THE ROLE OF AMPHIBIANS IN THE ECOLOGY OF PUERTO RICAN RAIN FOREST

George E. Drewry

The rain forest surrounding PRNC's El Verde Field Station supports abundant frog populations representing several species. Most are native to Puerto Rico and are restricted either to the island or to the forest itself. Nomenclatural problems seem to have been largely worked out by such workers as Stejneger, Schmidt, Rivero, Grant and Thomas. Ecological studies of one or more species have been made by Heatwole, Rivero, Majorca, Turner, Gist and others. This progress report describes a program to elucidate the role of frogs in the rain forest ecosystem and to describe the mechanisms by which species create and maintain separate specific roles. Ideally, such a study might document the patterns of distribution, speciation and adaptation of all the species and reveal the dynamics of each at the systems analysis level, considering all interactions within and between species and the relationships to predators, prey and other organisms.

Specific progress has been achieved within such an ambitious framework by dividing the problem into phases and correlating earlier descriptive and qualitative information into a program of experiments that reveal the quantitative aspects of the relationships. Frequent benefits resulted from integrating this study with ongoing research into other aspects of the rain forest ecosystem. For example, studies of radioisotope behavior have helped clarify the position of frogs in the animal food web structure. The use of radioactive tags now appears to be an ideal method of monitoring movements of individuals within the population and quantifying the effects of experimental manipulation.

Phase I. Classification and Geographic Distribution

This phase was descriptive and qualitative; construction of a theoretical framework is almost complete. Of 19 Puerto Rican amphibian species mentioned or described in the literature, and subsequently supported by taxonomic opinion, a few can be eliminated because their geographic range does not contact the rain forest. The bullfrog, Rana catesbiana Shaw and a still unidentified species of Hyla are recent introductions that have not yet been observed in the forest. The bullfrog is becoming common in freshwater lowland marshes, and the Hyla is reported by Dr. Manuel Vélez (personal communication) to frequent human dwellings in the vicinity of Isabela, P.R. The native Puerto Rican toad Bufo lemur (Cope) is nearly extinct, although García-Díaz (1966) has reported a rediscovery in western Puerto Rico. Finally, the ranges of Eleutherodactylus

cochranae Grant and E. cooki Grant have been examined by several workers; it appears that the former species is restricted to coastal plains, and that the latter is restricted to the Panduras mountain range of southeastern Puerto Rico, which at present has no rain forest. These species are being studied qualitatively but their quantitative ecology will receive less attention than the remaining species.

Bufo marinus (Linne) is an introduced toad that has become very common in Puerto Rico, and its preferred microhabitat of bare earth, pavement, gravel, short grass or cultivated fields makes it very compatible with the environment created on the island by man. It breeds in sluggish or still bodies of water, and has not been discovered to breed within the rain forest. It apparently penetrates the forest along roads or open trails to the distance a single individual can disperse between breeding seasons, when adults return to water. From our observations, this penetration is limited to between 2 and 3 miles.

The remaining 13 species are all members of the family Leptodactylidae, a prominent family in the new world tropics, particularly in the Caribbean area. The current classification, which could change as a result of this and other studies, consists of Leptodactylus albilabris (Guenther) and 14 Eleutherodactylus species. At any given elevation within the rain forest they can be divided into forest and forest edge species by surveying the distribution of calling males. This distinction is obscured somewhat by changes in the habitat of most species with increasing elevation, which will be described under the heading of phase II. Leptodactylus albilabris, Eleutherodactylus coqui Thomas, E. antillensis (Rheinhardt & Leutken) and E. brittoni Schmidt range over much of the island, while E. portoricensis Schmidt, E. richmondi Stejneger, E. hedricki Rivero, E. eneidae Rivero, E. wightmanae Schmidt, E. locustus Schmidt, E. gryllus Schmidt, E. karlschmilti Grant and E. unicolor Stejneger are restricted to montane forest.

The distinction between E. portoricensis and E. coqui was not recognized until the 1966 work of Thomas. This creates difficulties in interpreting the earlier work of Heatwole, Turner, Gist, Drewry and others who dealt with montane representatives of this complex. Both species are very common throughout the rain forest and later work to be described here fully supports the conclusions of Thomas. The possibility exists that an additional montane species is still being confused with E. coqui, but the weight of evidence so far indicates otherwise.

Phase II - Microhabitat

The evidence for microhabitat preference, like that for distribution, has been derived from the literature and from personal observation on numerous field trips, and is more qualitative than quantitative at this stage. Most of the conclusions rest on agreement of the animal's morphology with the data on where a species was common, where it was rare, and where it was not found after reasonable search. Some modification is still being introduced, but the major patterns seem to be established.

The most relevant aspect of morphology pertaining to microhabitat is probably the ratio of toe pad diameter to body weight, which appears to reflect the importance of vertical surfaces in the animal's way of life. This ratio is lowest in E. unicolor, E. richmondi and E. wightmanae, whose females were collected at ground surface. Males of the last two species often climb to a low perch on vegetation while calling, but even then a horizontal perch is normally selected. The highest ratios are obtained in E. cooki, E. karlschmidti, E. hedricki and E. coqui; both sexes of the first two species frequent the surfaces of boulders, while the last two are arboreal on tall plants and both have been collected as high as 40 ft. above the ground. The walls of buildings are also used by E. coqui and it is the only species commonly encountered in this microhabitat. Eggs of three of these species have been found attached to the underside of horizontal surfaces, which indicates climbing ability, while E. karlschmidti frequents inaccessible crevices between stream boulders (its eggs have not yet been observed). Toe pad diameters of the remaining species range between these extremes and correlate well with observed behavior. Other, more subtle correlations between microhabitat and morphology probably exist. For example, the color and texture of the skin may relate to the favored positions of daytime concealment. Another possible correlation is the fact that in rock dwelling species mentioned the eyes are larger in relation to body length than in species arboreal on low vegetation such as E. antillensis and E. brittoni, which may detect prey as much by vibration and touch as by vision.

Evidence that different features of the microhabitat are used for orientation by different species can be deduced from the varying behavior of species with altitude. Four vegetation zones have been described for the Luquillo forest with each bearing the name of the dominant species. Beginning at the highest elevation they are the mossy forest, the palm forest, the colorado (Cyrilla racemiflora) forest and the tabonuco (Dacryodes excelsa) forest. At certain localities in the palm forest one can encounter most of the frog species within a very short distance, although microhabitat segregation is noticeable even here. If one moves upward into the mossy

forest, first E. brittoni, then E. antillensis, drop out of the fauna and E. hedricki becomes rare, though most of the other species become more abundant. In moving downward the segregation is more pronounced, E. hedricki and E. portoricensis become more and more restricted to deep shade and less common at the forest edge. By contrast, E. antillensis and E. brittoni are excluded from the forest proper at lower altitudes, and E. locustus, H. gryllus become restricted to the forest edge, not appearing either in deep shade or surrounding grassland. Only E. unicolor is restricted to the very high elevations; it lives in intimate contact with the soil and drops out where the loose but water saturated and reduced soils typical of mossy forest give way to drier, slightly more compacted and often oxidized soils below 700 meters elevation. The other species range to the forest edge at all elevations.

Table 1 summarizes the microhabitat preferences noted for each species. Where behavior varies, any bias is probably toward the conditions in the vicinity of El Verde Field Station, where a large fraction of the observations have been made. Elevation of the station is 455 meters. The table is organized in approximate agreement with the phylogenetic evidence presented later (Phase IV).

Phase III. Reproductive Behavior

The third aspect of the natural history studied in the initial descriptive survey was reproductive biology. Reproduction in Bufo has been mentioned earlier and is well described in the literature, but the long breeding migrations apparently made by B. marinus from the rainforest deserve additional study.

Leptodactylus albilabris breeds in the typical manner for its genus, with pairs constructing foam nests in burrows, or natural cavities under banks and stones in muddy areas near shallow streams or puddles. Males call from such shelters, and are seldom encountered far from water. However, both juveniles and females forage for long distances into meadows and on the forest floor. The eggs hatch into tadpoles which either grow into large aquatic larvae (when water and food are plentiful) or metamorphose at a small size into terrestrial froglets (if conditions are dry). This pattern of development seems to represent an intermediate stage in the evolution of the unique condition found in the next genus.

Eleutherodactylus is one of the few amphibian genera that produces completely terrestrial eggs. The embryology was first described by Peters in 1877 from a Puerto Rican species (probably E. coqui). The tadpole stage is passed within the eggshell and at

Table 1. Known range and microhabitat preferences of Puerto Rican frogs.

Genus Species	Elevation limits (ft.)	Known range	Microhabitat
<u>Hyla</u> sp.	?	near Isabela, P. Rico	near or in dwellings
<u>Bufo marinus</u>	2000 max.	most of island	fields, lawns, roads
<u>lemur</u>	?	once widespread, now possibly extinct	probably lowland forest glades
<u>Leptodactylus</u> <u>albilabris</u>	3000 max.	most of island	muddy areas, females range widely on ground at night
<u>Eleutherodactylus</u> <u>richmondi</u>	400 min.	mountain forest	ground, often near stones, roots, logs, etc.
<u>unicolor</u>	2000 min.	eastern mountain forest	burrows in ground
<u>karlschmidti</u>	400 min.	mountain streams	stream boulders
<u>cooki</u>	400 min.	southeastern mountains	boulder grottoes
<u>coqui</u>	none	most of island	trees and shrubs in shade, on and in dwellings
<u>portoricensis</u>	300 min.	mountain forest	forest shrubs, palms, herbs
<u>antillensis</u>	3000 max.	most of island	shrubs, weeds in sunny areas
<u>brittoni</u>	2500 max.	most of island	weeds, tall grass in sunny areas
<u>wightmanae</u>	400 min.	mountain forest	forest floor, especially deep litter
<u>hedricki</u>	900 min.	eastern mountain forest	usually limited to tree holes
<u>eneidae</u>	900 min.	mountain forest	moss covered boulders and embankments
<u>locustus</u>	900 min.	eastern mountain forest	openings and edge of forest, especially fern thickets and mossy forest.
<u>cochranae</u>	300 max.	most of coastal plain	marshes, wet meadows, lawns
<u>gryllus</u>	900 min.	mountain forest	openings and edge of forest low canopies and bromeliads
<u>Rana</u> <u>catesbiana</u>	?	northeastern coastal plain	swamps and ponds

hatching the young are fully metamorphosed froglets with reabsorbed tails and functional lungs. Several writers have mentioned that a parent remains near the eggs during development, this has been incorrectly assumed to be the female. In the course of this survey the eggs of E. cooki, E. hedricki and E. coqui have been observed; the latter two have been seen repeatedly and the course of development followed. In all three the eggs have been attended by the male parent; in the latter two the eggs were attached to a wall of the shelter known to be routinely occupied during the day by that male.

A male, when in the shelter and not disturbed by the observer, kept some part of his body in contact with the egg mass, usually the lateral or ventral surface of the abdomen. If disturbed, he quickly left the shelter and hid nearby, but returned within a half hour or so and took up the same position. Eggs were observed to increase in diameter during development, even when the shelter walls were dry, indicating that water was absorbed from the skin of the male. Males were seen to travel at night to nearby sources of water where they drank by the usual amphibian method of skin absorption. Thus, in the species observed, such parental behavior is probably essential to normal development; in two cases where males were removed, the eggs failed to hatch. Hatching required approximately three weeks and the young remained in the shelter for about three days, sitting under or in contact with the male or with the collapsed egg skins, which remained moist. At the end of this time the young dispersed immediately, day or night, if disturbed; but undisturbed dispersal occurred at night and was not observed. Egg skins and remaining undeveloped eggs disappeared within a day after dispersal of the young; the exact method of removal has not been observed.

The species most thoroughly studied has been E. hedricki. Although rare by comparison with most of the species, adults of both sexes occupy treeholes, many of which are near eye level and are open enough for easy observation. Although, to our knowledge, no one has actually observed oviposition in Eleutherodactylus, Clayton Gist (personal communication) was able to bracket its occurrence closely by routinely observing for several nights a group of males whose shelters were known. He reports that a female was observed at 2100 hours in the shelter of a male known to have a week-old egg mass already developing. The pair were not in amplexus at that time, but by 0900 the following morning a new egg mass had been placed in contact with the old one. The female spent the day within the male's shelter, departing between observations at 1700 and 2100 the second evening. Males of this species continue to call from within and near the shelter while they attend eggs, and have been observed to accumulate as many as three egg masses at different stages of development.

Occasional observations of incubation by E. coqui are facilitated by their habit of adopting human artifacts, such as flowerpots, glass or metal cans and cardboard boxes as shelters. In the forest, detritus-filled axils of plant stems seem to be the most common shelters. Numerous coqui nests observed have always consisted of a single developmental stage. One male observed periodically through the course of four successive broods did not call while incubating except for a few occasions when new males began calling in his vicinity. At these times he emerged quickly from the shelter and called strongly until the newcomer stopped. Additional observations behavior in this species are reported under the heading of phase VII.

Egg care by E. cooki was observed once, and comparison with the observations of Juan Rivero (personal communication) indicates that it is typical. A male (verified by catching individual and observing vocal pouch development) covered with his body an egg mass attached in a shallow concavity on the underside of a large boulder some distance below the ground surface in a boulder grotto. When released, he hid in a crevice but was seen about an hour later on the eggs. He did not call during the time watched although two other males were calling at distances of about 10 feet. It is not known whether the site was a spot habitually frequented by the male.

Clearly these scattered observations need to be supplemented. The slow accumulation of data is due largely to the diffuse nature of terrestrial breeding, when compared to the large, usually seasonal aggregations of water breeding terrestrial frogs. Most Eleutherodactylus leave their shelters secretly and rapidly in the evening, and enter at dawn; territory-holding males seem to utilize the best concealed shelters available. The weight of authority stating that females are the attending parent (Schmidt, 1928) is an additional problem. Heatwole assumed (personal communication) that a clutch of E. richmondi eggs discovered in a decaying log were attended by the female, and did not attempt to determine the sex.

An entirely new set of hypotheses about the reproductive dynamics of Eleutherodactylus emerge from consideration of the terrestrial breeding pattern, the demonstrated male territoriality, and the implication of male attendance (from the only three species unequivocally studied). When males hold reproductive territory they increase the probability that their own genetic line will be propagated by mating with the nearby females; they also reduce the contribution of surplus males by suppressing their reproductive activity or forcing them to set up territories in marginal habitats. This appear to be the situation in E. hedricki, whose populations may be extrinsically limited by the availability of suitable treeholes and the ability of the very young to reach treeholes in the arboreal habitat of this species.

The young of *H. coqui*, however, seek shelter on or near the ground, and only large juveniles and adults are strongly arboreal. The apparent refusal of male coquis to accept new eggs while attending eggs, combined with continued defense of the acoustic territory, could therefore serve as an intrinsic, density-dependent mechanism for population limitation, forcing surplus reproductives of both sexes into marginal habitats, and keeping the territory free of competitive young at their most numerous, least mobile age. The following observations may be relevant to this hypothesis: (a) the populations of normal frog predators such as small mammals and snakes are unusually low in Puerto Rico; (b) frog populations of many species are extremely high; (c) vocal activity of frogs in Puerto Rico is greater than even the population densities would lead one to expect; (d) the diversity of calls produced by some species indicates that vocal functions other than simple mate attraction are being met.

A hypothesis receiving some attention from ecologists recently is that overlap in ecological niche between territorial species can favor the development of interspecific territorial behavior. Orians and Horn (1969) and Orians and Willson (1964) clearly outlined this hypothesis, and their studies of blackbirds with partially overlapping niches support the theory. They considered the visual defense of feeding territory from which the young were supported by the adults, and stated that the structural complexity of the habitat controlled the energetic expense of defense through the amount of patrol required. Most habitats are relatively transparent to sound, however, so relatively inexpensive modifications of acoustic territorial signals should enable them to carry interspecific messages. The learned mimicry of such bird species as the mockingbird probably functions in this regard, and much other mimicry may be uncovered by experiments that elucidate the exact nature of territorial, as distinguished from mate attraction, aspects of the calls. The converse of the above hypothesis could even be true in forms as strongly acoustic in orientation as frogs; that the demonstrable amount of interspecific territoriality indicates the amount of potential niche overlap between the beneficiaries, normally the young.

Phase IV - Vocal Organization

A survey of vocalizations was made in collaboration with Dr. A.S. Rand of the Smithsonian Tropical Research Institute. Calls were tape recorded, using several recorders at different times. Segments of activity 2.4 seconds in duration were analyzed with the Kay Electric Sound Spectrograph; one or more calls for most species can be included in a sample of this length. The frequency compo-

sition of each call and of whole assemblages were analyzed both with the sound spectrograph and with a B & K type 2107 Frequency Analyzer. Vocal organization over longer time intervals has been sampled from tapes and outdoor microphones using the analyzer, a diode demodulator and a galvanometric chart recorder. Early efforts to continuously monitor selected species with fixed comb filters tuned to dominant frequencies have yielded equivocal results because the species were discovered to shift dominant frequencies in the course of the year. A scanning and integrating system which will cover any desired portion of the audio spectrum in approximately one hour and continuously record a series of spectrum plots is currently being placed in operation. All but a few overlapping species can be picked out as separate peaks in each scan. These have differing activity cycles, so their periods of peak activity appear distinctly in time.

Figure 1 presents sound spectrograms of representative calls for each species; these are tracings from which background sounds not produced by the individual frog have been edited. Compression and mark levels of the machine were adjusted to print only components that were within 12 dB of the loudest component. Although weaker components are frequently useful for deducing attributes of the sound-producing apparatus, they have seldom been found to have biological significance. The calls presented in figure 2 are considered not to be functionally homologous to the regular calls and their functions will be discussed later.

Figure 3 presents vocal organization over longer periods of time for those species which have been adequately studied. Resolution on this scale is not always enough to show individual notes, and some calls composed of rapid sequences of notes are shown as single marks. Another compromise adopted in this presentation is selection of a vertical scale; an attempt is made to simulate oscillogram format, where mark width is equivalent to amplitude, but where notes represent different frequencies, as in the case of E. coqui, the wider marks denote the higher frequency. Mark width denotes duration in the case of E. eneidae, E. locustus, etc.

A working vocabulary has been developed for the verbal description of vocal parameters; it agrees in general with the usage of other bio-acoustical workers, but differ in slight details. The note, taken as the basic descriptive unit, represents the sound modulation produced by a single contraction of the thoracic musculature. Notes can usually be identified by observing the body wall and/or vocal pouch of a calling animal. In recorded sounds, notes can often be identified by observing the wave-form envelope on an oscilloscope. Because muscles cannot reach full tension instantaneously, the leading edges of notes are rounded, with a relatively gradual rise-time in intensity (note the exception below).

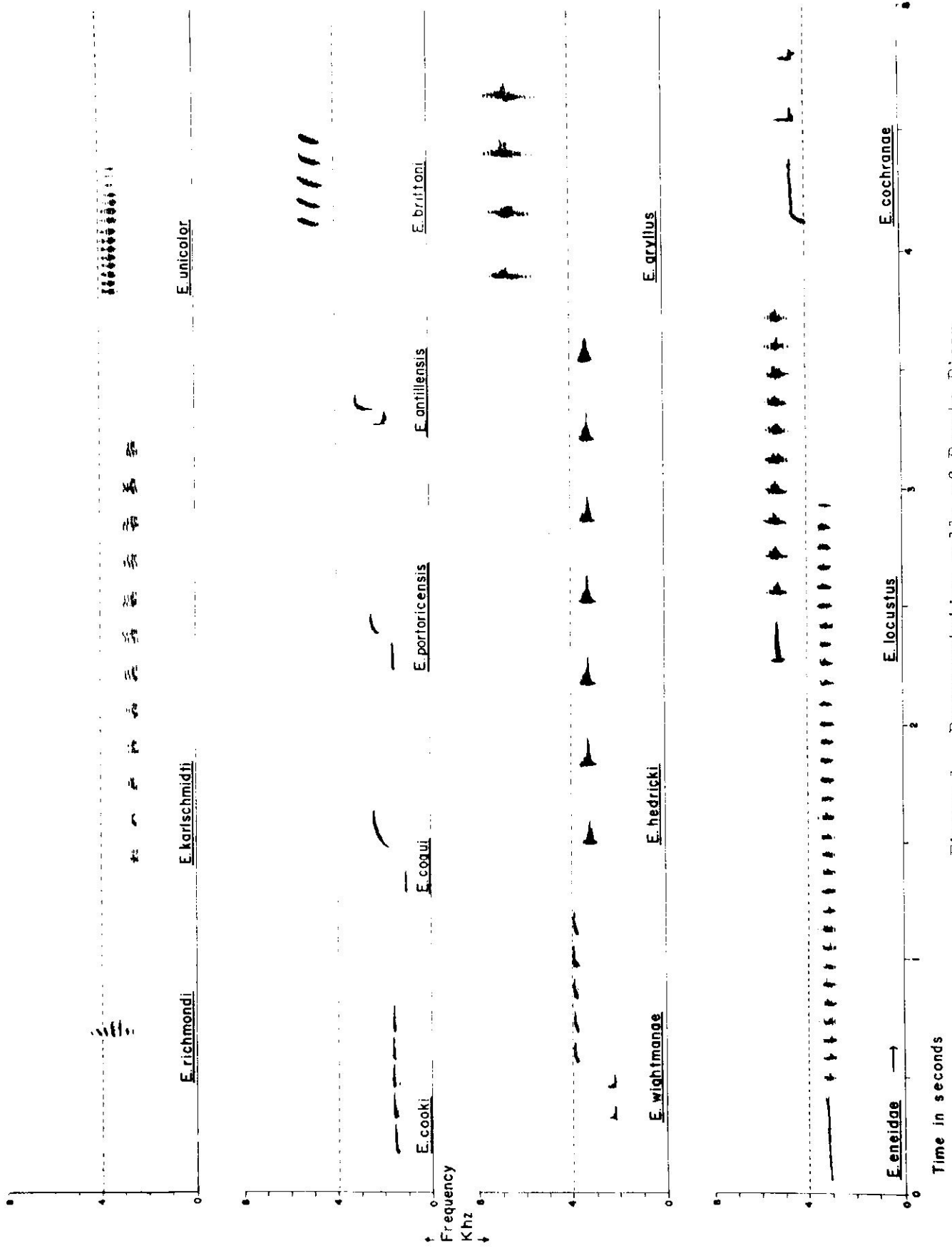


Figure 1. Representative calls of Puerto Rican Eleutherodactylus.

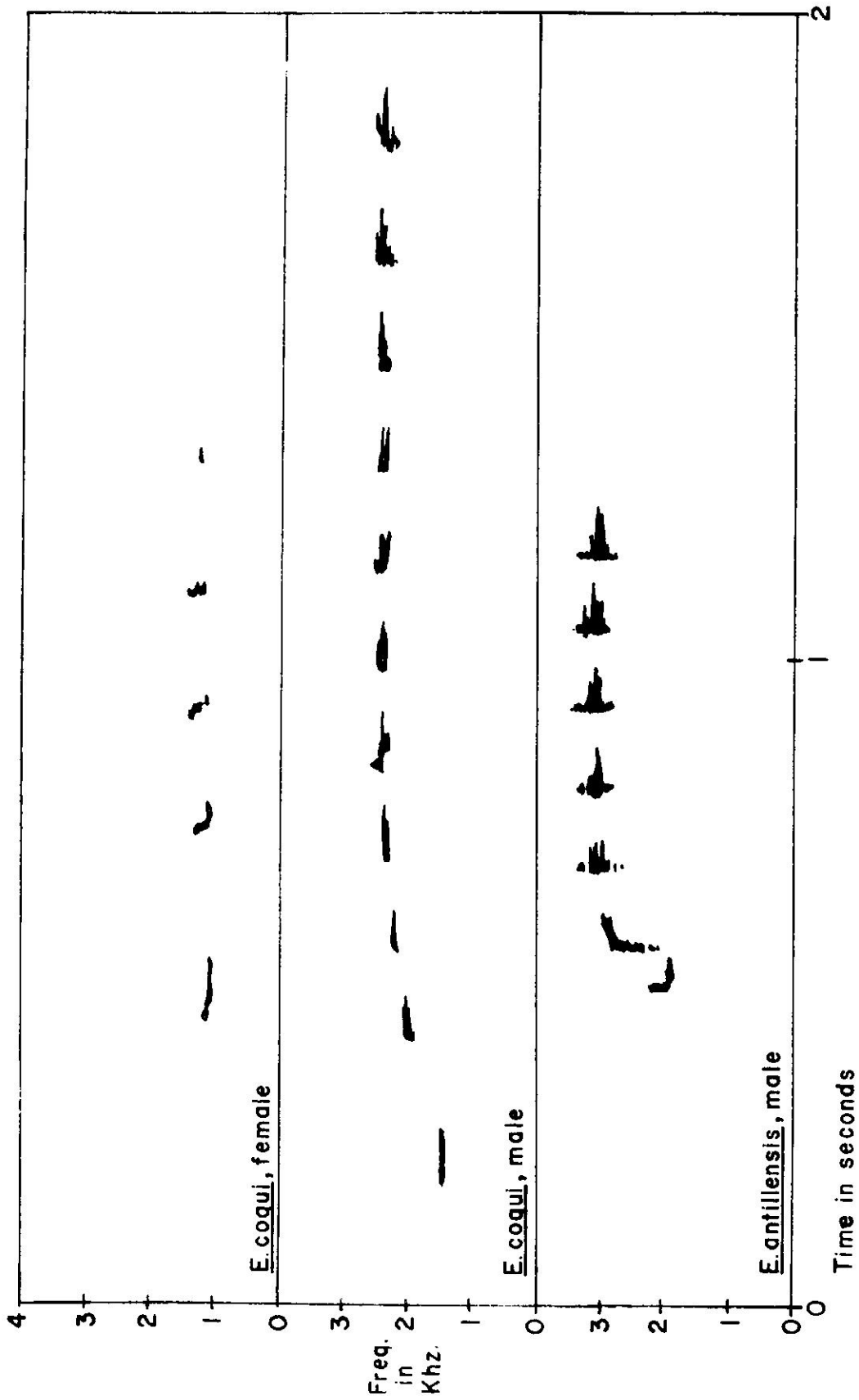


Figure 2. Occasional calls of E. coqui and E. antillensis.
 The calls of E. coqui are interpreted as warning calls.

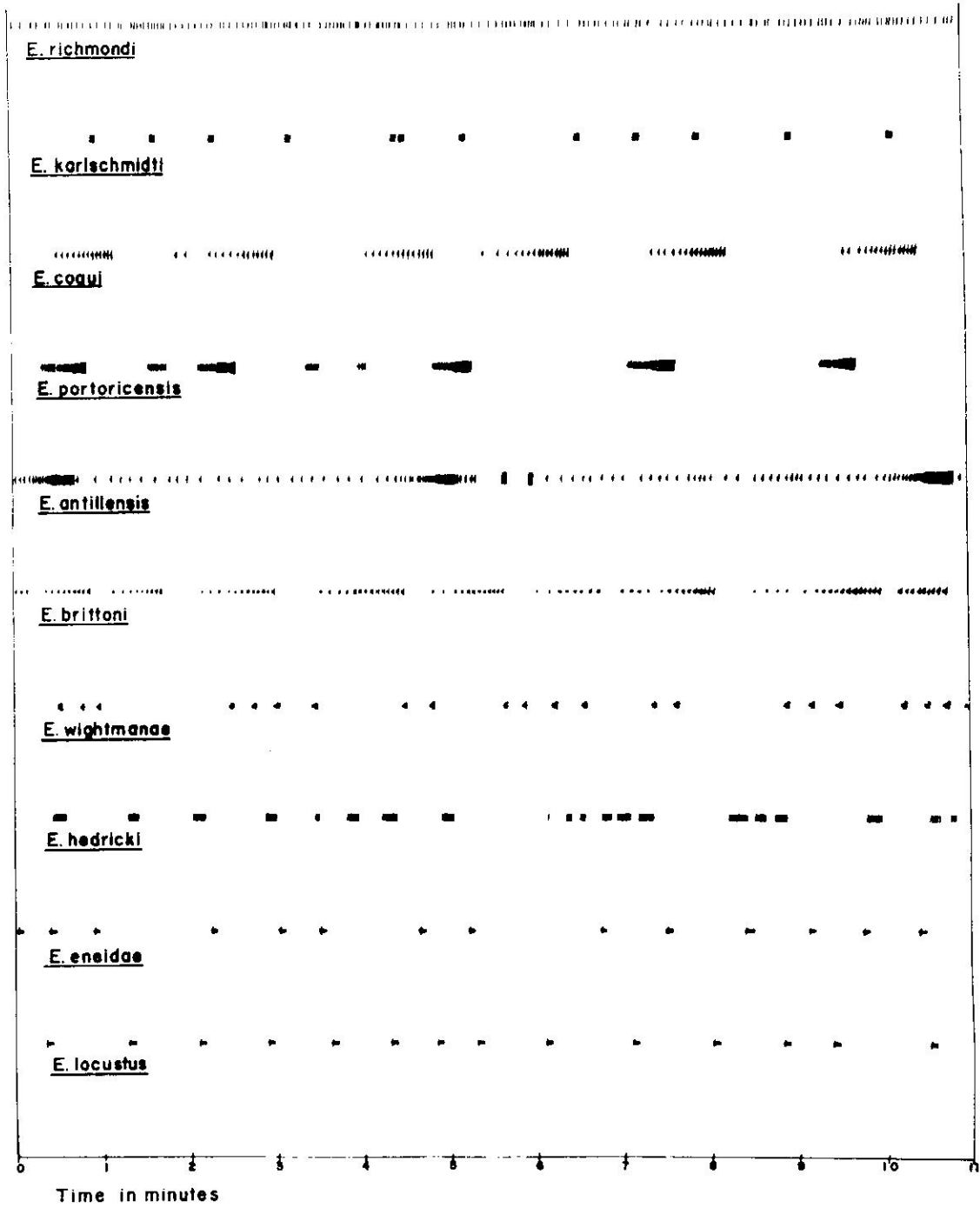


Figure 3. Calling patterns of ten species of Puerto Rican Eleutherodactylus. See text for additional explanation.

Many species use structures within the larynx or the glottis to introduce pulse modulation within the period of single notes (Martin 1967). The repetition rate of these pulses can reach an appreciable fraction of the vocal chord vibration rate. In some species the vocal chords themselves produce low frequency pulses which may or may not be further modulated by other structures; only the high order harmonics of the pulses are coupled to the air from the radiating surfaces of the vocal pouch or body wall. In other species the vocal chords vibrate at a high frequency and only the fundamental or a single low order harmonic is coupled out, producing clear whistles or peeps. Low frequency pulse modulation is always audible as a harsh grating or squeaking quality in the sound; it is identifiable in oscillograms as sharp rise-time pulses and in sonagrams as a series of spaced lines whose spacing defines the pulse frequency. High frequency vocal chord vibration with secondary pulse modulation appears in sonagrams as a central, strongly dominant frequency with more or less symmetrical sidebands above and below, while pulse modulation by the vocal chords themselves appears as more smoothly spaced harmonics whose amplitude distribution outlines the response characteristics of resonant structures in the acoustic pathway. Pulse modulation is evident in the calls of E. richmondi, E. karlschmidti and E. unicolor shown in figure 1. Their general relationship to one another is discussed later.

Low frequency pulse modulation is a potent source of confusion in discovering acoustic homologies, as slow pulses are analogous to fast note repetition rates, and considerable overlap occurs in anurans as a whole. Confusion is normally resolved by observation of calling animals. Frequency changes in successive pulses of sound often indicate that the animal is holding continuous muscle tension, while with each pulse air migrates from the lungs to the vocal pouch or mouth, changing the resonant frequency of these structures. A final confusion arises from the possibility that the pulse forming structures can close between notes, opening explosively at the beginning of each note to confer a fast rise time and produce a wide band noise to be shaped by the filtering action of resonant structures. The clicks produced by E. eneidae, E. locustus, E. cochranæ, E. gryllus and the longer notes of E. hedricki are believed to result from such action; the beginning of each note has a broad bandwidth but lacks distinguishable sidebands and appears to be a filtered noise, while the remainder of the note resembles the unpulsed introductory whistles. Thus, each click is a single pulse; it is also a single note as suggested by the lack of progressive change in dominant frequency and easily verifiable by observation. The clicks of E. gryllus exhibit a trend, however, toward pulse-modulation similar to that of E. richmondi; the pulse-forming structures apparently vibrate through the course of the note and superimpose pulse modulation ranging from slight to fully developed.

The changing dominant frequencies of E. coqui, E. portoricensis and E. antillensis represent more sophisticated vocal artistry than

the passive shifts that occur as a by-product of low frequency pulse modulation. These are two-note calls, as verified by observation of the body wall, in which vocal pouch resonance is changed by moving air in or out in synchrony with the laryngeal mechanisms, in a manner analogous to the playing of a trombone. A more exact idea of the precision involved is gained from imagining a hybrid instrument between a trombone and a single key accordion; the air volume of the instrument controls the resonant frequency and at the same time the air movement powers the reed. Thus, the reed must be keyed in and out at the exact times when the changing volume is right for the notes to be played. Individuals of these species are sometimes observed to miss the exact synchronization required and produce weak, off-key notes. They also engage in tune-up procedures reminiscent of human musicians.

Calls represent the next level of organization beyond notes, and the calls of several frog species consist of single notes. The unit of repetition defines calls where repetitive sequences of dissimilar notes occur, at least if the differences involve frequency or note duration. One difficulty in interpreting the present species occurs with E. brittoni, which produces sequential clusters of increasing numbers of notes. The number of notes at any given point in the sequence cannot be predicted exactly, however, which suggests that each cluster of notes is a separate call. The hypothesis is strengthened by the similarity to the call organization of E. antillensis in figure 2; other evidence for their close relationships is given later. The notes of E. hedricki are harder to interpret; a call may be one note or a sequence of notes. A later discussion of relationships suggests the latter. Despite the rapid note repetition rate of Leptodactylus albilabris, the unpredictability of note number indicates that each note is a single call. There is no sign of note organization in that species; an unvarying sequence of notes can extend for more than an hour.

The term "call group" applies to a series of calls separated from later, similar series by periods of silence. In those species exhibiting call groups, the number of calls per group cannot be predicted exactly. However, call rate does characterize each species and was the basis for discovering the distinction between E. coqui, and E. portoricensis (Thomas 1966). The function of call groups themselves will be discussed more fully in phase VII but it was noted in the survey phases that the beginning and end of the daily cycle of vocal activity involve periods of continuous calling in which call group organization is not apparent. At these times, males are apt to call from within, or very near to, their daytime shelters, where eggs in those species observed are deposited. The implication is that mate attraction may occur during these periods, leaving call group organization to be a territorial device. The hypothesis is strengthened by the fact that, as call group organization develops, the males climb vertical structures, sometimes high above their daytime shelters.

Available data on the organization of vocal activity in the diurnal cycle is diagrammed in figure 4. This preliminary information is pending the quantification of annual changes and a better understanding of climatic and altitudinal variables. As stated in the section on microhabitat, the species do not respond to the same variables in the same way. For example, the vocal activity of E. coqui seems to be more influenced by humidity than by temperature, while the reverse seems to be true of E. hedricki. Although both call actively on warm, wet nights, only E. hedricki is very active on warm dry nights, typical of late March and early April; occasional cool, wet nights in the same period reverse the relative activity.

Most of the species appear to be stimulated into activity by sound, including that of other species. The only one that appears to be negatively affected by other species is E. karlschmidti, which gives the subjective impression of filling silences, even at times during the day. This species and E. wightmanae synchronize calls closely with other conspecific males, and seem to be stimulated to call by flashes of light from passing automobiles, etc. Many of the species respond with calls to the onset of showers, even during the day. Heavy rain can depress calling activity in the smaller species such as E. brittoni and E. wightmanae; perhaps the mechanical effects of striking raindrops are responsible.

Not enough data is available to compare annual patterns in vocal activity. E. richmondi appears to be stimulated by low temperatures; it is maximally active on the coolest nights while most other species, except possibly E. karlschmidti and the inadequately studied E. unicolor, are silent. Again, the slope of correlation with most of the affecting weather variables differ between species. The Rain Forest Program Annual Report 1964 stated that temperature and humidity alone were inadequate to account for seasonal changes in the vocal activity of the E. portoricensis complex; hormonal changes are believed to be involved. The frequencies monitored in that study, incidentally, lie in the band of overlap between the second notes of E. coqui and E. portoricensis, and activity levels of both species were actually studied.

Appendix I provides a key to the vocalizations of common Puerto Rican frogs; only the Hyla and Bufo lemur were not studied in our survey of vocalizations and are omitted. An effort was made to make the key usable by non-specialists; terminology not found in standard dictionaries is avoided and no measurements are required. A tape of typical calls of each species has also been prepared.

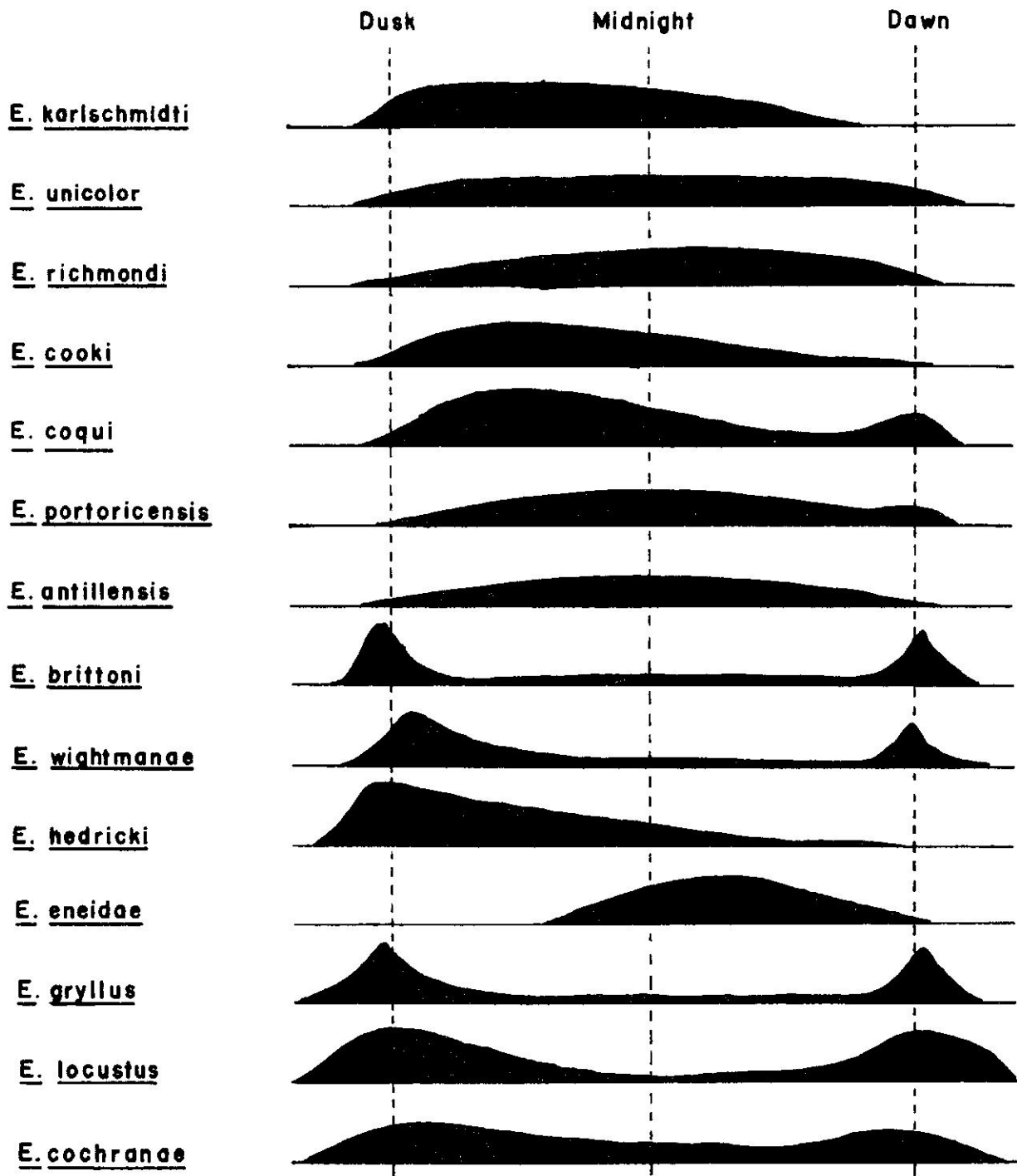


Figure 4. A diagrammatic approximation of diurnal vocal activity cycles in Puerto Rican Eleutherodactylus. The patterns show can be modified by several variables.

Phase V - Evolutionary Relationships

The survey also tried to establish the main features of evolution in the Puerto Rican Eleutherodactylus. The dynamic equilibrium, which is the real subject matter of ecology, can be most profitably interpreted when it is considered over an extended time, and, conversely, evolutionary trends are most meaningful in the ecological context.

A tentative pattern of relationships can be derived from the information in Table 2, which draws on morphological evidence, chromosomal studies, and the patterns of vocal organization. Comparative morphology of Eleutherodactylus can be seen in Appendix II, which is a guide compiled from our experience in field identification of species, and is also intended for use by non-specialists. Numerous errors are probably still present, and additions, corrections, or suggestions will be welcomed. The toads (Bufo), the bullfrog (Rana catesbiana) and Leptodactylus albilabris are not included; their identification should pose little problem, since all of them lack expanded toe pads, the bullfrog has webbed hind feet and the toads have dry, warty skin.

Differences in size of eyes and toe pads of Eleutherodactylus received considerable attention from early classifiers, but are not reflected in the patterns of relationship outlined in Table 2. This results from application of the theory that features of the greatest ecological importance are most rigorously subjected to the action of natural selection, i.e., structures and behavior used in foraging, concealment, reproduction, and locomotion within the microhabitat. Thus, they should tend to change most rapidly in evolutionary time, whether the changes are parallel, divergent or convergent. The vocalizations used to attract mates are particularly subject to pressures toward divergence, since failure to achieve recognizability can only result in the production of hybrids, which are apt to be non-viable, sterile or otherwise poorly adapted. The morphological features of color and pattern have long misled the prospective student of Eleutherodactylus classification; some of the species, including the common coqui, maintain systems of color polymorphism that fascinate geneticists, but only confuse the effort to identify species. If the indicated relationships are correct, polymorphism itself is partially independent of phylogenetic proximity, the most extreme species apparently being E. coqui and E. locustus.

Chromosome number is perhaps the most fundamental characteristic selected for inclusion in Table 2. This characteristic is relatively conservative, since it is not subject to strong selective pressures. Only two numbers appear in the Puerto Rican Eleutherodactylus fauna, 2N of 30 and 26, and neither the numbers nor the number of chromosome

Table 2. Tentative pattern of evolutionary relationships
in Puerto Rican Eleutherodactylus.

Species	Diploid Chromosomes	Canthus rostralis	Call ramps	Short term call synchro- nizations	hour- glass marks
<u>E. richmondi</u>	30	P	-	W	-
<u>E. unicolor</u>	30	P	-	?	-
<u>E. karlschmidti</u>	30	P	-	S	-
<u>E. coqui</u>	26	P	+	M	+
<u>E. cooki</u>	26	M	+	?	-
<u>E. portoricensis</u>	26	P	+	S	+
<u>E. antillensis</u>	26	P	+	S	-
<u>E. brittoni</u>	26	P	+	M	-
<u>E. wightmanae</u>	26	M	-	S	-
<u>E. hedricki</u>	26	R	-	S	-
<u>E. eneidae</u>	26	R	-	W	-
<u>E. locustus</u>	26	R	-	W	+
<u>E. cochranae</u>	26	R	-	?	+
<u>E. gryllus</u>	26	R	-	S	+

abbreviations used

- P prominent and straight
- R rounded and curved front to back
- M intermediate
- W weak
- S strong
- +
- typically present
- typically absent
- ? inadequately studied

arms based on centromere position indicate that one was recently derived from the other. Chromosome morphology is being studied in collaboration with Dr. James Bogart of the University of Texas, who has evaluated the chromosomes of several Central and South American Eleutherodactylus species. He has not found the 26 number there and though the evaluation is not completed, it appears that the 26 chromosome species from Puerto Rico are fairly consistent internally and not closely related to any other species in his study. Chromosome numbers of species on other islands have not yet been determined; the 26 number or derivatives from it may be associated with the auriculatus species group mentioned by several authors, with representative in most Caribbean islands. If so, this group might be elevated to the status of a separate genus.

Chromosome morphology between those species in Puerto Rico having 30 chromosomes is not particularly consistent and the many other differences suggest that they have had long independent histories. All however, have a strong pulse modulation in the calls, and tend to be more active in cooler weather than the other species; they also have a straight, fairly prominent canthus rostralis, and almost no color polymorphism.

The species having 26 chromosomes can be subdivided into two groups based on the shape of the canthus rostralis and the tendency to organize calls into call groups of progressively increasing call rate. The status of E. wightmanae is uncertain, and may occupy an intermediate position; its call has two frequencies (Figure 1), it has weak call group organization (Figure 3), and its call rate does not tend to increase within call groups. Call rate increase, termed "ramping", distinguishes five species; E. cooki may represent most closely the ancestral morphology and vocal organization. Three of the ramping species have regular two-note calls of obvious homology (Figure 1), and occasional calls (Figure 2), with slightly higher pitch and multiple notes that show homology to the regular calls of the other two species. The calls of E. brittoni are complex, increasing in both the number and rate of notes, but are probably derived from an ancestral pattern common to that of E. antillensis. These two species are very similar in morphology and habitat, and possibly diverged only slightly longer ago than E. coqui and E. portoricensis, which are the most similar species pair in the fauna. It is noteworthy that E. portoricensis places less emphasis than does E. coqui on the higher pitched note in its call, and could be in the long term process of abandoning it.

Not enough data is yet available to assure that non-ramping species constitute a coherent subgroup. To some extent, vocal organization characteristics cut across patterns of maximum morphological similarity. There is a clear link between E. locustus and E. cochranæ, which has been discussed by Rivero and Majorca (1963)

The same pattern of call organization and structure is shown by E. eneidae, i.e., no call groups and a whistled long note followed by a series of clicks, with little tendency to tight call synchronization between neighbors, but the latter species also has morphological affinities with E. hedricki. The notes of E. hedricki could be interpreted as lengthened clicks, minus an introductory whistle. In that interpretation a whole sequence of notes would constitute a single call. Each sequence is normally given in synchrony with neighboring individuals. The synchronization of E. wightmanae is clearly at the call, rather than the call group level, with large numbers of individuals calling almost as a single frog. The calls of E. gryllus, on the other hand, seem to be synchronized at the call group level. Their organization seems to be similar to, but slower than, that of E. brittoni. The study of E. gryllus has been hampered by the difficulty of placing a microphone so that calls of one individual are distinguishable from others. At the elevation of the El Verde Station, the species usually calls high in trees, and calling males appear to be clustered in the same or adjacent trees, with non-calling individuals frequently encountered far from the chorus. This is the only species where the males show any evidence of congregation. The closest morphological affinities of the species seem to be with E. locustus.

The survey of relationships will, hopefully, be strengthened by a survey of biochemical similarities, using modern techniques of chromatography and electrophoresis. Chemical characteristics probably have a whole spectrum of sensitivity to selective forces, and will need to be evaluated in conjunction with the other data. An effort will also be made to interest students of comparative osteology in the opportunities presented by a genus in such an apparently active stage of species formation.

The phylogenetic divergences and convergences in the frog fauna of Puerto Rico will be significant in the broader interpretation of the evolution of species diversity in the tropics. Island faunas have already contributed uniquely in this respect, because the ever present problems of island dispersal have filled a variety of ecological niches with closely related species, in which evolutionary trends are more easily discerned. The contribution of the finches of the Galapagos Islands to the evolutionary theories of Charles Darwin is a classic illustration.

Phase VI - Experiments in Niche Definition and Energy Flow

This and the remaining phase of the study are still in active stages of data collection and will depend on quantitative results and experimental procedures to test hypotheses already outlined, to

generate new ones, and to round out our understanding of the ecological roles played by frogs. A detailed study is now being conducted on the types of prey items found in the stomachs of each available forest frog species; a progress report is presented under a separate heading.

Frogs in the vicinity of all experimental sites involving gamma-emitting radioisotopes have been monitored for uptake. Since individual frogs tend to remain for long periods of time in one area, repeated measurements of the same individual have been obtained. Animals were captured, whole body live-counted for gamma radiation, marked by toe clipping if not previously marked, and released at the point of capture. Samples have been small, but enough data has been accumulated in three experiments for preliminary conclusions to be reported and much information relating to design of future experiments has been gathered.

In one experiment, ^{134}Cs , ^{85}Sr , ^{54}Mn were applied to 4 square meters of forest floor underlain by water-collecting lysimeters at various depths. (FRNC 119). Animals were first tested in this plot eight months after isotope application. At that time 2 resident frogs of the E. portoricensis complex (not separated then) and 3 resident Anolis gundlachi lizards were found to have body concentrations of ^{134}Cs approaching those in the plants rooted in the plots; isotope traces were found in lizards as far out as 15 meters, and in frogs, including E. richmondi and E. wightmanae, out about 8 meters. Animals taken in the first collection were sacrificed to determine which tissues retained ^{134}Cs (it was generally distributed in the body), but new animals moving into the area were found to increase concentration gradually. Follow up studies at about yearly intervals have shown lower concentrations in animals. In the last sample taken (spring 1970) two individuals of E. coqui showed average counts of 5 ± 4 cpm above background while a single E. portoricensis had 25 ± 4 cpm ^{134}Cs .

In a second experiment the epiphytic bryophytes, lichens and algae associated with tree trunks were labelled with ^{65}Zn . Three trees were used and the solution was applied to the first six feet of each trunk. Four specimens of E. coqui and one of E. hedricki, monitored over a two year period, reached an equilibrium concentration ranging from about 100 cpm per gram above background for a frog resident on one of the trunks to 20 cpm per gram in a frog living 2 meters away on a different trunk. Several individuals of the terrestrial species E. richmondi and E. wightmanae failed to show measurable uptake. Lizards showed much higher levels of uptake but none of the predatory vertebrates approaches the levels reached by snails of the genus Caracollus after even a single night of foraging on the trunks. It was concluded that the vertebrates did not ingest, or perhaps digest, the labelled plant material directly and probably received their intake of isotopes via prey that had fed directly or indirectly on the plants.

The third experiment is almost negative so far as isotope uptake by frogs is concerned, but the methods developed show promise for future studies. Large canopy trees were injected with gamma-emitting isotopes in two study plots about 80 meters apart. Scaffold towers adjacent to the trees were erected beforehand, and in an effort to increase frog populations in the areas, 28 nodes of bamboo were attached at 3 1/2 foot intervals to each of two diagonally opposite tower legs. The nodes were cut so as to be closed at both ends with a notch doorway near the bottom end. In four months the lower 22 shelters (38 1/2 feet) on each tower were colonized by E. coqui and E. hedricki of both sexes, with populations stabilizing at about 4 males and 4 to 6 females on each tower. The tree besides one tower was tagged with ^{85}Sr . At the other site one tree was tagged with a mixture of ^{85}Sr , ^{86}Rb and ^{54}Mn ; a second tree was labeled with ^{137}Cs . Details of these experiments are given in the Rain Forest Annual Report 1969. After 18 months ^{85}Sr and ^{137}Cs are the only isotopes detectable in the tree leaves. Canopy foraging lizards of two species in both areas showed measurable uptake of ^{85}Sr by the fall of 1969 but ground-dwelling species did not. The tower frog populations have been gamma-counted at intervals of about two months. In February 1970 a single female E. coqui living near the ground showed a barely measurable (less than 10 cpm) uptake of ^{137}Cs , after having been negative in December. At that time, litter-dwelling frogs were tested for ^{137}Cs , and three E. wightmanae had measurable levels, while two juveniles E. coqui did not. Most of the tower population of frogs remain uncontaminated by either isotope, as do the ground dwelling lizards.

Future tracer experiments to study energy flow appear promising and can provide data on both the fate of radionuclides introduced in the system and the sources of energy tapped by various branches of the animal food web. A proposed experiment would label living plant tissue with one isotope and the decomposing compartment with a second isotope. A second treatment would use the same isotope pair in reversed configuration. By monitoring the gradual mixing of these isotopes in living plants and in the litter layers, a source ratio could be developed with which to compare ratios in the predatory animals. Ratios in these animals might then provide an estimate of the relative proportion of prey that are herbivores and saprovores, and thus quantify the fraction of energy passing through the plant decomposer compartment before entering the animal food web. This fraction is expected to be high in the forest generally, although higher for the predators dwelling in the litter layer than in the arboreal species. Similar experiments using ratios of paired isotopes in reciprocal applications (as a control on differences in isotopes behavior) can be used to compare energy flows from decomposing fruits and leaves, leaves and stems, or many other combinations of ecosystem compartments. All of these would supplement direct analyses of stomach contents in defining differences in feeding niche.

Phase VII - Territoriality and Population Dynamics

Throughout the survey phases of this program, general evidence has indicated that male frogs of most species practice reproductive territoriality and the acoustical signals are used to coordinate territorial behavior. The exact definition and biological consequences of territoriality are still somewhat controversial, but in general, reproductive territoriality involves the ability of individuals or pairs to discourage or prevent reproductive activity by rivals within a more or less definable area in space and time. In theory, such control can convey several advantages. It can help to ensure continuity of an individual's genetic line by increasing his probability of breeding. It can also allocate and secure resources necessary for survival of the brood; in addition spreading the reproductive population throughout the suitable habitat and placing the optimum resources at the disposal of the most vigorous individuals and their progeny. An interesting and still controversial hypothesis is that inflexible reproductive territory size could place a fairly sharp upper limit on total population size. An additional requirement for this function would be that the amount of reproduction within each territory have an upper limit. With these ideas in mind, a series of experiments have been designed to uncover the nature, role, and extent of reproductive territoriality in Puerto Rican frogs.

The coqui is a logical first species to choose for intensive study at El Verde Field Station, since it is abundant and the only species whose nocturnal activities are carried on above ground, in apparently normal fashion, within the area of artificial light provided by the station. Early efforts to study this and other species by flashlight made slow progress. Behavioral sequences were often interrupted by the unaccustomed light and no framework of reference was available to interpret some disconnected sequences observed.

Special attention was paid to calls for several months and unusual ones were tracked down for identification of behavioral correlates. Various systems for recognizing individuals were also developed; some had distinctive color patterns, some were toe clipped, and one small group were for a month given waistbands of tiny color-coded beads. The latter method is ideal for quick recognition at a distance but requires further refinement. While behavior appeared normal and breeding occurred, lesions developed where the bands rubbed the skin.

The population density of coquis within the area of the light appeared to be somewhat higher than in the forest, probably due to concentration of insect prey. The small population beaded for easy identification consisted of ten adults, six males and four females,

all retreating at daybreak into crevices in the corrugated aluminum walls of a small tool shed adjacent to the laboratory building. No adults permanently entered or left the area during the month of intensive observation, although the population of juveniles appeared to wax and wane. Only one male, the largest, called regularly within the shed. However, on a few evenings one of the two next largest males called a few times, resulting in noticeable increase of vocal activity by the established male. These apparent challenges were not sustained or repeated on subsequent evenings. The largest pair of frogs bred once during the month and the second largest female presumably did: eggs were visible through her skin, she was absent from her accustomed perch for one night and reappeared the subsequent night minus the eggs. She must have moved into the nearby forest for mating; none of the nearby established males being monitored mated that night.

When undisturbed, each adult frog moved in a fairly predictable pattern during the night. The dominant male began calling a few minutes before dark and called from within, or at the entrance to, his daytime shelter until about an hour after dark. The other frogs emerged just before dusk and often moved soon after dark to a nearby outdoor sink whose normally moist bottom provided drinking water. Within an hour after dark, each frog usually appeared within a foraging (or calling) territory whose area was almost inversely proportional to the size of the frog but whose quality, in terms of insect concentration, was in direct proportion to size. The two lighted windows nearby were each occupied by one of the largest females, each female had a favorite perch in a corner from which she sprang to obtain insects alighting on the pane. The dominant male had a series of calling stations to each side and above his shelter, in right angle corners and other sheltered spots inside the shed; he rotated from one to another but moved immediately to the nearest station to any other male that began calling. The other five males and two females moved about more, but each was apt to be found in favored locations, often on a wall that was featureless to the human eye. The dominant male interrupted calling to take insects on dry nights and stopped calling well before midnight, often moving directly to areas of high insect concentration up to ten feet away from the shed, indicating that the terrain and its prey possibilities were well known. On wet nights he was seldom seen to feed, and called almost continuously.

The peculiar call shown at the top of figure 2 was heard several times. The four times it was successfully traced, the source proved to be one of the large females, three times in favored feeding areas and once in the shelter crevice. Each time, the female was facing a smaller frog of either sex that had entered the area. After repeating the call a few times, the larger animal butted the sides of the smaller

and called again. The intruder usually retreated a few inches after the first attack and more when it was repeated; the aggressor usually persisted until the intruder was several feet away, then returned quickly to the favored perch. Such defense of a feeding territory was observed only in the large females; smaller females and males fed in apparent peace, although preferred spots seemed to cover the available space fairly well. It is not yet clear whether dominance hierarchies existed, or if feeding territories were marked, possibly with odor. The call just described is termed a "warning call" from its timing; but it elicited no observable effect when played in the vicinity of frogs of either sex. Perhaps it serves merely to alert recipients. The observer has heard it in apparently complete darkness; light revealed the usual confrontation of two frogs.

A male warning call, shown in Figure 2, appears to differ from the female call mainly in frequency and intensity, both probably due to the presence and inflation of a vocal pouch. Male warning calls exhibit a range of increasing intensity and frequency, and blend at the low end of both with "warm-up" notes given in the daytime, especially near dusk and dawn, from the male shelter. Warm-up calls are repetitions of either or both of the two notes of the regular call and had no observable behavior correlate. A slightly more intense version at night is a good indication that the caller will soon stop calling, but it can usually be linked in time to a regular call being given nearby, and exhibits agonistic content by analogy to the next most intense version. This perhaps the most interesting of the male territorial calls; it is given by established males from the shelter or a regular calling station when a newcomer begins to call after the established males have grown silent for the evening. When newcomers persisted in calling the warning calls were usually followed up by butting attacks, exactly as in females, except that males were observed to travel 10 to 15 ft. to attack. Several established males sometimes arrived simultaneously and effectively silenced or drove away the newcomer. Warning calls were only given within the area of calling stations; if the newcomer was within such an area, warning calls were interspersed with attacks, while established males drinking or foraging away from their stations attacked without calling. The exact relationships here were worked out by playing calls with a tape recorder after the established males stopped calling; this often stimulated the non-established males to call, setting off the chain of events described. If no male called, the established males approached the tape recorder itself, attacking any other frog they encountered enroute, including each other. Males with eggs in their shelters seemed to have two options in responding to newcomers; they left the shelter and began regular calling or gave the warning call, followed soon by a butting attack. Note that all of these responses were given to non-established males. The response of non-calling established males to calling by other established males

was either indifference or an occasional low intensity warning call. Achievement of a calling territory clearly involves reciprocal recognition with a series of neighbors, at least at the population density of the observed group.

The highest intensity (and frequency) of male warning call is used when any other frog enters the male's shelter, particularly if eggs are present. This is a no-nonsense call, followed immediately by fierce pushing, and was once observed to be given to a lizard attempting entry. The call is most frequently heard at dawn on rainy mornings and apparently coincides with the presence of frogs that are moving into new areas and seeking shelter without knowledge of the pattern of habitation. As already mentioned, females call under this circumstance, and the calls were also given by males with shelters who lacked established call territories. Intruders sometimes responded by retreating before they were attacked, although again no response was obtained to the sound alone from a tape recorder.

At this point, the general behavioral framework of the adult coqui population appears to be established, although some experiments can further clarify details. Three types of territorial orientation are discernible: the shelter, which is defended by all adults; feeding territories, that can be based on past feeding success and can lie at a distance from the shelter, defended mostly by females; and call territories of males, which involve interactions with neighbors and have a small perimeter of calling stations but a much larger perimeter of area defended. Agonistic behavior affecting other frogs is observed in the defense of each type of territory, and similar, probably homologous, calls accompany each type of defense. In at least the dense populations studied, males lacking call territories outnumbered males with them and provided a reproductive reserve, being almost surely unable to breed before obtaining call territories.

Two supporting experiments are relevant here; one is a homing experiment and the other involves restricting movement by means of a cage. So far most homing studies have been performed with established males; they home reliably over distance up to 50 meters and some individuals returned from 100 meters when carried in the same direction as a previous 50 meter displacement. A few females and non-calling males have failed to home when carried 50 meters. Further studies are planned to test this type of behavior. Cage studies have provided two important clues to the reproductive territorial behavior. A cage made of 1/4 inch mesh, galvanized hardware cloth was provided with plants, shelter, and a small shaded light bulb to attract insects through the mesh. The cage was located about six feet from the shelter of an established male. One very large, previously established male and one non-calling young male were introduced to the cage. Although well nourished, neither male was able to establish regular calling territory within the cage in six months. Each caged male has challenged repeatedly,

more often than is usual for non-established males. The reason for more frequent challenges is probably that the cage protects them from attacks by the resident male. When either begins to call after the established male stops, he often calls for the remainder of the night, while the two nearest established males crawl repeatedly over the surface of the cage seeking entry routes and often butting one another, or return to their calling stations and finish out the evening in a calling trio with the caged male. There may be several reasons for the caged males' failure to establish regular calling early in the evening; they cannot retreat far enough from the established male; they cannot attract mates through the wire and, perhaps most important, they cannot engage in physical contests with outside frogs, in which the large size of one male might confer the required advantage. This male has, in the past two months, attacked the smaller male in the cage when it calls, indicating that at least one attribute of call territoriality has developed. No frogs showed evidence of physical damage from the attacks.

Vocal interaction between caged males and the established males outside the cage has clarified some of the acoustic mechanisms by which call territories are won and held. Observation has been supplemented with experiments where tape recordings played through a small outdoor speaker served as a stimulus and the responses of a selected male were recorded in correct time relationship on the other channel of a stereo tape recorder. Three general classes of responses can be distinguished: subordinate responses, equilibrium responses and responses to challenge by newcomers.

The early evening pattern of calling in E. coqui lacks the ramp pattern illustrated in figure 3, and is strongly suspected to represent the mate attracting system. At this time little or no interaction between adjacent males can be noticed, although challenges from within the call territory apparently stimulate early development of the ramp pattern. By the time the male moves to a call station outside his shelter, a plot of call rate versus time begins to show increases and decreases in rate, with pauses beginning to appear when the rate is low. Ramping in this species is very difficult to detect with the ear alone - we lack sensitivity to rate changes spread over periods of several minutes - but evidence that the frogs are very sensitive appears when we superimpose ramps of interacting individuals. Ramp structure is much easier to detect in E. portoricensis, which calls faster and whose individuals interact as large groups. Male coquis, on the other hand, interact in pairs or trios whose phase changes and ramp durations are synchronized but independent of other neighbors.

The responses termed "equilibrium responses", can proceed at two levels and usually involve two neighbors, each of whose calls are louder when measured at the calling site of the other than those of any other coqui. One level is ramp synchronization, in which peaks

and valleys of call rate remain in register, while calls usually shift slowly back and forth in phase, with first one animal and then the other leading. This sometimes gives way abruptly to note synchronization, in which one individual drops the second (high-pitched) note and locks his first note into fixed phase with the first note of the other. Depending on temperature, the lag between leading edges of first notes ranges from about 0.08 to 0.15 seconds, which probably represents the acoustic reaction time. The pauses during low call rate periods disappear in either type of synchronization, and rate fluctuations are still further reduced during note synchronization.

Exact equilibrium during note synchronization involves a balanced exchange of the leading position; one male holds the lead for about 10 calls (20 seconds) and executes a ramp, then as he slows the other male speeds up and obtains the lead. Sometimes the exchange point is marked by both frogs omitting the second note. Figure 5 illustrates a pattern of exchange in which balance was not exact; this exchange was interesting because the territorial history of the males was known. The male (no. 1) which held the lead most often had been established for over a year, while the other had been calling regularly for only two or three weeks. Responsibility for failure to smoothly exchange the lead is difficult to assign to either frog, but the longer established male appeared to interrupt often before the other relinquished the lead by slowing down. The first two arrows mark points at which neither male produced second notes; in each case male number 1 took the lead on the next note. There is other evidence of subordination in the responses of male number 2, and it is possible that the ratio of total second notes produced by each frog, 93:64 in this sequence, represents a numerical statement of relative dominance between this pair of males. Extremes of balance ranged from almost 30 minutes of smooth exchange to situations in which note synchronization was accepted by an established male but the other male was not allowed to hold the lead for more than one or two consecutive notes. Non-established males sometimes produced soft low-pitched notes in perfect synchrony with the lagging member of a pair of synchronized, established males, but any effort to take the lead by the third frog caused the other two to immediately abandon note synchronization. Three established males were once observed in balanced note synchrony for two complete rounds of the lead. Each male produced a ramp while the other two lagged in unison, and each waited two ramps before taking the lead; after this, one of the males did not obtain the lead again.

Insight into the function of two-note calls was gained from these observations, from experiments with whistled first or second notes and from tape recordings where frequency filters were used to remove one component. Only ramp synchronization could be obtained as a response to high-pitched notes alone, or to complete calls, which

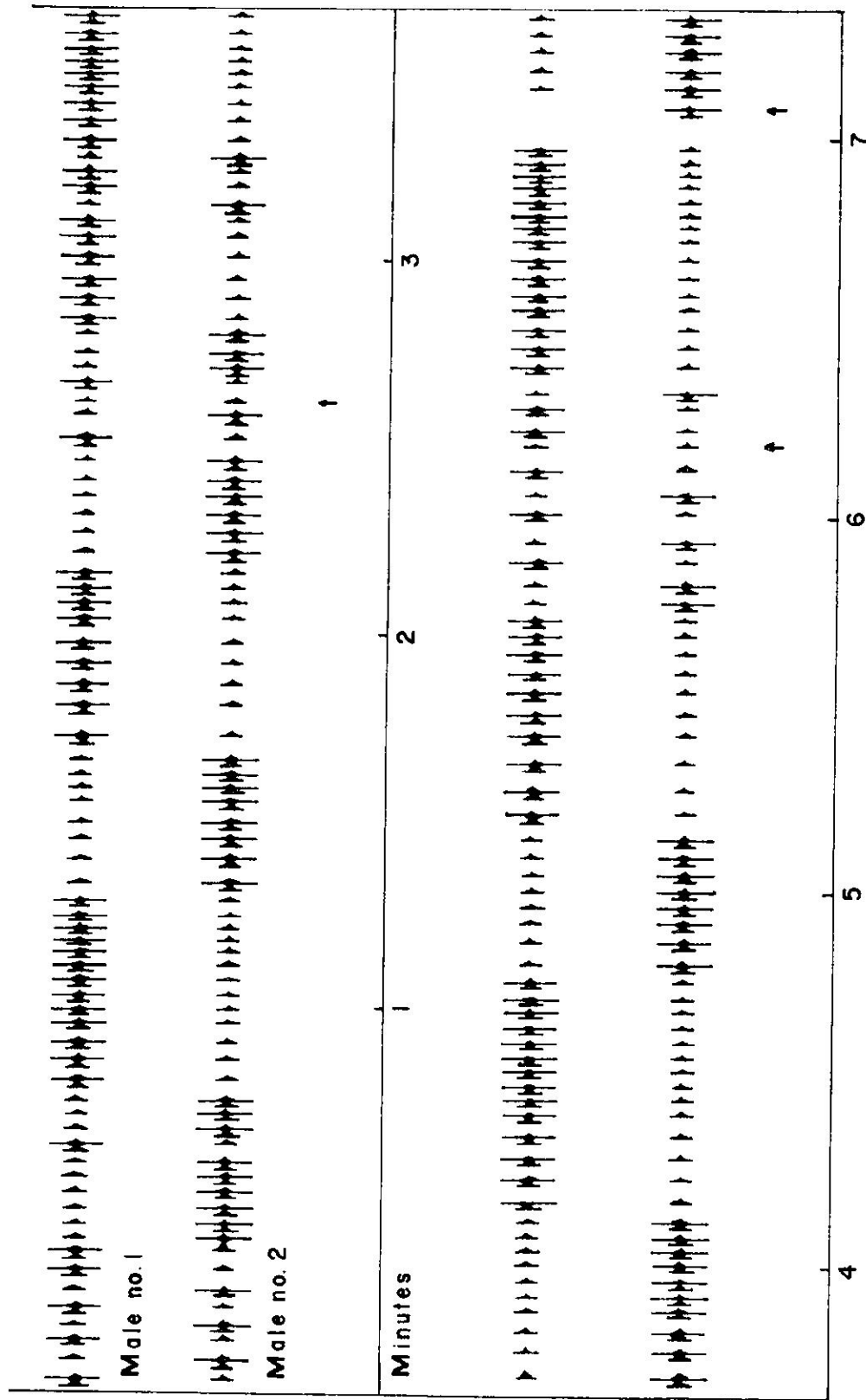


Figure 5. An acoustic interaction between two male coquis. The short marks are low-pitched first notes, longer marks represent high-pitched second notes. Arrows indicate departures from smooth exchange.

received the same response as inception of calling by non-established males. Presentation of the low-pitched first notes alone, however, often stimulated note synchronization, particularly when played near the end of the calling period. It was possible to set up equilibrium note synchronization with a filtered tape when intensities, times, and temperatures were carefully matched. Evidently an agonistic message is carried by the high-pitched notes, while the low-pitched first notes provide a stimulus for synchronized, balancing responses. Several lines of evidence converge to support this view, including the high pitch of warning calls and the response to non-established males, which include active call responses and the with-holding of note synchronization with its suppression of high notes. More evidence came from the responses to intensity of playback calls; when first notes alone were played, intensity was not critical over a wide range, but when second notes were made louder than those of a normal frog, response usually stopped altogether, sometimes for hours. These notes appear to have a cumulative effect on all males, which is probably somehow related to an integral involving intensity, repetition rate and time.

Additional subtleties were discovered in the more common type of vocal interaction, ramp synchronization. Besides a tendency to shift peak calling rate into register with the loudest neighbor, there appears to be an effort to increase average calling rate when interacting with an unfamiliar caller. Details of ramp synchronization interactions are still being studied, but the general pattern of this behavior can be outlined using data from a related species, E. antillensis. Figure 6 shows results of an experiment that tested reactions of a single male of that species to playback of an earlier recording of his own voice.

The heavy line in each pair of traces in figure 6 is the integral of call rate versus time in the tape played as a stimulus. The tape recording began at 10:00 p.m. when the temperature was 21°C and the male was interacting minimally with neighbors. The dashed line in figure 6 is a superimposed trace, recorded at the same hour the next evening, at the same temperature. This time the male was interacting with an established neighbor, and the pair of traces serve as a control for competitive response when the test stimulus is not heard by the male. Note that the call rate cycles are not repetitious enough for the minima and maxima to remain in any fixed relationship. The remaining three pairs of lines are responses to the stimulus tape at three different temperatures, each made at 10:00 p.m. on a different night. Temperature was 20°C in figure 6B, 21°C in figure 6C, and 22°C in figure 6D. The stimulus was presented from a small speaker placed seven feet from the animal. Volume was adjusted to give the same intensity measured at the animal as the animal's call gave measured at the speaker. The response was taken from a microphone placed as close to the animal as possible; this signal was filtered and demodulated into pulses which were fed to a pulse height discriminating circuit, which in turn delivered a pulse to a recording

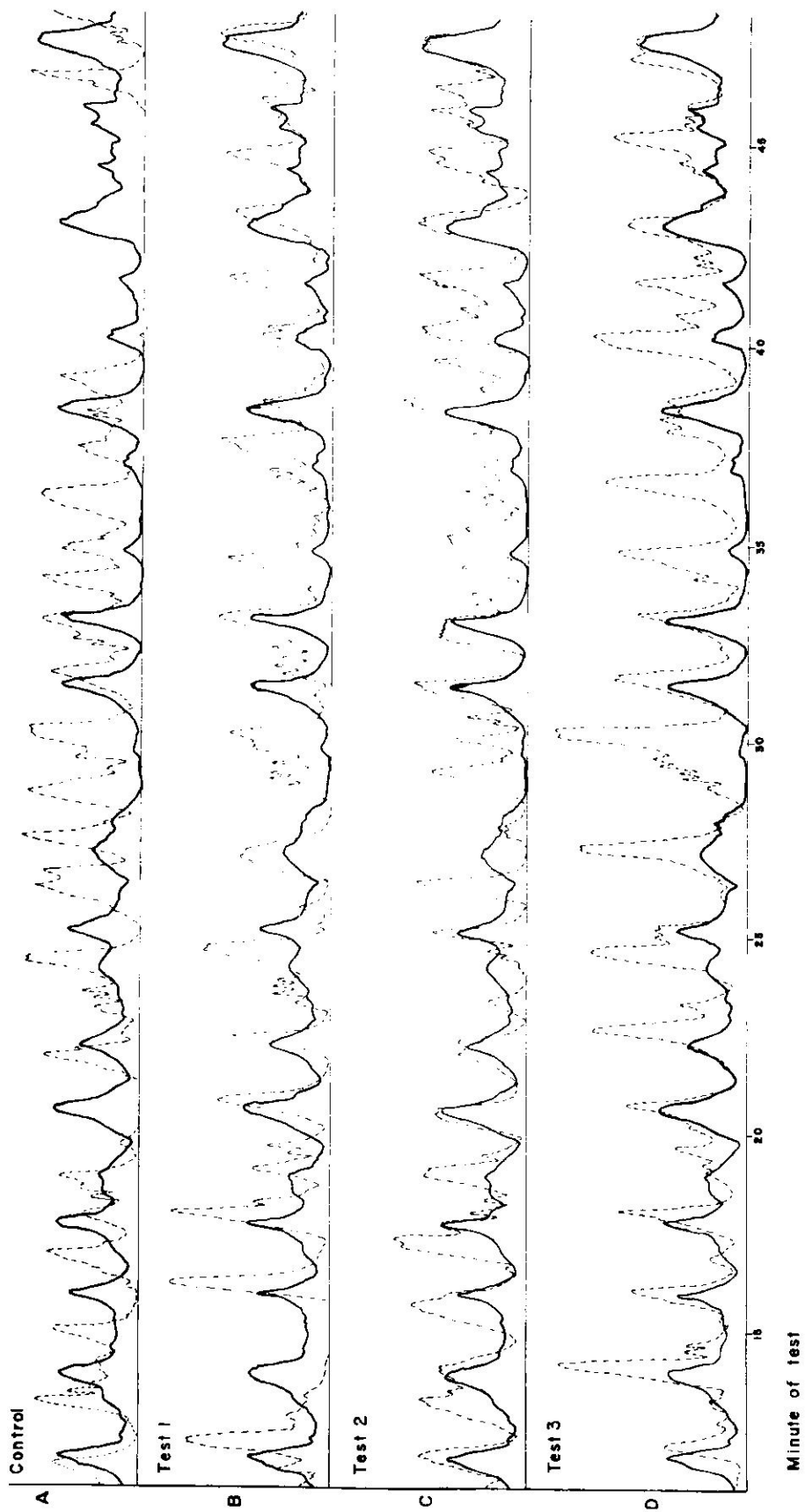


Figure 6. Ramp synchronization responses of *E. antillensis* to a recorded stimulus. Heavy line represents stimulus, dotted line is response. See text for additional explanation.

ratemeter each time a present threshold was exceeded. The stimulus was pre-recorded on the chart, and playback tape recorder and chart started synchronously. Each test lasted one hour; only 36 minutes of each is shown in figure 6, beginning 15 minutes into each test.

Some ramp synchronization is apparent in each of the three responses; the last two also illustrate an ability to sustain or restart ramps in response to stimulus ramps, which of course were not in any way reciprocally responsive. One degree of temperature difference either way apparently affected both ramp synchronization ability and absolute call rate. The figure was evaluated by measuring the area under each curve with a planimeter. Although absolute calibration of the areas was not made, the number of calls in the area of stimulus measured was about 1,714 while the greatest number of calls (response, figure 6D) was about 3,656. Table 3 breaks down call percentages into overlapping and non-overlapping sections of the curves; it can be seen that the percentage of stimulus calls not matched by responses was reduced much more effectively as response rate increased (first column) than the increase of response calls not matched by stimulus calls (fourth column). Even this breakdown does not give the complete picture of ramp synchronization, as figure 6 shows that buildup of response outside the stimulus curve is mostly synchronized with peak rates in the stimulus. Although it is not apparent in the figure, there is a strong subjective impression that males of E. coqui and E. antillensis try to reach a ramp peak just as the interacting male is subsiding from one, and that this effort is thwarted in responding to tape stimuli by unpredictability of the stimulus, a tendency to over-react to small rate increases in the stimulus, and some type of limit to overall area under the curve, apparently correlated with temperature. Responses were most appropriate on the right hand third of figure 6, where the stimulus was most regular in time. Response to a counter-responsive stimulus is now being studied by development of an artificial call-producing device under control of the investigator.

A theory attempting to explain the complexity of behavioral interaction in the species studied has been developed and is continuously being modified by additional evidence. Elements of the theory have been presented where most appropriate in the preceding Descriptions. The evidence for acoustic territoriality in at least E. coqui, E. antillensis and E. hedricki is considered conclusive at this point, and other species are nearing the stage of addition to the list. Nutritional requirements of the males is a poor explanation for genetic maintenance of such behavior, in the light of the fact that males are less territorial than females while feeding. Placement of the eggs in the center of each acoustic territory, however, does make evolutionary sense, because the eggs of other males cannot be placed within the defended perimeter and competition with the young by other young of

different genetic lineage and the same size can be minimized. Evolution appears to have favored the development of vocalization as a weapon for territorial defense. The evolution of vulnerability to this weapon should also have been favored, since the best reproductive prognosis could be obtained by locating a new territory outside the range of existing ones. This view represents acoustic warfare as almost an extreme of the process called ritualization, or the substitution of the physically harmless exchanges for dangerous ones, as vulnerability to vocal weapons is almost completely reproductive rather than physical.

The central role of daytime shelter observed in the life of *E. coqui* agrees with the observation that most dispersal involving a change of shelter occurs during rainy weather, while breeding occurred on dry nights as well. This, combined with the data on acoustic territoriality, provides an alternative explanation (to reproductive activity) for increased vocal activity on rainy nights. If most males seeking new territory disperse at these times, advertisement of existing territories would serve the joint functions of defense and the filling of suitable unoccupied areas. On dry nights, the early equilibrium indicated by note synchronization of established males permits a truce which allows foraging away from the calling stations, while in this species at least, violations of the truce by resident but non-territory holding males are suppressed by physical means. To round out the theory with predictions that can be tested experimentally, female movement to male's shelters for ovoposition should occur mainly in the early evening, while failures of non-established males to achieve acceptance of their calling efforts by the established males should be remembered through the day and inhibit early calling the next evening.

Table 3. Planimeter integration of areas under call rate curves in figure 6, given as percentage of total.

No.	Stimulus Curve		Over-lapping	Response Curve		
	Over-lapped	Not overlapped		Not over-lapping	Approx. no. calls	as % of stim
control	55%	45%	43%	57%	2,200	128%
test 1	72	28	50	50	2,470	144
test 2	88	12	49	51	3,050	178
test 3	93	7	43	57	3,660	214

Phase VIII - Interspecific Territoriality

Mentioned briefly above was the possibility that acoustic territoriality may extend across species lines, perhaps at reduced intensity. An interesting possibility is that the measurement of the intensity of interspecific interaction may provide an independent indication of the amount of overlap in ecological niche between the young of various species, which have been difficult to study by more direct methods. The existence of a well developed technology for the analysis and production of acoustic phenomena makes it feasible to try to manipulate male populations with sound and analyze the results.

Indirect evidence for the existence of interspecific acoustic territoriality comes from overlap or similarity between species in certain call parameters. Thus, the high-pitched second notes of E. coqui, E. portoricensis and E. antillensis, indicated in the last section to convey agonistic information, overlap considerably in frequency, while the low-pitched first notes do not. The occasional calls of the last species are still higher in pitch, and unlike the warning calls of the other two species, are interspersed freely in the pattern of two-note calls. In frequency and structure they approach the calls of E. brittoni, which is a not too distant relative occupying the same general habitat. The two-component calls of E. eneidae and E. locustus involve changing note duration rather than frequency and suggest that, in addition to potential exchange of territorial information with one another, communication with other species in adjacent microhabitats might be involved. In use of arboreal shelters, E. coqui overlaps with E. hedricki and the call of the latter bears a noticeable resemblance to the shelter-defending, warning call of the coqui.

Direct evidence of interspecific acoustic interaction was obtained when the response of a male E. antillensis to tape stimuli was tested. This male achieved ramp synchronization with taped, filtered, first notes of E. coqui, but in at least three experiments, responses ceased abruptly a few minutes after the start of a tape stimulus of filtered coqui second notes. Investigation showed that a neighboring male coqui was stopping the responses by butting the smaller E. antillensis male. On one occasion the invading coqui began calling from the site formerly occupied by the other male. The displaced male remained silent until the stimulus tape was finished, then began calling strongly from about a foot away on the same bush. The two males called from these positions for about an hour, and the entire exchange was recorded. The smaller male then leaped straight at the larger, which retreated the 11 feet or so into its own territory and began calling there. No further interactions were noted.

Analysis of this interaction was simplified by filtering the distinctive first notes of the two species. It was immediately apparent that many calls of the E. antillensis were phase-locked to the first notes of the coqui, and actually occurred between the more widely spaced coqui notes. Figure 7 reconstructs a typical 55 second sequence of the interaction. First notes were traced from the filtered chart, so their timing is accurately shown. Line A shows the coqui calls, line B the E. antillensis calls and line C the 200 millisecond delay on phase-locked calls. Line D was first thought to represent a coqui phase-locked response, but since lag in note-synchronized coquis has been found to also approximate 200 milliseconds, the lags in line D appear to illustrate a property of the E. antillensis response. This leaves no evidence that the coqui was responding at the note synchronization level, but the probability that timing in line B was due to chance is extremely low. Note that if the lags shown in line D were plotted after each call in line B, only those calls in line A that fell near the end of the lag period received a phase - locked response, including the last call in line A, which came after the speed-up of the E. antillensis ramp had inserted an extra call. This pattern of exchange was repeated with only minor variations throughout the hour of interaction.

This interaction suggests more than one hypothesis concerning territoriality in these species. One is that the butting attacks made by the coqui were actually stimulated by the taped coqui sounds, and that the male E. antillensis was attacked as the only visible frog in the area. The implication is that the latter male was being tolerated at a distance at which a conspecific male would not be. An alternative explanation is that the two males had reached equilibrium as if they were conspecific, and the taped sound upset the equilibrium. Although normal ramp structure and rates differ between the species, they were able to achieve note synchronization repeatedly by bringing the most similar rates into apposition. This indicates that ramping itself, already suggested to be territorial in function, also serves to permit some interspecific synchronization.

A small scale survey of spacing among males of these two species indicates that some interspecific distances may be slightly shorter than intraspecific minimum distances, but in general, average distances between neighbors appear similar regardless of species. The experiment is being enlarged to include all males of these and two other ramping species, E. portoricensis and E. brittoni in a selected area of forest edge. Males are being marked to identify the established individuals and separate them from newcomers which may or may not succeed in establishing territories. An effort is also being made to locate each male's shelter, in order to monitor reproductive success during the year. Radioactive tags attached to the calling males in the evening are expected to greatly simplify location of their shelters during the day.

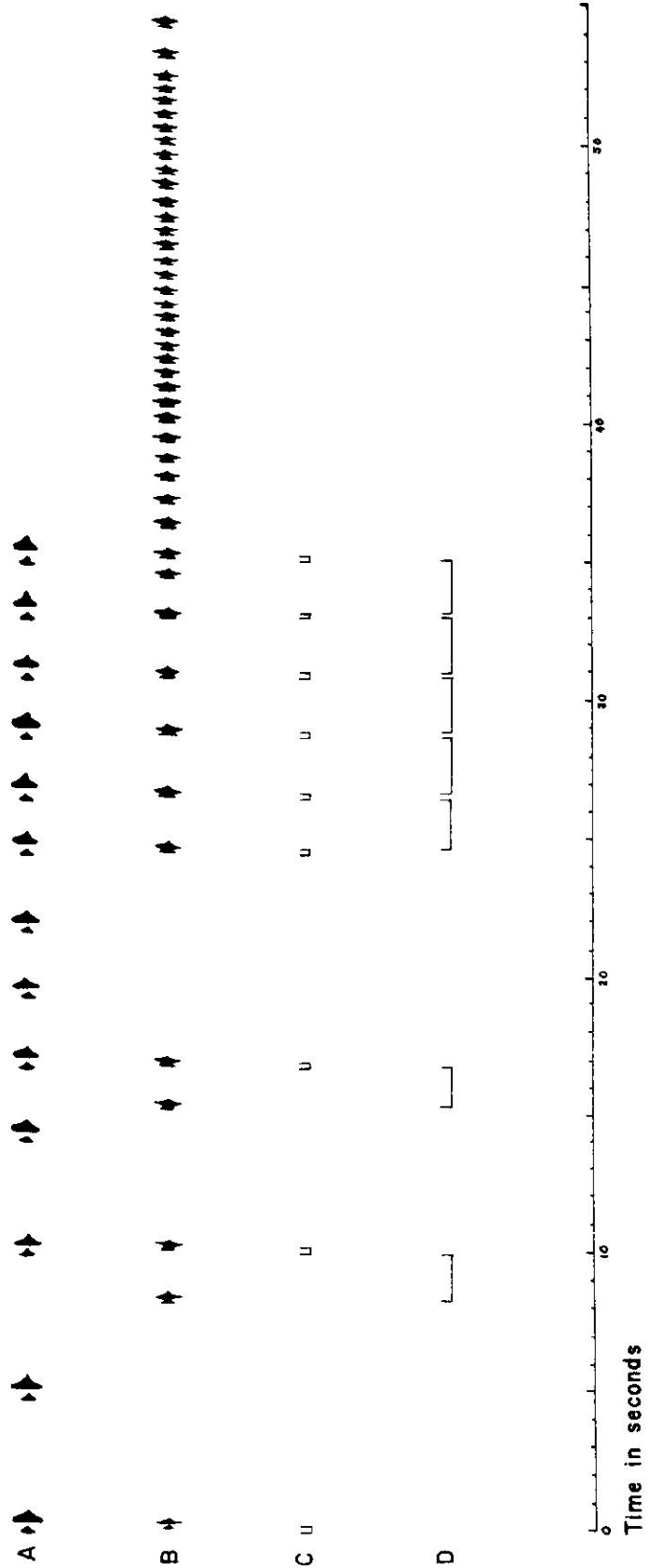


Figure 7. Interspecific vocal interaction between an invading male *E. coqui* and a resident male *E. antillensis*. Symbols as in figure 5.

When a baseline of normal spacing and activity patterns has been established in a mapped population, a number of experiments can be done. Some involve manipulation of the acoustic environment. It should be possible to "jam" the territorial communication systems in a limited area with competing sounds. Properties such as frequency and timing of effective interfering sounds can tell us much about the bandwidths and species separations in these systems. Similarly, it should be possible to create artificial territories with a loudspeaker playing either tape recorder calls, electronically synthesized artificial calls, or calls transferred from another frog elsewhere. If territorial maintenance should require appropriate responses to the real frogs, it is even possible with equipment on hand to arrange reciprocal intercom connections between physically distant frogs, thereby having full control over intensities, frequencies and other transmission properties.

Spacing is also subject to experimental manipulation, as demonstrated already in the tower populations of E. coqui and E. hedricki. Here, the bamboo shelters voluntarily adopted by the frogs have been successfully moved with the residents inside. The possibilities for study of interspecific relationships in this vertical gradient are being explored. It is possible that similar techniques can be developed for holding semi-captive populations of the other species as well, and moving them about as desired.

In summary, the composite populations, numbering up to nine or ten species where different habitats meet, provide one of the most challenging ecological, behavioral and evolutionary problems in the rain forest ecosystem. The characteristics of the animals make them ideal for field study. Both in their ecosystem roles and in their potential for illustrating basic ecological principles, they appear to hold a fundamental position in Puerto Rico.

References

- Blair, W.F. 1958. Mating call in the specification of anuran amphibians. *Amer. Naturalist* 92: 27-51.
- Drewry, G.E. In Press. Factors affecting activity of rain forest frog populations as measured by electrical recording of sound pressure levels. In "A Tropical Rain Forest" H.T. Odum ed.
- Emlen, S.T. 1968. Territoriality in the bullfrog, Rana catesbiana. *Copeia* 1968 (2): 240-243.

- Falls, J.B. 1963. Properties of bird song eliciting responses from territorial males. Proc. 13th Int. Ornithol. Congr. 1:259-271.
- García-Díaz, July 1967. Rediscovery of Bufo lemur (Cope) and additional records of reptiles from Puerto Rico. Misc. Pap. Mus. Biol. Univ. Puerto Rico, 10: 1-6, fig.
- Grant, Chapman. 1931. A new frog from Puerto Rico. Copeia 2: 55-56.
- Grant, Chapman, 1932a. A new frog from Puerto Rico. J. Dept. Agr. Puerto Rico 16 (2): 145-148.
- Grant, Chapman, 1932b. A new frog from the Virgin Islands. J. Dept. Agr. Puerto Rico 16 (3): 325-326.
- Heatwole, H. 1962. Contributions to the natural history of Eleutherodactylus cornutus maussi. Stahlia Misc. Pap. 2: 1-11.
- Heatwole, H. 1963. Ecologic segregation of two species of tropical frogs of the genus Eleutherodactylus. Carib. J. Sci. 3 (1): 17-23.
- Heatwole, H., N. Mercado and E. Ortiz. 1965. Comparison of critical thermal maxima of two species of Puerto Rican frogs of the genus Eleutherodactylus. Physiol. Zool. 38 (1): 1-8.
- Heatwole, H., F. Torres, S. Blasini de Austin and A. Heatwole, 1969. Studies on anuran water balance. I. Dynamics of evaporative water loss by the coqui, Eleutherodactylus portoricensis. Comp. Biochem. Physiol. 28: 245-270.
- Littlejohn, M.J. and A.A. Martin, 1969. Acoustic Interaction between two species of leptodactylid frogs. Anim. Behav. 17: 785-791.
- Orians, G.H. and M.F. Willson, 1964. Interspecific territories of birds. Ecology 45: 736-745.
- Orians, G.H. and H.S. Horn, 1969. Overlap in foods and foraging of four species of blackbirds in the potholes of central Washington. Ecology 50: 736-745.
- Peters, W. 1876. Über eine von Hrn. Viceconsul L. Krug und Dr. J. Gundlachi an der Insel Puertorico gemachte Sammlung von Säugethieren und Amphibien, so wie über die Entwicklung eines Batrachiens, Hylodes martinicensis Dum. Bibr. ohne Metamorphose. Monatsber. d.K. preuss. Akad. d. Wissensch., Berlin, pp. 703-714, 914.

- Rivero, J.A. 1956. Two new species of Eleutherodactylus from Puerto Rico. *Breviora* (103): 1-6.
- Rivero, J.A. 1963. Eleutherodactylus hedricki, a new species of frog from Puerto Rico (Salientia, Leptodactylidae). *Breviora* (185): 1-7.
- Rivero, J.A. and H. Mayorga. 1963. Notes on the distribution of some Puerto Rican frogs with a discussion on the possible origin of Eleutherodactylus locustus. *Carib. J. Sci.* 3 (2 & 3): 81-85.
- Rivero, J.A., J. Maldonado and H. Mayorga, 1963. On the habits and food of Eleutherodactylus karlschmidti Grant. *Carib. J. Sci.* 3 (1): 25-27.
- Sampson, L.V. 1904. A contribution to the embryology of Hylodes martinicensis. *Amer. J. Anat.* 3: 475-504.
- Schmidt, K.P. 1927. A new tree frog from Puerto Rico. *Amer. Mus. Novit.* No. 279, pp. 1-3.
- Schmidt, K.P. 1928. Amphibians and land reptiles of Puerto Rico with a list of those reported from the Virgin Islands. *Sci. Surv. Porto Rico and the Virgin Islands, New York Acad. Sci.* vol. 10, part 1, pp. 1-160.
- Stejneger, L. 1904. The herpetology of Puerto Rico. *Rept. U.S. National Mus.* (1902): 549-724.
- Thomas, R. 1966. New species of Antillean Eleutherodactylus. *Quat. Jour. Florida Acad. Sci.* 28(4): 375-391.
- Turner, F.B. and C.S. Gist. In Press. Observations of lizards and tree frogs in an irradiated Puerto Rican rain forest. In "A Tropical Rain Forest". H.T. Odum ed.
- Wahl, M. 1969. Untersuchungen zur Bio-Akustik des Wasser froches Rana esculenta (L) *Oecologia (Berl.)* 3: 14-55.
- Wiewant, Thomas A. 1969. Vocalization, aggressive behavior, and territoriality in the bullfrog, Rana catesbiana, *Copeia* 1960 (2): 276-285.

Appendix I. A Key to the Calls of Common Puerto Rican Frogs

- 1 Regular call consisting of 2 notes, the second higher-pitched than the first; extra syllables added near beginning and end of activity cycle, and two frogs sometimes synchronize closely, one omitting second note; all have occasional calls of 5 to 10 shorter, usually higher-pitched notes 2
- 1' Call seldom composed of 2 notes, if so they are same pitch 4
- 2 No pause between notes; noticeable consonant sounds at start and end of notes ("burdick"); occasional calls interspersed among regular calls; widespread, habitat weedy or shrubby areas receiving sun in daytime; call rate accelerating to 2.5 calls per second or higher
..... Eleutherodactylus antillensis (Rheinhardt and Leutken)
- 2' Noticeable pause between notes; no consonant sound after note; occasional calls most common at dawn and dusk, not interspersed with regular calls; habitat shady in daytime 3
- 3 Accent usually on first note; prone to repeat first note and/or drop second; call higher-pitched and shorter than next species (plaintive "birdie"); habitat restricted to montane forest; call accelerating to 2 calls per second; individuals call synchronously so all silent at once
..... E. portoricensis Schmidt
- 3' Accent usually on second note; prone to repeat both first and second notes; notes well spaced ("co-qui"); widespread in forest and shady areas throughout island, often in or on human dwellings; call rate accelerating to 1 call per second or so; silences not synchronized E. coqui Thomas
- 4 Call introduction a single, clear, high-pitched whistle, usually, but not always, followed by sharp clicks or a buzz. 5
- 4' Call not starting with an isolated whistle 7
- 5 Call, unless interrupted, a whistle followed by more than four clicks or a buzz; montane species 6
- 5' Call a clear whistle, early in the evening often followed by 1 to 4 clicks, which are left off later; chorus sounds like a rusty swing; habitat lawns, meadows and marshland at low elevations E. cochranae Grant
- 6 Clicks of call well separated, 5-8 per second ("pic-pic-pic"); call less than 2 sec. long; often calls before twilight and after dawn; habitat forest edge and fern growth at middle elevations and common everywhere at high elevations
..... E. locustus Schmidt

- 6' Clicks more rapid, approaching a buzz, 10-15 per second; call lasting more than 2 sec.; most active from 10 p.m. to dawn; habitat forest, most common on earth banks and mossy boulders E. eneidae Rivero
- 7 Call composed of 3 to 10 high-pitched whistles; several individuals often call simultaneously; first 1 or 2 notes lower in pitch and difficult to hear; lives in forest litter layer but sometimes climbs to call; a small species E. wightmanae Schmidt
- 7' Notes hard to describe as whistles and all same frequency... 8
- 8 Same note repeated rapidly and monotonously for indefinite periods of time ("quick-quick-quick"); characterized by some as sounding under water; stopping abruptly or giving explosive, trilled note ("Brrr") when startled or approached by another frog; calls from concealment in muddy or marshy areas Leptodactylus albilabris (Guenther)
- 8' Call pattern not indefinitely regular or otherwise not as above 9
- 9 Individual calls lasting for more than 4 seconds; low-pitched melodius notes repeated so fast they blend into a tremolo (or trill) not easily distinguished from common call of Puerto Rican screech owl; given near edge of standing or slow-moving water Bufo marinus (Linne)
- 9' Call duration less than 4 seconds 10
- 10 Regular call a single note, harsh and given at long, irregular intervals ("tick"); in cool weather calling more often and sometimes giving double or multisyllable note; habitat forest floor, sometimes under stones outside forest edge E. richmondi Stejneger
- 10' Call more than one note and/or repeated frequently 11
- 11 Sound of call like winding a watch held against the ear or running fingernail along teeth of a stiff comb; restricted to very high elevations; calls from burrows in earth
..... E. unicolor Stejneger
- 11' Not as above 12
- 12 Calls high-pitched chirps or squeaks with six or fewer notes per call; very small frogs 13
- 12' Calls lower-pitched, usually six or more identical notes per call 14
- 13 Notes well spaced, very high-pitched squeaks, first call in a sequence usually one note, subsequent calls often adding notes to a maximum of 4 or 5; a montane, arboreal species that usually calls from 6 ft. to treetop level
..... E. gryllus Schmidt

- 13' Notes rapid in clear, trilled chirps; sequences often begin with 2 note chirps and build up to 5 or 6; a widespread meadow and roadside species seldom calling more than 6 feet above the ground E. brittoni Schmidt
- 14 Notes harsh and penetrating, usually given in the vicinity of fast water or waterfalls in mountain streams
..... E. karlschmidti Grant
- 14' Notes clear tones. If call does not fit one of next two species check occasional calls in couplets 1 and 8 and suspect bird or insect sounds15
- 15 Bell-like "ping-ping-ping"-given from treeholes in montane forest; often attributed to birds when heard in daytime E. hedricki Rivero
- 15' Lower pitched ("cu-cu-cu") emanating from boulder piles and grottos in the southeastern Panduras Mountain Range; calls attributed to spirits by the local inhabitants
..... E. cooki Grant

Synoptic Key to Calls

1	two-notes calls	2
1'	otherwise	4
2	"burdick", occ. calls mixed in, sunny habitat	<u>E. antillensis</u>
2'	occ. calls not mixed in, shady habitat	3
3	"birdie", silences synchronized, 2 calls/sec maximum	<u>E. portoricensis</u>
3'	"co-qui", silences not synchronized, 1 call/sec. maximum	<u>E. coqui</u>
4	Call start an isolated whistle, usually followed by clicks	5
4'	otherwise	7
5	more than 4 clicks, montane	6
5'	4 to no clicks, lowland	<u>E. cochranae</u>
6	clicks slow, less than 1.5 sec. long, crepuscular	<u>E. locustus</u>
6'	clicks fast, longer than 2 sec. graveyard shift	<u>E. eneidae</u>
7	3-10 high whistles, first low, calls synchronized	<u>E. wightmanae</u>
7'	otherwise	8
8	"quick -quick-quick"- monotonous, indefinite, alarm call "brrr"	<u>L. albilabris</u>
8'	otherwise	9
9	call longer than 4 sec., tremolo, near water	<u>B. marinus</u>
9'	shorter than 4 sec.	10
10	single "tick", long irregular interval	<u>E. richmondi</u>
10'	otherwise	11
11	sound like winding watch, high elevation	<u>E. unicolor</u>
11'	otherwise	12
12	chirps or squeaks, tiny frogs	13
12'	otherwise, usually 6 or more notes/call	14
13	separate squeaks, montane, arboreal	<u>E. gryllus</u>
13'	trilled chirps, widespread, meadow species	<u>E. brittoni</u>

14	notes harsh, lives near mountain streams	<u>E. karlschmidti</u>
14'	notes not harsh	15
15	"ping-ping-ping", treeholes, montane forest	<u>E. hedricki</u>
16	"cu-cu-cu", boulder grottos, southeastern mountains	<u>E. cooki</u>

Appendix II Field Identification Guide to
Puerto Rican Eleutherodactylus

Use list A as a cross indexed key, taking characteristics in order and following instructions. Additional characteristics are presented in list B. Underlined numbers in list A refer to species in list C. List C indexes characteristics from list A and B that help confirm an identification, the most valuable are followed by an asterisk. Dead specimens can be identified but require more cross checking after colors fade (skip over item 3, 5b, 5c and 5e).

List A

No.	Look at	If	Go to	Cross check
1.	Hind toes	a. webbed	<u>1a</u>	
		b. not webbed	2	
2.	Dark lateral stripe	a. from nostril to vent, upper border light, dots below	<u>2a</u>	
		b. from nostril over shoulder, well past front legs	7	7a
		c. absent or short	3	
3.	Iris color	a. brick red, at least above	<u>3a</u>	6a, 12a
		b. pearly white above, darker below	<u>3b</u>	6a, 11d
		c. golden	5	5b, 8b
		d. yellow above, reddish below	6	2a, 7a, 8b
		e. gray, strongly marbled	9	9c, 10a
		f. gray or other dark color, marbled weakly or not at all	4	10b
4.	Nostril position	a. near tip of snout	5	
		b. halfway from tip of snout to eye	<u>4b</u>	
5.	Underside of abdomen	a. as dark as back or nearly so	5d	4b, 10a
		b. green, or blue green	<u>5b</u>	5e, 6b
		c. yellow, usually yellower than throat	<u>5c</u>	6c

List A (cont.)

No.	Look at	If	Go to	Cross check
5.	(cont.)	d. with irregular white patches	5e	3b, 10a
		e. skin translucent, a pair of white lines show through	9	5b, 9d, 10b
		f. skin opaque, color ivory, silver, gray or brown	6	
6.	Canthus rostralis (bone ridge from nostril to eye)	a. fairly straight and prominent	6d	3a, 3b, 4b
		b. curving and weak	9	5b
		c. intermediate, hard to determine	8	8a, 5c
		d. with a thin white line on dorsal edge	6e	3a, 3b
		e. with a dark lateral face	7	3a, 3b
7.	Top of snout (viewed from above)	a. almost as wide as jaw	<u>7a</u>	2b, 3d
		b. much narrower than jaw	8	
8.	Dorsal coloration (head and back)	a. highly uniform, 13a and 16 usually true	<u>8a</u>	
		b. a pattern or mottled coloration present	<u>8b</u>	3b, 9
9.	Distance between eyes	a. less than $3/4$ eye-ball diameter	10	
		b. more than $3/4$ eye-ball diameter	9c	11b
		c. less than twice largest toe pad width	<u>9c</u>	3e, 9d, 5b
		d. more than twice largest toe pad width	<u>9d</u>	5b
		e. more than nostril-eye distance	4	9c, list B
		f. less than nostril-eye distance	1	list B
10.	Silver spots on underside	a. numerous, several on edge of lower lip	<u>10a</u>	3e, 5d
		b. scattered, few or none on edge of lower lip	<u>10b</u>	3f, 5e

List B

No.	Look for	If	Cross check
11.	Melanophores on underside of abdomen (dark pigment cells, use magnification if available, most useful for young frogs)	a. much less dense than on throat b. almost absent c. present between iridophores (silver) d. absent between many iridophores e. absent in large patches	5c, 11b 5b, 5c 8b, 10b 3b, 5c 3b, 10a
12.	Inside surfaces of hind legs (hidden when leg folded)	a. dark with light spots b. solid red or brown	3a 3b, 8b
13.	Width of largest toe pads as a fraction of distance between nostrils	a. more than $3/4$ b. more than $1/2$ c. less than $1/2$	8a 1a, 8a, 8b, 9d 2a, 4b, 5c
14.	Light hourglass pattern on back	a. when young b. throughout life	3b, 8b 5b, 9d, 10b
15.	Dark W-shaped mark on back of head	a. when young b. throughout life	3b, 8b 9c, 10a
16.	Eyes inside jaw line in dorsal view (looking down on head)		8a
17.	Iridophores (silver pigment spots) absent from underside		5b
18.	Median or paired white lines down back and/or hind legs		(see list C)
19.	Adults usually less than one inch long		(see list C)

List C

<u>No.</u>	<u>Species</u>	<u>Should also have</u>	<u>Usually have</u>	<u>Can have</u>
<u>1a</u>	<u>E. karlschmidti</u>	5f, 6a, 13b*	3f, 9f, 11a	
<u>2a</u>	<u>E. richmondi</u>	3d*, 6a, 6d*, 6e*, 13c*	9f, 11a, 11e, 18*	
<u>3a</u>	<u>E. antillensis</u>	5f, 6a*, 6e*, 11c, 12a*	6d, 9f, 19, 11b	9e, 18
<u>3b</u>	<u>E. portoricensis</u>	5d*, 5f, 6a*, 6e*, 11d*, 12b*	6d*, 9f, 13b	9e, 14a, 15a, 18
<u>4b</u>	<u>E. unicolor</u>	5f, 6a, 8a, 9e*, 13c*, 19*	3f, 5a*, 6e	
<u>5b</u>	<u>E. gryllus</u>	6b*, 17*, 19*	3c*, 5e*, 11a, 11b, 14b*	6e, 18
<u>5c</u>	<u>E. wightmanae</u>	5f, 6c, 11a, 13c*	3f, 6b, 9f, 19	3c, 6e, 18
<u>7a</u>	<u>E. brittoni</u>	3d*, 5f, 6a*, 6e*, 19*	2b*, 9f	6d, 9e, 18
<u>8a</u>	<u>E. cooki</u>	5f, 13a*	3f, 6a, 9f, 16*	6e
<u>8b</u>	<u>E. coqui</u>	5f, 6a*, 6e*, 11c*, 12b*	3c*, 9f, 13b	3d, 5c, 6d, 9e, 14a, 15a, 18
<u>9c</u>	<u>E. hedricki</u>	3e*, 5f, 6b*, 13b*	9e, 15b*	6e
<u>9d</u>	<u>E. cochranae</u>	6b*, 19*	3f, 14b*	5e, 6e, 18
<u>10a</u>	<u>E. eneidae</u>	3e*, 5f, 6b*, 9a*, 9f, 19	5a, 5d, 11e, 15b*	6e, 18
<u>10b</u>	<u>E. locustus</u>	6b*, 9a*, 9f, 19	3f, 5e*, 11c, 14b*	5a, 6e, 11e, 18

FEEDING BEHAVIOR OF THE FROGS AND LIZARDS IN THE TROPICAL
WET FOREST - PRELIMINARY REPORT

Robert J. Lavigne and George Drewry

The following is the preliminary report on the analysis of the contents of the digestive systems of frogs and lizards in the tropical wet forest in the vicinity of the El Verde Field Station. This report covers the period October 8, 1969 to February 15, 1970 during which time 140 stomachs were dissected representing nine species of frogs and three species of lizards. Eight of the species of frogs belong to the genus Eleutherodactylus which predominates in the frog fauna of Puerto Rico. Since many of these species apparently compete with each other on several behavioral levels, it is of great interest to learn how they divide the available food and if their food preferences reflect their observed behavior in the field.

Procedure

Because lizards are known to be active diurnally and frogs, nocturnally, the basic assumption was made that this was the time period during which they fed and captures were made accordingly. Both frogs and lizards were identified in the field as many distinctive color patterns are lost when the organisms are preserved. Time of day and height of reptile when captured were recorded. In the case of frogs it was also noted whether or not the frog was calling.

While in many cases the effect of digestive enzymes in the posterior half of the digestive tracts precluded identification of the remains of devoured organisms below the family level, the remains in the stomach were usually identifiable to species due to the method of preservation used. The procedure was to pith the frogs and lizards in the field and then immediately inject Turtox Insect Preservative into the peritoneal cavity. This caused the cessation of digestive enzyme activity, thus increasing the chances of identifying the insect remains. The animals were then transferred to the laboratory where they were tagged and immersed in a jar of Turtox Insect Preservative.

Insect identifications were based on comparison with available material in a collection maintained on the site. Because of the poor state of taxonomy of some tropical insect groups, it is often easier to obtain field information concerning the habits of certain insects than it is to attach a scientific name to the species.

Consequently, many of the species in the collection, with distinct sets of characteristics, have been assigned letter designations until such time as scientific names become available. These letter designations, therefore, are used occasionally in tables and text.

Food Consumption Related to Taxonomic Category of Predator and Prey

Categorization of prey into taxonomic groups has been used in several recent food studies of tropical frogs (Heatwole, 1963) and of tropical and temperate lizards to provide ecological niche separation for sympatric species or to indicate resource partitioning by allopatric species (see references). Milsted (1957) suggested that it was sufficient in lizard studies to separate food items to the ordinal level and in subsequent studies insect determinations were carried to the ordinal and in some cases family level. Where this was inadequate to separate lizard species, prey volume was introduced as an additional variable. Although habitat specialization was indicated by these techniques we believe valuable information was missed through insufficient nomenclatural separation. Additionally, this approach does not provide information on the habits of the prey species. We feel that the most effective understanding of niche specialization will come by measuring with both entomological and herpetological criteria.

In order to provide comparison of our data with that obtained by various herpetologists we have initially delineated the prey at the class or ordinal level. This information is presented in Table 1 which is arranged according to species and sex of adult frogs. Because present sample sizes differ according to species, the table provides the average number of each prey category per individual frog. The data indicate that, in general, the first four species consume a greater number of non-insect organisms. Interestingly enough these four species plus E. eneidae and Leptodactylus albilabris make up the "forest complex", whereas the rest of the species belong more properly to the open area and forest edge fauna. While this information is interesting, it can not be used to delineate any specificity of niche feeding by predators. It does not tell us whether the insects in the order Hymenoptera were bees and wasps or ants and, if the latter, whether they were arboreal ants or those confined to the litter. Similar data is presented for juvenile frogs in Table 2 and for lizards in Table 3. Data are not yet sufficiently complete to allow conclusions to be drawn.

Because of the way in which frogs feed, i.e. taking up a foraging site and awaiting the arrival of prey, it follows that organisms which aggregate in one spot, such as ants and termites,

Table 1. Comparison of feeding habits of adults frogs (number/individual) by sex based on organisms collected from their stomachs and categorized on the basis of the systematic categories of Order or Class.

	Eleutherodactylus																			
	coqui		portoricensis		richmondi		wightmanae		eneidae		antillensis		brittoni		locustus		Leptodactylus albilabris			
N	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀		
	12	6	8	8	5	5	11	2	5	13	7	3	3	3	3	1	3	1	3	
Araneida	0.4	0.6	0.3	0.5	0.6	0.4	0.2	0.5	0.2	0.6	0.3	0.5	0.6	1.3	1.0	0.3				
Acarina	0.6	0.3	-	3.1	0.4	0.4	2.0	-	0.2	0.5	0.4	1.6	-	3.6	5.0	-				
Chilopoda	0.05	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-				
Diplopoda	0.1	0.15	-	0.1	0.3	-	0.4	0.5	-	-	-	-	-	-	-	-				0.3
Chelonehthida	0.05	-	-	-	-	-	0.2	1.0	-	-	-	-	-	-	-	-				
Isopoda	-	-	-	0.1	0.1	0.2	0.1	-	-	0.07	-	0.25	-	-	-	-				
Mollusca	-	-	-	0.1	0.1	0.2	-	-	-	-	0.1	0.15	-	-	-	-				
Collembola	-	-	-	-	-	-	-	-	0.4	-	-	0.15	0.3	1.3	3.0	-				
Orthoptera	0.5	0.9	-	0.4	0.4	-	0.1	-	0.2	0.2	0.1	-	-	0.3	-	-				
Psocoptera	0.05	0.25	-	0.1	-	-	0.1	-	0.2	0.07	-	-	-	-	1.0	-				
Coleoptera	0.2	0.25	0.5	0.5	0.7	1.8	0.9	-	-	0.15	0.3	-	-	0.3	-	-				
Hemiptera	0.6	0.5	0.3	0.4	0.1	-	0.6	1.5	0.4	0.6	0.4	0.8	-	-	-	-				
Hemiptera	0.05	0.15	-	0.2	-	-	0.2	-	-	0.15	0.3	0.25	0.3	-	-	0.3				
Diptera	0.7	4.1	1.3	1.1	1.0	0.4	0.6	-	1.8	1.8	3.0	1.15	4.6	3.3	3.0	-				
Hymenoptera	7.6	6.8	3.5	5.1	1.0	1.0	10.0	11.0	3.0	1.8	6.7	1.8	2.0	3.3	4.0	3.0				
Lepidoptera	0.5	0.5	0.5	0.6	-	-	0.2	-	-	1.5	4.6	0.7	1.3	0.3	-	-				
Iscoptera	-	-	-	-	-	-	1.3	0.5	-	-	-	-	-	-	-	-				
Thysanoptera	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-				

Table 2. Comparison of feeding habits of juvenile frogs (number/individual) based on organisms collected from their stomachs and categories on the basis of the systematic categories of Order or Class.

N	<u>Eleutherodactylus</u>						
	coqui	portoricensis	richmondi	antillensis	locustus	gryllus	
	2	7	1	3	2	1	
Araneida	-	0.9	1.0	-	-	-	-
Acarina	7.5	0.4	1.0	4.3	2.5	1.0	-
Chilopoda	-	-	-	-	-	-	-
Diplopoda	-	-	-	-	-	-	-
Chelonethida	-	-	-	-	-	-	-
Isopoda	-	0.1	-	-	-	-	-
Mollusca	-	0.1	-	-	-	-	-
Collembola	-	-	1.0	-	1.5	-	-
Orthoptera	-	0.1	-	0.3	-	-	-
Psocoptera	-	-	-	-	-	-	-
Coleoptera	-	0.4	-	-	1.5	-	-
Homoptera	-	0.9	3.0	0.3	-	2.0	-
Hemiptera	-	0.1	-	-	-	-	-
Diptera	4.5	1.0	-	4.0	1.0	3.0	-
Hymenoptera	6.0	20.1	3.0	7.3	5.5	1.0	-
Lepidoptera	2.5	0.1	-	2.6	-	-	-
Isoptera	-	-	-	-	-	-	-
Thysanoptera	-	-	-	-	-	-	-
Trichoptera	-	0.1	-	-	-	-	-

Table 3. Comparison of feeding habits of lizards (number/individual) based on organisms collected from their stomachs and categorized on the basis of the systematic categories of Order or Class.

N	Anolis gundlachi			stratulus			evermanni			juvenile gundlachi			juvenile evermanni			
	♂	♀	+	♂	♀	+	♂	♀	+	♂	♀	+	♂	♀	+	
	3	3	3	1	3	3	3	3	3	3	2	2	1	1	1	
Araneida	0.3	2.0		1.0	1.0		0.6									1.0
Acarina	-	-		-	4.5		0.5				0.5					-
Chilopoda	-	-		-	-		-				-					-
Diplopoda	-	0.3		-	-		-				1.0					-
Chelonethida	-	-		-	-		-				-					-
Isopoda	-	0.3		-	-		-				-					-
Mollusca	-	-		-	-		-				-					-
Collembola	-	0.3		-	-		-				-					-
Orthoptera	1.0	1.0		-	-		0.3				-					1.0
Psocoptera	-	-		-	0.5		1.3				-					-
Coleoptera	0.3	8.6		-	0.5		-				2.0					6.0
Homoptera	0.6	1.6		1.0	2.0		1.6				1.0					5.0
Hemiptera	0.6	2.3		3.0	-		-				-					1.0
Diptera	1.3	1.0		-	1.5		1.3				1.0					2.0
Hymenoptera	4.6	28.0		29.0	27.0		12.0				4.5					43.0
Lepidoptera	0.3	1.0		1.0	1.0		0.3				2.0					-
Isoptera	-	1.0		-	-		-				0.5					-
Thysanoptera	-	-		-	-		-				-					-
Trichoptera	0.6	-		-	-		-				-					-

will be consumed in considerably larger numbers than non-aggregating insects. On this basis we are inclined to give different weights to these two groups when interpreting our data. Lizards, while actively seeking prey exhibit a similar pattern of behavior. A single observation of a female Anolis stratulus digging up ants from a nest and an additional observation of selective feeding on worker termites to the exclusion of soldiers by A. gundlachi suggest that these insects will have to be weighted when interpreting lizard data as well. This is another area where nomenclatural categorization to the ordinal level will not provide us with sufficient information to make correct assumptions.

Food Consumption Related to Habitat

One goal of this study is to assign ecological niches to the various species encountered, both predator and prey and predator stomach analysis provides us with a good tool. Toward that end a system of correlation has been adopted which works as a circular but open train of associations. Initially, field observations provided us with varying amounts of evidence on habitat restriction by certain species, and such evidence continues to accumulate. Apparent absence of any species from a habitat, such as forest edge or forest, a microhabitat such as the surface of leaves, trunks, the litter layer, etc., or a temporal period such as day or night, when the same species is present elsewhere, is noted. The presence of an organism is also noted but is given somewhat less weight than its absence in a particular environment. For example, field observations indicate what prey species should not be available in a given niche as well as where frogs are presumably feeding. If then, as in the case of E. portoricensis which were collected on the vegetation, insects known to be confined to the litter, are found in the female stomachs, it indicates foraging behavior was not completely observed. Good observations receive additional testing in the field and a nucleus of species "reliable" with respect to certain ecological behavior patterns is built up. Organisms associated with "reliable" species in the stomachs of the predator with high quantitative correlation can then be assigned to the same habitats and subsequently these habitats are checked for their presence. This continual cross-checking steers the investigation toward the weak links in the chain.

Table 4 and Figure 1 together illustrate a stage in the separation of arboreal and litter species both within and near the forest edge. Candidates for the list of prey indicators (Table 4), based on direct observation, were screened with respect to the feeding behavior of E. coqui, which had been observed to feed high in the vegetation, and E. wightmanae, which had not. Several candidates not listed

were tentatively discarded. In comparing this list with that of prey items taken by E. antillensis and E. locustus, we discovered even sharper preferences for the same prey categories in these forest edge species. Thus significant occurrence of a prey species in E. coqui and E. antillensis combined with relative absence in E. wightmanae and E. locustus indicate that the prey is arboreal and vice versa. The occurrence of the same prey in E. portoricensis alone would tell us little about vertical distribution of prey because feeding occurs in both niches. The list of reliable prey species suggests that E. portoricensis females do a significant amount of feeding in the litter, even though most of the individuals collected have been taken from the vegetation.

Figure 1 contains a great deal of information, and requires additional explanation. The aim is to present profiles of predator feeding behavior in which biases due to uneven or still inadequate sample size are minimized. Instead of constructing conventional bar graph profiles in which the two categories of indicator species from Table 4 are presented side by side or as wedges of circles, the two profiles point toward each other. The clear area between opposing bars indicates the proportion of prey whose vertical distribution is either unknown or complicated by orientation along other gradients. Width of bars is a rough indicator of the prey sample size. Also, it is probable that collection toward a goal of equal prey samples will provide more informative profiles than would the goal of equal representation by each predator species. This technique requires, of course, that a different graph be constructed for each ecological distinction made. Reversing positions of predators and prey would provide profiles of prey behavior.

Table 4. Categories of prey regarded as indicators of habitat types

Habitat A Litter Complex

- | | |
|-----------------|------------------------------------|
| 1. Acarina | 7. root coccids |
| 2. Mollusca | 8. <u>Forcipomyia</u> larvae |
| 3. Isopoda | 9. <u>Scolytidae</u> F |
| 4. Diploda | 10. <u>Strumigenys rogeri</u> |
| 5. Chelonethida | 11. <u>Strumigenys gundlachi</u> |
| 6. Collembolla | 12. <u>Xestocephalus maculatus</u> |

Habitat B Vegetation Complex

- | | |
|-----------------------------------|-----------------------------------|
| 1. Tipulidae | 7. <u>Ugyops occidentalis</u> |
| 2. Lepidopterous larvae | 8. <u>Cyrtoxiphia gundlachi</u> |
| 3. Lampyridae (winged) | 9. <u>Abelona</u> sp. |
| 4. Dolichopodidae | 10. <u>Anaulocomera</u> sp. |
| 5. <u>Iridomyrmex melleus</u> | 11. <u>Wasmannia auropunctata</u> |
| 6. <u>Myrmelachista ramulorum</u> | 12. Adult Fulgoridae |
-

will be consumed in considerably larger numbers than non-aggregating insects. On this basis we are inclined to give different weights to these two groups when interpreting our data. Lizards, while actively seeking prey exhibit a similar pattern of behavior. A single observation of a female *Anolis stratulus* digging up ants from a nest and an additional observation of selective feeding on worker termites to the exclusion of soldiers by *A. gundlachi* suggest that these insects will have to be weighted when interpreting lizard data as well. This is another area where nomenclatural categorization to the ordinal level will not provide us with sufficient information to make correct assumptions.

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Habitat B Vegetation Complex

- | | |
|-----------------------------------|-----------------------------------|
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| 2. Lepidopterous larvae | 8. <u>Cyrtoxiphia gundlachi</u> |
| 3. Lampyridae (winged) | 9. <u>Abelona</u> sp. |
| 4. Dolichopodidae | 10. <u>Anaulocomera</u> sp. |
| 5. <u>Iridomyrmex melleus</u> | 11. <u>Wasmannia auropunctata</u> |
| 6. <u>Myrmelachista ramulorum</u> | 12. Adult Fulgoridae |
-

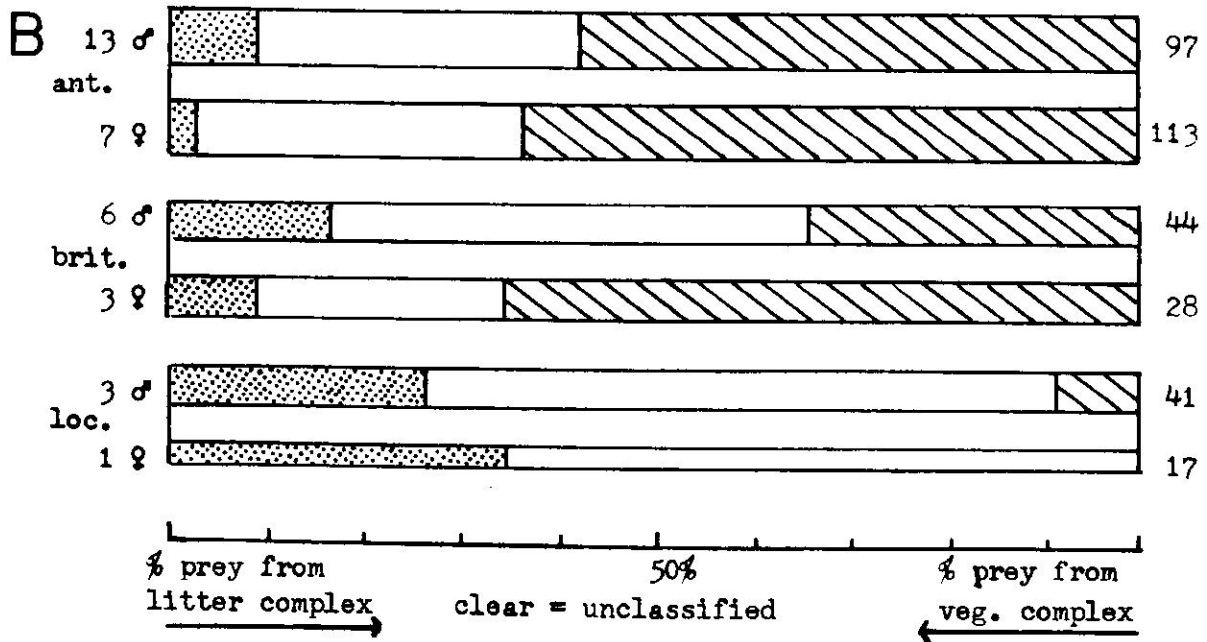
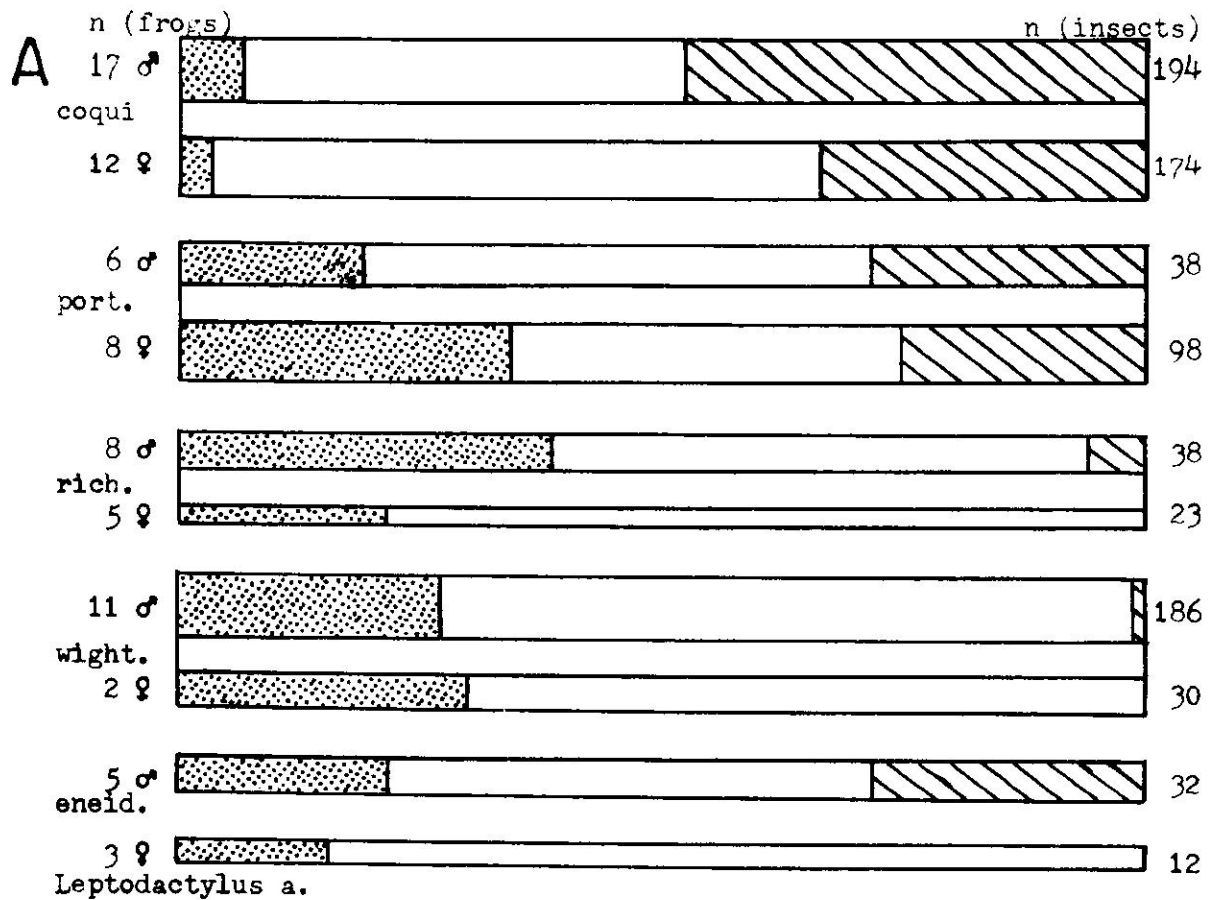


Figure 1. Profiles of predator feeding behavior.

Collection Times

During the early phases of this study lizards were collected during midafternoon, at which time their stomachs were full. Later collections, not as yet classified, have involved other times of day and night. Except for those species that sleep high in the trees, the Anolis are easily collected at night. Although 2 species are often active near the station lights all anoles observed at night away from the station have been inactive.

Initial frog samples were taken between the hours of 2100 and 0100. The following trends were noted: (1) Stomachs of most female frogs tended to fill earlier than those of males (2) E. wightmanae males were the only ones with full stomachs when collected while calling, the remainder of males tended to have partly empty stomachs during the period of calling, whether or not they were calling when collected (3) juveniles, which seemed to become more available for capture after 10 p.m., had full stomachs when collected, except for a single E. coqui taken with half empty stomach at 11 p.m.

Perch Height when Collected

At this time we lack sufficient data to establish whether or not male frogs forage at the same sites and times that they call. Again, within range of the field station lights, E. coqui males have been observed to do so, but this may not be typical. Individuals of both sexes of E. coqui tend to establish territories within the areas of high insect density near lights, and these territories persist for long periods of time even though lights are left off. There is little reason to believe that female frogs are not foraging at the sites where they are collected, but there is reason to believe a collection bias exists toward finding animals above the ground, particularly near eye level of the collector.

References Cited

- Hillman, Peter E. 1969. Habitat specificity in three sympatric species of Ameiva (Reptilia: Teiidae). *Ecology* 50: 476-481.
- Heatwole, H. 1963. Ecologic segregation of two species of tropical frogs of the genus Eleutherodactylus. *Carib. J. Sci.* 3(1): 17-23.
- Medica, P.A. 1967. Food habits, habitat preference, reproduction and diurnal activity in four sympatric species of whiptail lizards (Cnemidophorus) in south central New Mexico. *Bull. So. Calif. Acad. Sci.* 65: 251-276.
- Milstead, W.W. 1957. Some aspects of competition in natural populations of whiptail lizards (genus Cnemidophorus). *Texas J. Sci.* 9: 410-447.
- Pianka, E.R. 1967. On lizard species diversity: North American flatland deserts. *Ecology* 48: 333-351.
- Pianka, E.R. 1969. Sympatry of desert lizards (Ctenotus) in western Australia. *Ecology* 50: 1012-1030.
- Rand, A.S. 1964. Ecological Distribution in Anoline Lizards in Puerto Rico. *Ecology* 45: 745-752.
- Rivero, J.A., J. Maldonado and H. Mayorga, 1963. On the habits and food of Eleutherodactylus karlschmidti Grant. *Carib. J. Sci.* 3(1): 25-27.
- Schoener, T.W. 1968. The Anolis lizards of Bimini: resource partitioning in a complex fauna. *Ecology* 49: 704-726.

THE ECOLOGY OF ANTS OF LUQUILLO FOREST IN THE VICINITY
OF EL VERDE FIELD STATION - PRELIMINARY REPORT

Robert J. Lavigne

Much of the information gathered about the ants of Puerto Rico deals with the more common species found near the coast and on coffee plantations. Wheeler (1908) gathered some ecological data as well as describing several new species during his month's stay on the island. An additional species was described by Mann (1920) and more was added to our taxonomic knowledge by Wheeler (1934). Additional ecological information on the known species of ants was presented by Smith (1936) who spent a year in the western half of the island. He unfortunately did not have the opportunity to collect in Luquillo forest. In his two volume work on forest insects, Martorell (1954) only dealt with two species, i.e. Camponotus ustus and Myrmelachista ruculorum. Wolcott (1948) summarized available information on the habits of Puerto Rican ants, emphasizing their importance in the diets of Anolis lizardis, pulchellus, cristatellus, krugii and stratulus.

Since the compiled data show that ants may constitute more than a third of the diet of many species of frogs and lizards; at least on a numerical basis, and since many ant species are involved (Table 1), it would seem to be of interest to compile ecological information which might answer some important questions. A primary question is why certain species of ants appear only in the digestive tracts of particular species of frogs and lizards. Equally interesting is why many ant species appear in the stomachs of frogs which are most active at night whereas ants are generally considered to have diurnal habits.

Prior to the present study the list of ants collected near the El Verde field station included 22 species as identified by W. L. Brown of Cornell University (see insect checklist, Rain Forest Project Annual Report, 1967, p. 97). An additional 8 species have been encountered during the past year; all have been identified to genus and most to species. A key to the species distinguished is included as Appendix I. This key differs from Smith's (1936) key in that characters of the antennae and mandibular dentition have been emphasized to aid in identification of isolated heads as usually found in the stomachs of ant predators. Code names using the abbreviation "Form" for Formicidae and letters of the alphabet relate all species to previous diversity studies and field notes of George Drewry, resident director of the El Verde Field Station.

Observations of ant activity have been made in the field whenever time permitted. Notation was made as to time of day or night when ants were active as well as the type of activity in

Table 1. Colony size and site utilized by certain ant species in the tropical wet forest.

Ant Species	Colony Size				Site Chosen	
	queens	workers	eggs	larvae		
Solenopsis sp. (JJ)	5	39	8	12	1	component of decaying Cecropia limb
Solenopsis sp. (JJ)	-	13	-	7	4	between 2 leaves in litter on boulder
Solenopsis sp. (JJ)	4	36	20	32	4	within decayed palm nut
Solenopsis sp. (JJ)	6	52	6	67	17	Euterpe globosa within decayed palm nut
Solenopsis sp. (JJ)	2	78	77	107	50	within decayed palm nut
Strumigenys rogeri	1	105	2	15	2	component of decaying Cecropia limb
Strumigenys rogeri	3	+ 2 winged 8	reproductives 1	14	3	component of decaying Cecropia limb
Solenopsis sp. (GG)	5	113	77	101	40	component of decaying Cecropia limb
Solenopsis sp. (GG)	3	62	13	32	9	within decaying palm nut
Trachymesopus stigma	-	6	-	16	5	Euterpe globosa in soil beneath rock
Paratrechina sp. (EE)	1	5	4	5	0	component of decaying Cecropia limb
Paratrechina sp. (EE)	-	55	-	22	1	in dehiscid seed coat of Sloanea berteriana with soil
Formicid Z	-	1903	6	307	38	decaying seed pod of Inga vera
Formicid Z	-	41	2	63	112	within hollow seed coat of Dacryodes excelsa
Formicid Z	-	58	-	105	112	with hollow seed coat of Dacryodes excelsa
Formicid Z	-	145	-	135	387	with hollow seed coat of Dacryodes excelsa
Iridomyrmex melleus	1	816	528	731	5	component of decaying Cecropia limb

which they were engaged. Mention has been made in another report of the attraction of some species to fallen fruits of forest trees (Table 1, p.87). These observations along with other have indicated that the following species are active diurnally: Form Z, Form EE (Paratrechina sp.), Cyphomyrmex rimosus, Pheidole moerens, Form HH (Pheidole sp.), Form GG (Solenopsis sp.), Form JJ (Solenopsis azteca pallida), Myrmelachista ramulorum and Mycocepurus smithi. The workers of Iridomyrmex melleus and Brachymyrmex heeri are active both diurnally and nocturnally, often being taken on the same tree. They have been captured crawling on the vegetation as late as 11 PM. Form CC (Paratrechina sp.) workers have only been observed to be active at night crawling on tree trunks. While workers of Odontomachus bauri can be collected singly from within the litter or under bark of trees during the day, they have only been observed on rocks after dark. The winged reproductives of this species fly at night, coming to lights.

Of equal interest is the knowledge as to where these ants establish their colonies. A Iridomyrmex melleus colony was located under the bark of a living manzanilla tree, Sapium laurocerasus, at a height of four feet while another was found within a dead branch of an unidentified tree. A Form CC colony was discovered living in a large hole in the trunk of a living Roystonea borinquena tree at a height of about four feet. Colonies of Pheidole moerens and Trachymesopus stigma have been found under rocks in the forest trails.

Apparently the high incidence of rain in a tropical wet forest discourages some species from nesting in the soil and artifacts of the decaying forest are utilized instead by the ants. The empty seed coats of Dacryodes excelsa, Euterpe globosa and Sloanea berteriana, serve as nest sites as well as the individual pith chambers of decayed Cecropia peltata limbs. The ants species able to use these artifacts, because of their small colony size, are Form JJ (Solenopsis azteca pallida), Form GG (Solenopsis sp.), Strumigenys rogeri, Form EE (Paratrechina sp.) and Form Z. Data on size of colonies and colony site are presented in Table 1.

When more information has been accumulated on the activities of forest ants, it will be possible to plug the data into the general food web and hopefully answer the questions posed.

References Cited

- Mann, W. 1920. Additions to the ant fauna of the West Indies and Central America. Bull. Amer. Mus. Nat. Hist. 42(8):404.
- Martorell, Luis F. 1945. Formicidae, p. 557-60 IN A survey of the forest insects of Puerto Rico. Univ. of Puerto Rico J. Agr. 39(3&4): 69-608.
- Smith, M.B. 1936. The ants of Puerto Rico. Univ. of Puerto Rico J. Agr. 20(4): 819-875.
- Wheeler, W. Morton. 1908. The ants of Porto Rico and the Virgin Islands. Bull. Amer. Mus. Nat. Hist. 24(6): 117-158.
- Wheeler, W. Morton. 1934. Neotropical ants collected by Dr. Elizabeth Skwarra and others. Bull. Mus. Comp. Zool., Harvard 77(5): 157-240.
- Walcott, G.N. 1948. Formicidae; ANTS, p. 810-839 IN The insects of Puerto Rico-Hymenoptera. Univ. of Puerto Rico J. Agr. 32(4): 749-975.

Appendix I

Key to Formicidae (workers) of Luquillo Experimental Forest by Robert J. Lavigne (identifications by W.L. Brown, Cornell University, Ithaca, N.Y; David R. Smith, Systematic Entomology Laboratory, USDA and Robert J. Lavigne, University of Wyoming, Laramie, Wyoming).

- 1 Mandibles linear, inserted close together at middle of oral border and extending parallel well beyond border of head 2
- 1' Mandibles broadly flattened apically, arising from corners of head and when closed barely extending beyond anterior border of head 6
- 2 Abdominal pedicel consisting of a single scale; antennae 12 segmented 3
- 2' Abdominal pedicel consisting of two segments; antennae 6 segmented 4
- 3 Keel of scale elongated into a single sharp point dorsally; mandibles with 3 large blunt teeth apically followed by row of tiny teeth; large black species; length 8 mm (Form E) Oiontomachus haematodus (L.)
- 3' Keel of scale broad dorsally, bifurcate; mandibles with 3 teeth, apically followed by 2 tiny teeth; brown species; length 2.5 mm (Form FF) Anochetus mayri Emery
- 4 Inner border of mandibles lacking teeth other than apical pair but bearing a series of minute denticles; brown species; length 1 mm (Form S) Strumigenys eggersi Emery
- 4' Inner border of mandibles with 2 or more teeth in addition to apical pair 5
- 5 Inner border of mandibles with 2 large teeth in addition to apical pair; orange species, length 2 mm (Form F) Strumigenys rogeri Emery
- 5' Inner border of mandibles with row of small teeth; dark brown species; length 1.5 mm (Form T).... Strumigenys gundlachi (Roger)
- 6 Frontal carinae expanded laterally concealing antennal insertion; antennae 11 segmented; mandibles with 5 teeth; orange species; length 2 mm. (Form G)..... Cyphomyrmex rimosus (Spinola)
- 6' Frontal carinae not expanded laterally 7
- 7 Abdominal pedicel consisting of one expanded scale or scale is vestigial or absent 8
- 7' Abdominal pedicel consisting of two distinct segments 23

- 8 Scale of pedicel vestigial or absent 9
- 8' Scale of pedicel distinctly separated from gaster
resulting in a semivertical to vertical scale 11
- 9 Compound eyes absent; mandibles with 6 teeth on inner
border 4 of which mesh with the 5 teeth on the apical
border of clypeus; antennae 11 segmented; orange species;
length 2.3 mm (Form JJ) Amblyopone sp.
- 9' Compound eyes present, antennae 12 segmented; mandibles
with 4 teeth apically followed by row of minute teeth,
3rd tooth smaller than other three 10
- 10 Antennal scape not reaching posterior border of head; yellow
species; length 1.5 mm (Form Q) Tapinoma littorale Wheeler
- 10' Antennal scape reaching beyond posterior border of head; head
and thorax dark brown, legs and gaster pale yellow; length
2 mm (Form LL) Tapinoma melanocephalum (Fab.)
- 11 Abdominal pedicel segment, when viewed laterally, is
vertically erect and as high as dorsum of prothorax; antennae
12 segmented 12
- 11' Abdominal pedicel segment when viewed laterally, inclined
and less than height of dorsum of prothorax; antennae
variable 15
- 12 Compound eye of normal proportions, 26-30 facets; head
and body bare dorsally except for scattered setae; mandibles
with 6 teeth of approximate equal size; brownish yellow
species; polymorphic; length of smallest worker, 3.3 mm,
that of largest worker, 6.1 mm; (Form C)
..... Camponotus ustus Forel
- 12' Compound eye minute, composed of 10-12 facets 13
- 13 Mandibles with six large teeth of approx. equal size; each
middle and hind tibiae with two spurs; dark brown species;
length 2.1 to 3.5 mm (Form L) ... Trachymesopus stigma (Fab.)
- 13' Mandibles with 3 large teeth, a small 4th and large 5th;
all teeth much smaller than those of Trachymesopus; workers
less than 3 mm in length 14
- 14 Antennal pedicel black and almost reaching posterior border
of head; additional antennal segments, all tibia and tarsi
brownish orange; dark brown to black species; length 2.6 mm
..... Hypoponera trigona opacior (Forel)
- 14' Antenna brownish orange throughout, pedicel misses
reaching posterior border of head by approximately one-sixth
of its length; brownish orange species; length 2.0 mm
..... Hypoponera ergatandria (Forel)

- 15 Antennae with 9 segments; mandibles with 5 teeth, 1, 2, and 4 being larger than 3 and 5 16
- 15' Antennae with 12 segments; mandibles variable but not as above 17
- 16 Terminal 3 antennal segments forming a club; clypeus extends posteriorly between antennal insertions; head reddish brown to black; thorax, orange, gaster black; length 2.25 mm (Form M) Myrmelachista ramulorum Wheeler
- 16' Terminal 7 antennal segments grey, not forming a club, basal segments yellow; clypeus not extending between antennal insertions; tiny yellow species; length 1.2 mm (Form P) Brachymyrmex heeri Forel
- 17 Mandibles with 2 apical teeth followed by a series of tiny teeth; head and body lacking setae; terminal 7 antennal segments grey, basal segments yellow; orange species; length 2.5 mm (Form A) Iridomyrmex melleus Wheeler
- 17' Mandibles with 5 or 6 teeth 18
- 18 Length of antennal scape more than twice as long as distance from antennal socket to vertex 19
- 18' Length of antennal scape at most 1 1/2 times distance from antennal socket to vertex 20
- 19 Mandibles with 5 teeth of approx. equal size; setae of head white; black species; length 3 mm (reaches edge of forest not yet recorded within) (Form HH) Paratrechina longicornis (Latreille)
- 19' Mandibles with 6 teeth, 1, 2, 4 and 6 being larger than 3 and 5; setae of head black; yellowish brown species; length 3.5 mm (Form CC) Paratrechina sp.
- 20 Compound eye minute, composed of 10-12 facets; setae of head and body black; mandibles with 6 teeth, 1, 2, 4 and 6 being larger than 3 and 5; yellow species (Form DD) Paratrechina microps (M.R. Smith)
- 20' Compound eye of normal proportions, 26-30 facets; mandibles with 6 teeth, 1, 2, 4 and 6 being larger than 3 and 5 21
- 21 Head and thorax brownish orange, gaster black; length 2 mm (Form R) Paratrechina poss. vividula (Nylander)
- 21' Head and body same color throughout 22
- 22 Excluding basal setae, dorsal surface of mesotibia with 2 rows of 3 setae; head, body, femur and tibia dark brown; stouter species with heavier setae; length 2.5 mm (Form KK) Paratrechina steinheili (Forel)
- 22' Excluding basal setae, dorsal surface of mesotibia with 1 row of 4 or 5 setae and one row of 3 setae; head, body, femur and tibia light brown; length 2 mm (Form EE) Paratrechina sp. near vividula (Nylander)

23	Epinotal spines present	24
23'	Epinotal spines absent	31
24	Antennae with 11 segments	25
24'	Antennae with 12 segments	27
25	Posterior corners of head with single spines; thorax and keel of first abdominal pedicel segment spinose; mandibles with 5 teeth; orange species; length 2.5 mm (Form AA)	
 <u>Mycocephurus smithi</u> var. <u>borinquensis</u> Wheeler	
25'	Posterior corners of head without spines	26
26	Epinotal spines large and conspicuous; frons striated from antennal foveae to posterior border of head; mandibles same color as head, with 5 teeth; yellowish orange species; length 1.75 mm (Form Z)	
 <u>Wasmannia europunctata</u> (Roger)	
26'	Epinotal spines barely protruding; frons not striated; mandibles pale yellowish with 4 small brown teeth; head and gaster dark brown, thorax orange, legs almost transparent; length 2 mm	Form RR
27	Frontal carinae raised and distinct reaching posterior border of head; head striated and reticulated; mandibles with 7 teeth; reddish brown species; length 2.75 mm (Form W)	
 <u>Tetramorium guineense</u> (Fab.)	
27'	Frontal carinae, if distinct, not reaching posterior border of head, mandibles variables	28
28	Distance between epinotal spines less than length of one; mandibles with 5 teeth; antennae, head, gaster and femora blue black; thorax orange; tibia and tarsi yellow; length 3 mm (Form OO)	
 <u>Macromischa isabellae</u> Wheeler	
28'	Distance between epinotal spines approx. 1 1/2 times length of one; mandibles with 2 teeth apically followed by 2 rows of small teeth	29
29	Frons not reticulated or striated; epinotal spines reduced in size and barely protruding; dark brown species with yellowish brown legs; length 2.25 mm (Form H)	
 <u>Pheidole subarmata borinquensis</u> Wheeler	
29'	Frons reticulated or striated; epinotal spines prominent; workers less than 2 mm in length	30
30	Striations of frons almost reaching posterior border of head; compound eyes normal; head and gaster dark brown; thorax orange; legs brownish yellow; length 1.6 mm (Form K)	
 <u>Pheidole moerens</u> Wheeler	

- 30' Striations of frons not extending backward much beyond antennal sockets although area is reticulated; compound eyes slightly reduced in size; head, body and legs orange; length 1.2 mm (Form BB) Pheidole sp.
- 31 Antennae with 12 segments; mandibles with 4 teeth, the last one, on ventral angle, small 32
- 31' Antennae with 10 segments, mandibles with 4 teeth, the last one, on ventral angle, small 33
- 32 First pedicel segment evenly rounded; head and gaster reddish brown, thorax and legs orange; length 1.75 mm (Form MM) Monomorium floricola (Jerdon)
- 32' First pedicel segment flat dorsally dropping off rapidly both front and back; body black throughout; length 2.3 mm (has not yet been collected within forest but reaches edge) (Form SS)..... Monomorium carbonarium subsp. ebeninum Forel
- 33 Apical border of clypeus with pair of distinct teeth 34
- 33' Apical border of clypeus lacking teeth; uniform yellowish brown throughout except apical segments of gaster darker; length 1.5 mm (Form JJ)
..... Solenopsis azteca pallida Wheeler
- 34 Length of smallest worker greater than 2 mm, that of largest worker 3.6 mm; polymorphic; brown species with apical segments of gaster light (Form U)
..... Solenopsis geminata (Fab.)
- 34' Length of workers 1.75 mm or less 35
- 35 Head and gaster dark brown; thorax and legs orange; slightly darker than 6 segments basal to it; length 1.75 mm (Form WW) Solenopsis sp.
- 35' Uniform coloration throughout or with apical segments of gaster lighter 36
- 36 Dark brown species with apex of gaster yellow; monomorphic; length of workers 1.5 mm (Form GG) Solenopsis sp.
- 36' Yellow throughout; polymorphic; length of workers 1.2 mm or less (Form UU) Solenopsis corticalis Forel

THE ROLE OF INSECTS IN THE FOOD WEB OF THE
'TROPICAL WET FOREST' - PRELIMINARY REPORT

Robert J. Lavigne

The ecological niches occupied by insects in the tropical wet forest are poorly understood. In Puerto Rico the only broad study connecting forest plants and insects is that of Martoreil (1945) who summarized existing knowledge. Only scattered information has since appeared in the literature. Various trapping procedures used in the past (PRNC Rain Forest Project Ann. Rep. 1967, p. 78) have indicated generalized habitats for certain groups such as those associated with dead animals. Because of the necessity to delineate the litter environment from that of the vegetation in a concurrent frog study, a project was initiated to ascertain the specific niches occupied by insects.

The preliminary phase of this study is concerned primarily with those insects utilizing the fallen fruits of forest trees as breeding environments. The fruits of the various trees differ in the amount of flesh surrounding the seeds on the ground as well as the rate of decay of the dehiscent seed coat in those species in which this occurs. The seed of some species such as Byrsonima coriacea, Genipe americana and Manilkara balata are surrounded by thick fleshy pulp while those of Andira inermis and Dacryodes excelsa have thin fleshy seed coats. Other species such as Inga vera and Inga fagifolia produce pods in which the seeds remain protected until the pod rots. The tough outside coats of some species, such as Sloanea berteriana, Guarea trichilioides and Paullinia pinnata dehisce on the trees allowing the seeds to fall naked. The fruit of Euterpe globosa is properly termed the palm nut since the seeds are tightly compacted. Thus the fruits of the different trees become available to the insect fauna in different ways.

As seen in Table 1, a variety of insects have been found to be associated in some manner with fruiting bodies. Code names using an abbreviation for the insect family in combination with a letter of the alphabet relate all species to previous diversity studies and field notes of George Drewry, resident director of the El Verde Field Station. Interestingly, the majority of reared insects have belonged to the order Diptera and represents eleven separate families. The evidence, so far, is insufficient to ascertain whether there are restricted host-insect relationships but at least three species, Chironomid KK, Limonia willistoniana and a species belonging to the family Drosophilidae have been reared from two different hosts.

Thus far, only three species of ants (Formicidae), Pheidole moerens, Solenopsis sp. (JJ) and Formicid Z, have been consistently associated with several fruits. Such evidence would seem to indicate that, at least part of their sustenance is derived from this source.

Table 1. Insects reared from or associated consistently with tree fruits found in the forest litter, El Verde Field Station, P.R.

Tree fruit	Insects <u>Diptera</u>	<u>Coleoptera</u>	<u>Other Insects</u>
Andira inermis	Tipulidae Limonia willistoniana* Limonia domestica* Ceratopogonidae Forcipomyia gemialis* Sciariidae Sciariid B* Sciariid E* Stratiomyidae Nothomyia nigra* Chironomidae Chironomid DD Chironomid OO Psychodidae (undet.) Phoridae (undet.)	Scarabaeidae Canthochilum histeroides Endomychidae Endomychid B Staphylinidae Staphylinid C Curculionidae Curculionid C	
Sloanea berteriana	Chironomidae Chironomid KK* Psychodidae (undet.)	Nitidulidae Nitidulid A	Hymenoptera Formicidae Paratrechina sp. (EE) Formicid Z
Byrsonima coriacea	Drosophilidae (undet.)		Hymenoptera Formicidae Cyphomyrmex rimosus
Paullinia pinnata			Hymenoptera Formicidae Mycocepurus smithi
Euclea capitata		Nitidulidae Nitidulid A	
Manilkara balaia	Drosophilidae (undet.)*	Nitidulidae Nitidulid A	Hymenoptera Formicidae Pheidole moerens Solenopsis sp. (JJ) Formicid Z
Genipa americana		Nitidulidae Nitidulid A Staphylinidae Staphylinid G Staphylinid N	
Euterpe globosa		Scolytidae Scolytid F*	
Dacryodes excelsa	Drosophilidae (undet.)* Psychodidae (undet.)* Chironomidae Chironomid KK*	Nitidulidae Nitidulid A Endomychidae Endomychid B	Hymenoptera Formicidae Solenopsis sp. (JJ) Pheidole moerens
Eugenia stahlii			Orthoptera (undet.)*
Inga fagifolia	Tipulidae Limonia willistoniana* Chironomidae (undet.)*		Hymenoptera Formicidae Pheidole moerens Solenopsis sp. (JJ) Formicid Z
Inga vera	Phoridae Phorid Q* Cecidomyiidae Cecidomyiid F* Lonchaeidae Lonchaea sp. (B)* Drosophilidae (undet.)* Ceratopogonidae (undet.)* Mycetophilidae Boletina sp. (L)*	Nitidulidae Nitidulid B	Lepidoptera microlepidop (undet.)* Hymenoptera Formicidae Formicid Z Myrmelachista ramulorum Pheidole sp. (HH)
Guarea trichillioides	Sciariidae (undet.)* Drosophilidae (undet.)	Staphylinidae Staphylinid C Staphylinid G Staphylinid K Staphylinid L Staphylinid M Endomychidae Endomychid B Scarabaeidae Canthochilum borinquensis Nitidulidae Nitidulid A	Hymenoptera Formicidae Formicid Z Solenopsis sp. (OO) Solenopsis sp. (JJ) Solenopsis corticalis Pheidole moerens

*reared from fruit

Among the Coleoptera, four families (Scarabaeidae, Endomychidae, Staphylinidae and Nitidulidae) are commonly found feeding within the collected fruits. The greatest variety of species belong to the family Staphylinidae but it is only in the dehisced seed coats of Guarea trichilioides that all 5 species collected thus far, have been taken. Nitidulid sp. A seems to have the widest host range, which is not surprising since members of its family are commonly associated with decaying fruit.

Several members of the family Scolytidae, the bark beetles are known to occur in the forest but little is known concerning their life histories. However, a large percentage of the fruit of the palm, Euterpe globosa, are commonly infested by Scolytid F. The palm nuts are bored into by the adult beetles after the nuts have fallen into the litter. As a result of the infestation, the entire interior of the nut is consumed. As many as 18 adults, 22 eggs, 16 larvae and 12 pupae have been recovered from a single palm nut. A single lepidopterious larvae (species undetermined) may be coexisting in the same nut as the beetles.

More should be learned about the population cycles of insects in the rain forest. As the various species of trees come into fruit, it will be of great interest to compare faunas for the different times of the year.

References Cited

- Martorell, Luis F. 1949. A survey of the forest insects of Puerto Rico. Univ. of Puerto Rico J. Agr. 39 (3&4): 69-608.

PROPERTIES OF AQUATIC COMMUNITIES IN CONTAINER
HABITATS - PRELIMINARY REPORT.

John F. Addicott¹

Introduction

The purpose of this work was to investigate some of the properties of the communities in aquatic container habitats. Some of the properties in question are as follows: Does the diversity of prey organisms vary with the density and diversity of predators in some predictable fashion? Are certain container habitats acting as near or far islands with respect to the source of colonization, and can the community structure be explained or the probability of future structure predicted?

Container habitats are those in which water accumulates, usually in small amounts, in a depression formed exclusively by plant tissues. Examples of these are tree-holes, bromeliads, pitcher plants, *Heliconia* bracts, and in general any depression formed at a leaf or bract axil. In recent years these habitats have gained increasing attention from ecologists who have studied them from both a population and community viewpoint (Laessle, 1961; Maguire, 1959, 1963a, 1963b; Maguire and Belk, 1967; Maguire, Belk and Wells, 1968). This interest has stemmed mainly from the realization that these habitats are very frequently small (less than 20 ml.) and abundant, and that therefore replication in the field is available. Where rainfall is relatively predictable, such as in the low and middle elevations of Atlantic Costa Rica, and where it is constant, a minimal variability of the physical conditions in the habitat through time and between similar habitats in space can be expected. This lessens the probability that successional changes in community structure will be the result of physical changes in the habitat. This in turn makes easier the analysis of community interactions. Another advantages of this habitat for community ecology is that few kinds of organisms utilize it: osmotrophic and holotrophic protozoans, rotifers, nematodes, other worms, mites, and insect larvae, particularly mosquito and syrphid larvae.

The purpose of this part of the work was to evaluate and extend the ideas that Paine (1966) presented relating the diversity and density of predators to the diversity of prey. The bract contents of *Heliconia bihaie* (?) were used for this evaluation. On the basis of the work of Maguire et al. (1968) mosquito larvae (*Culex americanus*) were considered as the predator and all protozoans as the prey. The

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use of protozoans as an ecological unit is justifiable on the following basis: the mosquito larvae seem to recognize all members of the group as potential prey organisms; there are only two trophic types represented among the protozoan sampled from this habitat, osmotrophs and bacterial feeders; the protozoans are the only group found in the bracts which has a high enough population turnover rate to be able to respond quantitatively to the predation pressure. The nature of the interaction between predator and prey is hypothesized to be the following. Where mosquito larvae are absent or very rare in a bract, competition among the protozoans for the available food resources should result in a low diversity of protozoans, with the better competitors becoming very abundant and the majority of species being rare or absent. Likewise at very high densities of mosquito larvae diversity of the protozoans should be low due to the elimination of all but those species which are able to divide very quickly or are able to find some micro-environment within the bract which is relatively free from predation pressure. At intermediate densities of mosquito larvae it is hypothesized that the effect of predation will be to lower the competitive superiority of the better competitors, thereby allowing more species and a more even distribution of abundances among species to exist. Therefore, when some measure of the diversity of prey is plotted against some measure of predation pressure, a unimodal humped curve should result. The hypothesis can be extended to predict the effects of added diversity among the predators and the addition of further trophic levels to the system, but since this could not be tested during this part of the work, it will not be discussed.

Methods

The study was carried out between June 16 and June 18, 1969 at the El Verde Research Station, in the Luquillo National Forest in Eastern Puerto Rico (the logistic support of the Puerto Rico Nuclear Center is gratefully acknowledged). The contents of individual bracts of Heliconia bihai (?) were removed using a plastic turkey baster and rubber bulb, and placed in wide mouth jars, which had been rinsed with 70% ETOH and allowed to dry. These samples were then carried immediately to the lab for analysis. The analysis consisted of taking two small subsamples, one from the top the other from the bottom of the bottle, for censusing which protozoans were present. From the entire sample the mosquito larvae were counted and the presence of other organisms recorded. For simplicity the diversity of protozoans was calculated as the number of species present; density of mosquito larvae was calculated on the basis of the total number of larvae present, regardless of the distribution of instars among this number.

Results

Figure 1 shows the results of samples taken from 35 bracts on 6 inflorescences. Use of the non-metric E and H test (Eyeballing and Handwaving test) shows that the hypothesized relationship between prey diversity and predator density was not demonstrated.

Discussion

A number of improvements of the methods involved in this part of the work can be made. A better measure of predation pressure is desirable. This could be accomplished in the following manner: in the lab determine the relative feeding rates of each instar of the mosquito larvae (I-IV); ensure that all mosquito larvae are removed from the bract during sampling; (which was not the case in this study); classify each larva as to its instar. Second, with the maximum number of protozoan species found being 6, the measure of diversity of protozoans was relatively crude and insensitive. Subjective estimates of the biomass of each species (abundant, common, rare, present, absent) would enable the use of a more sensitive measure of diversity. Third, a larger sample size of bracts would include a greater range of density of mosquito larvae, thus filling out the right hand tail of the curve which was missing from this study. Fourth, the hypothesis as it has been presented predicts conditions in an equilibrium condition, whereas there is every reason to believe that such conditions were not always encountered. The predation pressure upon the protozoans was probably changing rapidly from day to day as the distribution of individual larvae among instars changed. Some bracts may have been encountered in which colonization was not yet complete, or in which more species were present than could be supported at equilibrium and due to a time lag all species were still present. The effect of most of these problems probably can be analyzed during a longer term study in which individual bracts are repeatedly sampled. Another approach would be to control the densities of mosquito larvae and allow the system to move towards an equilibrium. This latter approach will be attempted in the near future using pitcher plants instead of Heliconia bracts.

Literature Cited

- Buskirk, W.H. 1969. Microinvertebrate Faunas of Heliconia Flower Bracts. OTS Research Report. Unpublished.
- Hairston, N.G. 1958. The Species Problem in Paramecium. Evolution
- Kudo, R.R. 1966. Protozoology. C.C. Thomas. Springfield, Illinois. 5th ed.
- Laessle, A.M. 1961. A Micro-limnological Study of Jamaican Bromeliads. Ecology 42: 499-517.
- Maguire, B., Jr. 1959. Aquatic biotas of teasel waters. Ecology. 40: 506.
- 1963a. The Passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. Ecol. Mono. 33: 161-185.
- 1963b. The exclusion of Colpoda (Ciliata) from Superficially Favorable Habitats.
- Maguire, B., Jr. and D. Belk. 1967. Paramecium transport by land snails. J. Protozool. 14: 445-447.
- Maguire, B., Jr., D. Belk and G. Wells. 1968. Control of Community Structure by Mosquito Larvae. Ecology 49: 207-210.
- Paine, R.T. 1966. Food Web Complexity and Species Diversity. Amer. Nat. 100: 65-75.
- Shandon, 1932. Food of the Protozoa. Cairo.

STUDIES ON THE CHEMISTRY OF THE TROPICAL
MARITIME ATMOSPHERE

1. COMPOSITION OF AEROSOLS AND PRECIPITATION
OVER PUERTO RICO

Anders Andren and Robert C. Harris¹

Introduction

In the tropical rain forest ecosystem chemical input through impaction of atmospheric aerosols on the forest canopy and precipitation is a major component of the chemical mass balance. The Luquillo Experimental Forest of eastern Puerto Rico is exposed to a flow of relatively uncontaminated maritime air and studies by Jordan (1969) have demonstrated that a large percentage of the calcium, magnesium, sodium and potassium in the system is derived from atmospheric precipitation. Studies by Duce *et al.* (1965) have shown that the halogen composition of marine aerosols is different from the sea water from which they are derived with many of the minor elements being highly enriched in the aerosol phase.

The objectives of the present study are to determine the total atmospheric input of chloride, sodium, potassium, calcium, magnesium, strontium, fluoride, sulfate and phosphate into the rain forest. The input mechanism will be determined by studies on the relative importance of dry impaction of aerosols on the forest canopy versus input by precipitation. Aerosol size distributions over the rain forest and variations in aerosol composition as a function of particle size will be investigated.

Experimental Methods

Analytical procedure - The rain water samples were all concentrated under ultraviolet lamps ten to a hundred fold. Sodium, potassium, calcium, magnesium and strontium were all determined using a Beckman atomic absorption unit. The chloride and fluoride ion concentrations were determined by Orion specific-ion electrodes. Sulfate was determined by a turbidimetric method. Phosphate was determined by the molybdate blue method.

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Sampling Procedures

All rain and aerosol samples were collected at the tower situated in the El Verde Experimental Forest station at an altitude of 500 m. The rain was collected in 20 cm x 40 cm polyethylene cans rinsed clean with distilled water. The amount of rain was recorded for each shower as well as direction of the wind and time of collection. The aerosol samples were collected with a six stage Anderson cascade impactor at the same location. The water soluble aerosols were then washed off with five to ten ml of distilled water and analyzed as described above.

Results

The results of the rain samples are given in Table 1. The ion ratios for these samples are presented in Table 2. The sea water ion ratios and the enrichment factor are also included for comparison. A large variation in absolute concentration is observed. This is not surprising when they many parameters which might effect the chemical composition of the rainfall are considered among which are the conditions of the sea off the coast of the island, the age of the shower when it passes over the collection site, the wind speed and direction, and the path over land that the shower has followed before reaching the sampling location. The washout effect can be seen in samples Y-1 and Y-2 which were from rain that occurred after several days of no rain. The Cl/Na ratio and the Mg/Na ratio are both very close to that of sea water whereas the other ions seem to be enriched with respect to sodium.

The results for the aerosols are presented in Table 3. The most note worthy trends are as follows:

1. All have an initial enrichment with respect to sodium relative to sea water ratios.
2. There is a maximum enrichment in the 2 u to 6 u size range.
3. Sulfate is much more enriched in the aerosol particles than in the rain water.
4. The total ratios of aerosol particles approximate the ratios in the rain water except the sulfate/chloride ratio.

Similar results have been found for sodium and chloride in Hawaii (Junge, 1957) but the other ions have not been investigated in the same manner and thus no comparisons can be made for other areas.

Table 1. Elemental Composition of Rain in Puerto Rico

Sample No.	Date	Local time	Altitude	Amt. of rainfall	Cl (PPM)	Na (PPM)	K (PPM)	Ca (PPM)	Mg (PPM)	F (PPM)	SO ₄ ⁻ (PPM)	PO ₄ ⁻ (PPM)	Sr (PPM)
L-1	20 Aug. '69	1400-1600	650 m	0.21"	1.85	1.10	0.17	0.375	0.100	0.34	0.7	----	----
Y-1	26 Aug. '69	1300-1400	500 m	0.13"	3.54	1.51	0.10	0.893	0.125	0.043	2.0	0.015	----
Y-2	27 Aug. '69	0900-1000	500 m	0.09"	3.92	1.69	0.58	0.648	0.159	0.043	1.2	0.005	----
Y-3	27 Aug. '69	1100-1400	500 m	0.82"	1.98	1.05	0.10	0.140	0.064	0.027	0.6	----	0.018
Y-4	28 Aug. '69	2400-0900	500 m	0.29"	1.70	0.83	0.12	0.160	0.053	0.021	0.6	----	----
Y-5	29 Aug. '69	0800-1300	500 m	0.24"	3.03	1.63	0.15	0.700	0.130	0.030	0.7	----	----

Table 2. Ion Ratios in Rain from Puerto Rico

Sample No.	Cl/Na	K/Na	Ca/Na	Mg/Na	F/Cl	SO ₄ /Cl	Sr/Ca
L-1	1.68	0.16	0.34	0.09	0.018	0.38	0.13
Y-1	2.34	0.07	0.58	0.08	0.012	0.57	----
Y-2	2.32	0.34	0.39	0.09	0.011	0.30	----
Y-3	1.88	0.09	0.14	0.06	0.014	0.30	----
Y-4	2.05	0.15	0.19	0.08	0.012	0.30	----
Y-5	1.86	0.09	0.43	0.08	0.009	0.23	----
S.W.					6.7		
Ratio Ave.	1.80	0.0036	0.038	0.12	10 ^{-5x}	0.13	0.031
Ratio	2.02	0.15	0.35	0.08	0.013	0.35]	0.13

Table 3. Elemental Composition and Ion Ratios of Aerosols from Puerto Rico

Sample No.	Diameter Range (μ)	Cl	Na	Ca	Mg	SO ₄	Cl/Na	Ca/Na	Mg/Na	SO ₄ /Cl
		ug/m ³	ug/m ³	ug/m ³	ug/m ³	ug/m ³				
1Y-1	<8	0.63	0.17	0.045	0.015	----	3.70	0.26	0.088	----
1Y-2	5-10.5	0.50	0.30	0.056	0.015	----	1.67	0.19	0.050	----
1Y-3	3-6	1.37	0.47	0.170	0.048	1.49	2.92	0.36	0.102	1.09
1Y-4	2-3.5	0.78	0.55	0.280	0.082	2.60	1.42	0.50	0.149	3.33
1Y-5	1-2	0.85	0.55	0.186	0.074	3.94	1.55	0.34	0.135	4.63
1Y-6	<1	0.37	0.28	0.056	0.022	----	1.32	0.20	0.079	----
Total		4.5	2.32	0.793	0.256	78.03	1.93	0.34	0.11	1.79

Discussion

The ion ratios exhibit several important features. The Cl/Na ratios show an enrichment factor of 1.12 in rain water and 1.07 in aerosols indicating that some fractionation occurs at the sea surface. The same is true for the other elements except for Mg which shows almost the same value as that of sea water. Dry fallout could be quite an important factor for sulfate removal since the SO₄/Cl ratio for rain is 2.7 and that of aerosols is 13.8. Fluoride is enriched 540 times in rain and is quite an important mechanism for input of this element into the ecosystem. Additional samples have recently been collected and the authors hope to establish a regular sampling program in the near future.

References

- Duce, R.A., Winchester, J.W. and Van Nahl, T. (1965) Iodine, bromine, and chlorine in the Hawaiian marine atmosphere, *J. Geophys. Res.*, **70**: 1775-1799.
- Jordan, C.F. (1969) Isotope cycles, in: *The Rain Forest Project Annual Report, Puerto Rico Nuclear Center, Univ. of Puerto Rico*, p. 2-64.
- Junge, C.E. (1957) Chemical analyses of aerosol particles and of gas traces on the island of Hawaii, *Tellus*, **9**: 528-537.

KARYOTYPIC STUDIES OF BATS OF THE FAMILY PHYLLOSTOMATIDAE

Robert J. Baker¹

From 16 July to 24 July 1970, Mr. Genaro Lopez and I visited the Puerto Rico Nuclear Center's El Verde Field Station in El Yunque National Forest. Our visit was part of a National Science Foundation sponsored research project (GB-8120) entitled "Karyotypic Studies of Bats of the Family Phyllostomatidae". Permission to use the facilities was kindly granted by PRNC and the Terrestrial Ecology Program director. Collections were made by "mist nets" placed above the forest canopy and the forest floor.

The karyotypic preparations were made by a modification of the in vivo bone marrow culture method. Each live animal was weighed, and then injected intraperitoneally with a 0.025% Vinblastine or colchicine solution at a rate of 0.01 ml per gram of body weight. After two hours the animal was sacrificed and approximately 2/3 of the humerus was removed without damaging the proximal end. The flesh and a chip of bone were removed from the proximal end of the humerus to expose the red bone marrow cavity. The shaft was flushed with 3 ml. of a 1.0% sodium citrate solution, pipetting vigorously to break up any cell clumps. The resultant cell suspension was allowed to set for 10-12 minutes after which time it was filtered through two layers of cheese cloth and centrifuged at 500-1500 RPM for four minutes.

As much of the supernatant fluid as possible was discarded exercising caution so as not to disturb the button of cells. To the precipitate 3 ml of freshly prepared Carnoy's fixative (3 parts absolute methanol and 1 part glacial acetic acid) were added. Floating materials and lipids may be removed at this stage. The cell button was gently disrupted with a pipette until a homogenous cell suspension was obtained. After allowing the cells to fix for 10-12 minutes, the suspension was centrifuged for 4 minutes and the supernatant was discarded. The cells were resuspended in 1.0 ml of fixative and centrifuged as before. This step was repeated three times. After final washing the cells were resuspended in 1.0 ml of fixative.

Three to four drops of the cell suspension were placed on a clean slide and ignited. When the fire extinguished itself, the residue was promptly slung from the slide. Usually, four slides from each specimen were made. The dry slides were stained with Giemsa's stain (1 part Giemsa's stock solution to 8 parts distilled water) for 15 minutes. The slides were then passed through two baths of acetone;

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Brief comments on the chromosomes of each species follow.

Pteronotus parnellii (Gray)

$2n=38$, FN=60. The chromosomes of this species have been studied from specimens from Mexico (Baker, 1967) and from specimens from Trinidad (Baker, In Press) and no geographic variation was found. The chromosomes of the Puerto Rican specimens were indistinguishable from those previously reported for species (Baker, 1967).

Pteronotus fuliginosa (Gray) Figure 1a

$2n=38$, FN=60. This species which is endemic to the Greater Antilles has a karyotype identical to that reported for the other three species of Pteronotus which have been studied (Baker, 1967).

Monophyllus redmani Leach Figure 1b

$2n=32$, FN=60. All autosomes are biarmed and metacentric or submetacentric. The one of the smallest pairs has a distinct secondary constriction on the long arm. The X is a submetacentric and the Y a minute element. The closest living relative of this genus is Clossophaga of which three species have a karyotype much like that reported for this genus (see Baker, 1967).

Artibeus jamaicensis Leach Figure 1c

$2n=30^{00}3100$. This species has been studied from a variety of localities (see Baker 1967, Hsu et al 1968, and Baker, In Press) and all males examined have had two Y chromosomes. This is true of the Puerto Rican specimens. There also appears to be no variation in the autosomes.

Brachyphylla cavernarum Gray Figure 1d

$2N=32$ FN=60. All of the autosomes are biarmed and metacentric or submetacentric in nature. The X is a submetacentric and the Y is a minute element. There is a secondary constriction on one of the smallest pairs of autosomes.

Sternoderma rufum St.-Hilaire Figure 1e

$2N=30^{00}3100$. All of the autosomes are biarmed and, except for two pair of subtelocentrics, all are metacentric or submetacentrics. The X is a subtelocentric and the Y's are two small acrocentrics, one of which is slightly larger than the other.

Erophylla bombifrons (Miller) Figure 1f

2N=32, FN=60. All of the chromosomes of females are biarmed elements and most are metacentric or submetacentric in nature. One of the smallest pairs has a distinct secondary constriction on the long arm. Since only females were collected the sex chromosomes could not be determined.

Eptesicus fuscus Palisol de Beauvois

2N=50, FN=48. The chromosomes of the two Puerto Rican specimens were like those described for this species from the United States and Mexico (Baker and Patton, 1967). All autosomes were acrocentric and the two X chromosomes were submetacentric.

Molossus molossus

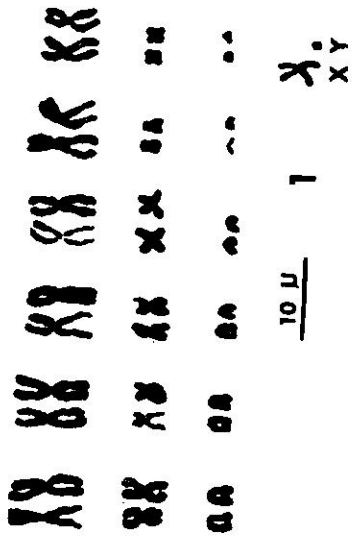
2N=48, FN=56. The autosomes consist of a large pair of submetacentrics, three medium pairs of submetacentrics, a pair of medium sized subtolocentrics, and a graded series of 18 pairs of acrocentrics. One of the two largest pairs of acrocentrics has a secondary constriction very near the centromere. The X is a submetacentric and the Y is a small, but by no means minute, acrocentric.

Discussion

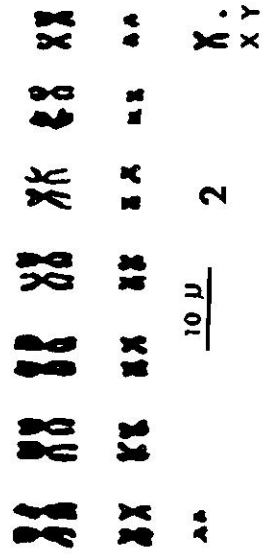
The collections made for this study revealed some ecological aspects of the rainforest bat fauna. Tamsit and Valdivieso (1970) did not list any insectivorous forms for the El Verde field station area. We collected 3 different genera of insectivorous bats, Pteronotus parnelli (8 specimens), Eptesicus fuscus (5 specimens) and Molossus molossus (1 specimen). Many Eptesicus were observed flying over the nearby stream and above the roads, and Molossus were frequently observed flying high over the forest. Fewer Pteronotus were observed but the number collected suggests that they are not uncommon to the forest.

Since to our knowledge the air above the canopy had not been netted or extensively collected, about thirty feet of net was placed for one night between existing towers. The collection yielded 2 specimens of Brachyphylla cavernarum, 4 specimens of Artibeus jamaicensis and 2 specimens of Stenoderma rufum. Of interest is the fact that Brachyphyllum had not been previously reported from the Luquillo Forest, although it is known to be abundant near the numerous caves of the limestone regions of the island.

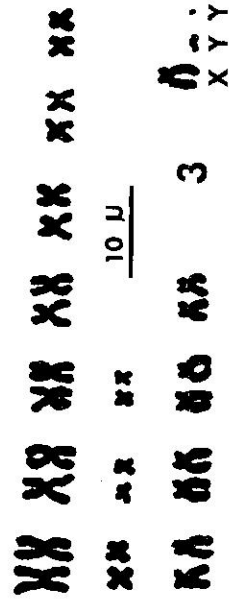
On four separate nights nets were placed within the forest in situations judged to be optimal based on netting results in other tropical forests. At no time were as many as 8 specimens obtained in a 30 foot section of net. It is thus possible that a larger number of bats fly in the upper canopy than near the forest floor.



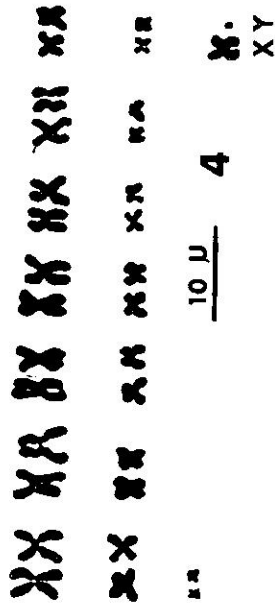
1a Pteronotus fuliginosa



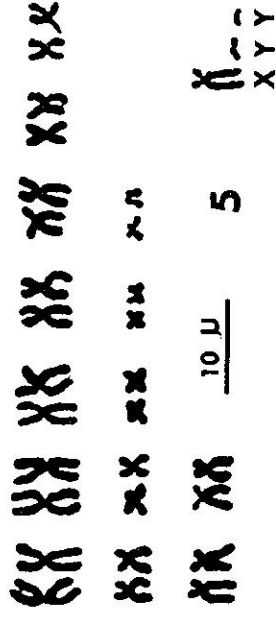
1b Monophyllus redmani



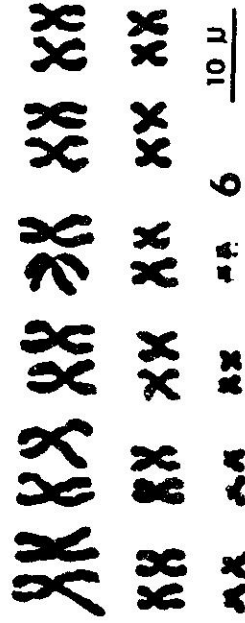
1c Artibeus jamaicensis



1d Brachyphyllium cavernarum



1e Stenoderma rufum



1f Erophylla bombifrons

Figure 1 Representative karyotypes of the Family Phyllostomatidae

one of acetone and xylol (1:1) and two of xylol, and then mounted under a 22x40 mm coverslip with Permount. A minimum of 25 spreads were counted from each specimen examined. All specimens were prepared as museum skins and skulls and deposited in the collection of animals, Department of Biology, Texas Tech. University.

Results

A total of 65 specimens were studied involving nine species, eight genera and three families. A summary of these data are shown in Table 1.

Table 1. Chromosomal Data For Bats From Puerto Rico

	2N	FN	X	Y	oo	♀♀
Family Phyllostomatidae						
<u>Pteronotus</u> <u>parnellii</u>	38	60	SM	A	3	1
<u>Pteronotus</u> <u>fuliginosa</u>	38	60	SM	A	1	0
<u>Monophyllus</u> <u>redmani</u>	32	60	SM	A	5	2
<u>Artibeus</u> <u>jamaicensis</u>	30-31	56	ST	A-A	4	1
<u>Brachyphylla</u> <u>cavernarum</u>	32	60	SM	A	7	4
<u>Stenoderma</u> <u>rufum</u>	30-31	56	ST	A-A	12	4
<u>Erophylla</u> <u>bombifrons</u>	32	60	?	?	0	11
Family Vespertilionidae						
<u>Eptesicus</u> <u>fuscus</u>	50	48	?	?	0	2
Family Molossidae						
<u>Molossus</u> <u>molossus</u>	48	56	SM	A	0	7

Even though the Puerto Rican populations are isolated, the karyotypes do not vary within species (Artibeus jamaicensis, Pteronotus parnellii and Eptesicus fuscus) which occur both on the mainland and on Puerto Rico. Further, the karyotype of Monophyllus is much like that of its closest relative, Glossophaga (Baker, 1967) which occurs on the mainland. Pteronotus fuliginosa also has a karyotype like that of other species within the same genus. These data suggest that there has been little or no karyotypic evolution within these forms since reaching this island.

Another genus, *Stenoderma*, is found to have the XX/X₁Y₂ sex determining mechanism. This brings the number of genera to 6 (*Stenoderma*, *Ametrida*, *Artibeus*, *Choeronyctus*, *Carollia*, *Enchisthenes*) which have this type of sex determining mechanism (see Baker In Press).

Literature Cited

- Baker, R.J. 1967. Karyotypes of Bats of the Family Phyllostomidae and Their Taxonomic Implications. *The Southwestern Naturalist* 12(4): 407-428.
- Baker, R.J. In Press. Karyotypic trends in bats. In: *Biology of Bats*. William A. Wimsatt, Editor. Academic Press, New York.
- Baker, R.J. and J.L. Patton. 1967. Karyotypes and Karyotypic Variation in Vespertilionid Bats. *J. Mammal.* 48: 270-286.
- Hsu, T.C., R.J. Baker, and T. Utakoji. 1968. The Multiple Sex Chromosome System of American Leaf-Nosed Bats (Chiroptera, Phyllostomidae). *Cytogenetics* 7: 27-38.
- Tamsitt, J.R. and Dario Valdivieso. 1970. Observations on Bats and their Ectoparasites. *A Tropical Rain Forest*. H.T. Odum, Ed. (In Press).

ARRESTED SUCCESSION IN TROPICAL, TERRESTRIAL ECOSYSTEMS

Jack Ewel

Introduction

Tropical terrestrial ecosystems exposed to low temperatures or low moisture are less structurally complex than systems which are not stressed in this manner, such as the wet lowlands. Also, agricultural systems of low structural complexity (e.g., corn fields) have been notably unsuccessful in hot, wet, tropical areas, but work well in drier or colder environments. The examples of successful, permanent agricultural systems in the wet tropical lowlands are ones of relatively complex structure, such as cacao and rubber plantations, and dooryard gardens in which many species of varying size are grown in a small area.

Stress constitutes an energy drain on the system resulting from work done to permit survival in an adverse environment. Under stress conditions a relatively high proportion of the energy available to a system is channeled into mechanisms which permit the system to withstand the stress, whereas systems not subjected to stress put proportionally more energy into structural complexity.

In agricultural systems, man attempts to maintain limited structure and accomplishes this through energy inputs in the form of weeding, cultivating, and applying herbicides and pesticides. In low stress conditions, relatively high inputs of this form are required to avoid complexity, whereas limiting water and temperature are two natural stresses which perform the same function. What is the relative caloric cost of limiting the development of structural complexity in low stress environments compared with areas of high natural stress? Measurements will be made to achieve the following:

- 1) Measurement of the rate at which systems exposed to different environmental stresses begin to return to their original levels of structural complexity after their complete destruction.
- 2) Measurement of the energetic cost of preventing this return.
- 3) Development of an index of structural complexity which will be applicable to a wide variety of tropical, terrestrial ecosystem.
- 4) Comparison of the energetic cost of limiting structure via a direct energy inputs vs. a specialized fossil fuel input, herbicide.

- 5) Simulation (digital or analog) of the response of various tropical systems to stress and prediction of the inputs necessary to prevent various ecosystems from becoming structurally complex.

PROCEDURES

Field Studies

Sites - Study areas have been selected in five tropical environments: two sites in low stress zone, two sites along a moisture gradient with mean annual temperature held approximately constant, and one site at low temperature where moisture is non-limiting. Data on location and gross climatic factors for each of the five areas are shown in Table 1. While the procedures and methods are applicable to all sites, only the Quebrada Jimenez site in Eastern Puerto Rico will be described in this report.

The Quebrada Jimenez site is located in the Luquillo Experimental Forest and is approximately 10 km. from the PRNC site at El Verde. The site has been classified as a low stress environment with respect to rainfall and temperature. The rainfall distribution in the area is relatively uniform. The vegetation at Jimenez had been sprayed with herbicides four years ago. The canopy, at 19 M, was open and the ground covered by a dense understory of Palicourea sp. and Psychotria sp. about 3 M tall. The soil is a moderately well drained, heavy latosol and the topography is somewhat irregular, with slopes averaging 20 to 30%.

Experimental Design - A split-plot design is being used, with four replications per location. Each replication consists of two mainplots. One of these was cleared, while the other was cleared and sprayed with herbicide. This initial treatment is intended to provide a measure of the role of coppicing in early recovery. Each mainplot, in turn, consists of six subplots, two of which will be selected for measurement and retreatment at each of three times. One of the selected subplots will be cut and the other herbicided.

Techniques - July, and September through December, 1969 were spent selecting and preparing the five sites. An area of 100 by 58 m was surveyed at each location, with the long dimension running, insofar as possible, parallel to the contours. After the existing vegetation was felled, the 48 subplots, each of which is 3 by 6 m, were laid out. A 10 m buffer zone was left around the inside border of the plot. All cut vegetation and litter was removed and discarded into the 4 m buffer zone between adjacent subplots. In the case of the Ojo de Agua plot, biomass was removed only to the surface of the deep organic layer, since this seemed to be the principal rooting medium. Due to time and labor

Table 1. Location and climate of the field sites.

	North Latitude & Elevation	Gross Climatic Stress	Life Zone (sensu Holdridge, 1967)	Mean Annual Biote mp. (est.)(°C)	Mean Annual Precip. (mm)(est.)	Date of Initial Treatment
Guánica, Puerto Rico	17° 50' 60M	low rainfall	Subtropical Dry Forest	24	700	Oct. 18
Guanacaste, Costa Rica	10° 30' 80M	moderately low	Tropical Dry Forest	26	2000	Dec. 23
Ojo de Agua, Costa Rica	9° 40' 2900M	low temperature	Tropical Montane Rain Forest	8	3000	Dec. 15
Quebrada Jiménez, Puerto Rico	18° 25' 320M	none?	Subtropical Wet Forest	23	4000	Oct. 17
Rincón de la Osa, Costa Rica	8° 40' 30M	none?	Tropical Wet Forest	26	4600	Dec. 5

limitations it was not possible to fell the larger overstory trees at the Quebrada Jimenez, P.R. site, these were girdled and poisoned. A recent inspection indicates, however, that this treatment killed only about 20% of the trees, so an immediate retreatment is planned.

Following a final recleaning of all subplots, herbicides were spray - applied to one randomly selected mainplot of each replication as follows:

<u>Dow Chemical Trademark</u>	<u>Name of Herbicide</u>	<u>Application Rate (g/m²)</u>
Tordon	4 - amino - 3,5,6 - trichloropicolinic acid	0.067
	2,4 - dichlorophenoxyacetic acid	0.250
Dowpon	2,2 - dichloropropionic acid	1.749

The above doses are two to three times higher than those usually applied in normal agricultural use. Tordon and 2,4 - D are dicot killer, while Dowpon kills monocots. The date on which herbicide was applied at each site, thus the time from which all regrowth is dated, is indicated in the final column of Table 1.

Preceding the retreatment of each subplot, the accumulated structure will be evaluated by measuring:

- 1) Number of stems, separated by origin (seedling or coppice)
- 2) Heights
- 3) Per cent cover
- 4) Visible light transmission
- 5) Number of species
- 6) Leaf area index
- 7) Above-ground biomass

These measurements, excluding leaf area index and above-ground biomass, will also be made in nearby steady-state forests on similar sites.

Evaluating Energy

A generalized energy flow diagram of the systems, using the terminology and symbols of Odum (1967 a&b), is shown in Figure 1. The drain caused by the necessity of developing and maintaining mechanisms to endure environmental stress is shown within the plant population as part of the potential generating work. This acts as a feedback through a work gate on the energy flow within the plants, and its associated energy drain would increase as environmental stress increases.

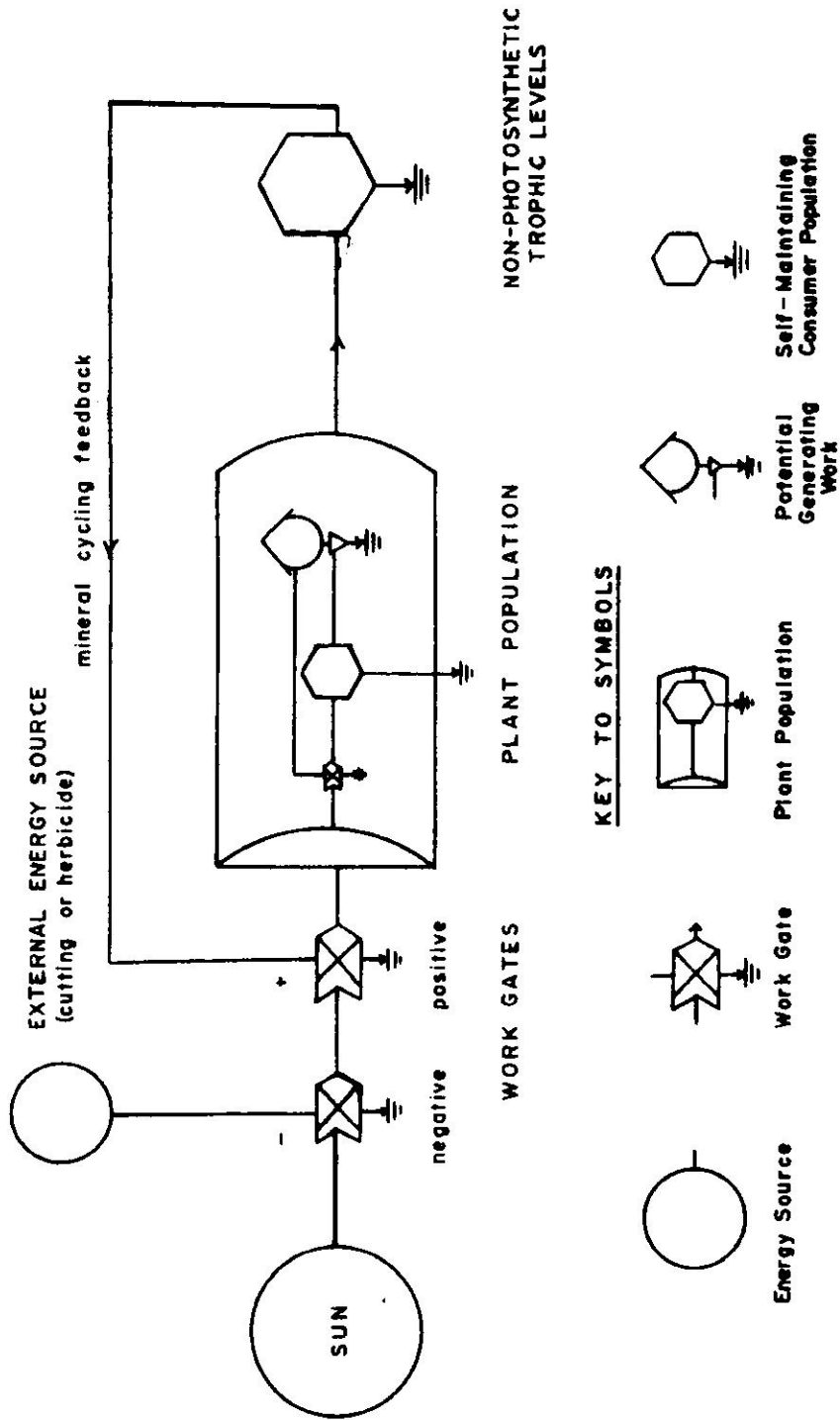


Figure 1 Simplified energy flow diagram for a tropical terrestrial ecosystem, showing external energy source used as negative work gate. (Symbols after Odum, 1967a & b)

Table 1. Location and climate of the field sites.

Location	North Latitude	Elevation (m)	Gross Climatic Stress	Life Zone (sensu Holdridge, 1967)	Mean Annual		Date of Initial Treatment
					Biotemp. (est.) (°C)	Precip. (mm) (est.)	
Guánica, P. Rico	17° 50'	60	low rainfall	Subtropical Dry Forest	24	700	Oct. 18
Guanacaste, C.R.	10° 30'	80	moderately low rainfall	Tropical Dry Forest	26	2000	Dec. 23
Ojo de Agua, C.R.	9° 40'	2900	low temperatures	Tropical Montane Rain Forest	8	3000	Dec. 15
Quebrada Jiménez, P.R.	18° 25'	320	none?	Subtropical Wet Forest	23	4000	Oct. 17
Rincón de la Osa, C.R.	8° 40'	30	none?	Tropical Wet Forest	26	4600	Dec. 5

The cutting and herbicide treatments are shown as an outside energy source acting through a negative multiplier (work gate) on the energy flow into the plant populations. In the case of cutting, this will directly break the flow by removing the plants. The herbicide, however, acts by channeling the metabolic energy of a plant into its self-destruction; this would be represented more accurately as a source acting as a positive multiplier on the flow between the energy receptor and respiratory network of the plants. The action of hormonal herbicides apparently results from ethylene, which is produced in toxic levels (Hallaway and Osborne, 1969).

The energy spent by a man to cut the vegetation represents the direct cost of preventing the development of structure and can be evaluated by knowing an individual's weight and the exact time spent at a given task (e.g., see Brody, 1945). The added energetic value of cutting with a machete can be determined by measuring the time required for the same individuals to clear smaller subplots of similar vegetation by hand. At least two individuals will be evaluated at each task in order to estimate the differences within workers.

The application of an herbicide represents a specialized form of fossil fuel input to limit structural development. The labor used to apply it can be evaluated as above, while the energetic value of the herbicide can be measured by determining its caloric value, coupled with the foliar cost of its production. The energetic value of a dollar spent in a fossil fuel economy has been estimated by Odum (1967a).

Data Processing and Simulation

Some or all of the seven kinds of measurements used to evaluate structural complexity will be combined, if possible, to produce a numerical index of structure. Since all of the measures are to some extent interdependent, multivariate analysis will be used to determine the contribution of each factor to stand structure.

Information theory offers another possibility for combining the seven measures into a useful index, provided suitable weighting factors can be determined for each of the variables.

The two measures of the cost of preventing structure can then be tested as responses to 1.) initial clearing by cutting vs. herbicide, 2.) time, 3.) effect of repeated treatment, and 4.) environmental stress. It should then be possible to model the cost of maintaining a low-structure system as a function of stress, of form of energy input to control structure, and of time. Further modeling may make it possible to predict structural response to stress in combinations other than those included in the field study.

Acknowledgement

This work was jointly sponsored by grants from the Organization of Tropical Studies and an AEC Contract AT(40-1)-3666 with the University of North Carolina under the direction of H.T. Odum.

The investigator wishes to acknowledge the assistance and logistical support provided by the Terrestrial Ecology Division of the Puerto Rico Nuclear Center.

Literature Cited

- Brody, S. 1945. Bioenergetics and Growth. Rheinhold, N.Y. 1023 p.
- Hallaway, M. and D.J. Osborne. 1969. Ethylene: a factor in defoliation induced by auxins. Science 163: 1067-1068.
- Holdridge, L.R. 1967. Life Zone Ecology. Tropical Science Center, San José, Costa Rica, 206 pp.
- Odum, H.T. 1967a. Energetics of world food production. Chap. 3 IN: The World Food Problem, Vol. III, Report of the panel on the world food supply. The White House, Washington, D.C. 332 pp.
- Odum, H.T. 1967b. Work circuits and system stress. pp. 81 - 133 IN: Symposium on primary productivity and mineral cycling in natural ecosystems. University of Maine Press. 245 pp.

PROGRESS REPORT, FIELD STUDIES ON THE PUERTO RICAN
TODY, TODUS MEXICANUS

Kay Kepler

My graduate research (Cornell University) involves the documentation of the natural history of the family Todidae, with emphasis on the Puerto Rican Tody, Todus mexicanus. This small family of birds (5 species) is one of two families unique to the Caribbean. By arrangement with Puerto Rico Nuclear Center, I have included the study area of the El Verde Field Station as one research site. During the period Sept. 1968 to March 1970 I have made approximately 40 visits, studying population densities, territoriality, reproductive biology and feeding behavior.

Linear densities of todies near El Verde Field station average 12.5/Km. This area is situated in the so-called tabonuco forest zone. By comparison, tody densities decrease with increasing elevation, averaging 5.81/Km in the colorado forest zone, 2.3/Km in the sierra palm forest zone and 0.85/km in the dwarf forest zone. In lowland scrub forest densities average 20/Km and in coffee plantations 25/Km.

The 160 meter fence and surveyed grid pattern of the gamma irradiation study area facilitate measurements of area density and territory shape and size. Approximately 18 pairs of todies utilize the forest within the fence, giving an area density of approximately 225 pairs per Km² (Figure 1). Comparison with Recher's 1965 census figures for the same area indicate little change since that time.

The exact territories occupied by each pair are receiving major emphasis at El Verde, utilizing the grid for reference. It appears that pairs remain mated throughout the year and use the same area from year to year. Territorial defense and other aggressive behavior is under study. The presence of the Black-whiskered Vireo (Vireo altiloquus) at El Verde elicits certain patterns of aggressive behavior not seen at higher elevations where this species is absent.

Nests of the tody consist of burrows in vertical earth banks; a number of suitable banks occur along the El Verde trails. Much of my nesting information is being obtained here. A nesting peculiarity observed near the field station in 1969 consisted of 4 birds feeding young in the same burrow. It is probable that 2 of these were immature offspring from the previous year.

Another facet of my study is feeding behavior. Information of foraging height, percentage of time spent feeding on insects from

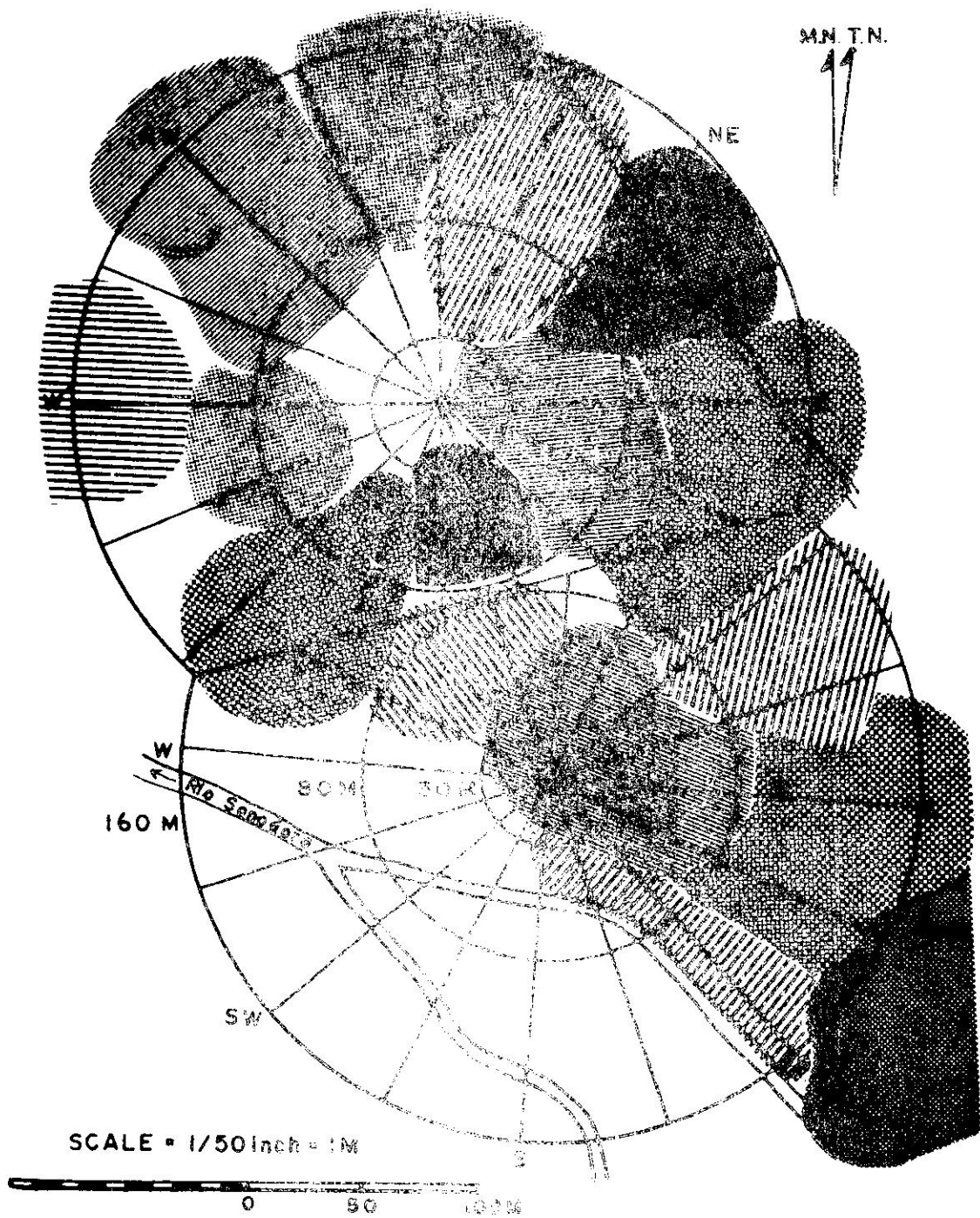


Figure 1 TODY TERRITORIES (TENTATIVE) 1968-1970

the air, from leaf surfaces and from other sources, and, when possible, type of food taken is compared from the 15-16 meter canopy at El Verde through the range down to 1.5 meter canopies at high elevations.

The study is only a small part of the avifauna of the Luquillo Forest, and incidental to its study, information has been obtained at El Verde station on other species of native birds and migrant warblers, in an attempt to understand the avian ecological relationships of the rain forest. I would like to express my gratitude to Puerto Rico Nuclear Center for permission to study in the area.

THE TERMITES AT EL VERDE: 1969 SURVEY

Elizabeth A. McMahan¹

In July 1969 McMahan, with the help of L.A. Burns, recensused the termite populations of the Radiation, South Control, and North Cut Centers at El Verde. The work was a continuation of a study begun in the summer of 1966 and carried through the summer of 1967 and 1968 (See PRNC, Rain Forest Annual Reports for 1968 and 1969. Also Odum, in press, A Tropical Rainforest). It attempts to evaluate the effects of gamma radiation (93 days in the spring of 1965) on the termites in the Radiation Center.

Two termite species of family Termitidae are prevalent in the centers: Nasutitermes costalis, which builds large carton nests on the forest floor, and Parvitermes discolor, which is not a nest builder but lives within decaying logs and stumps. Both species build carton tunnels to food sources (dead limbs, etc.). One colony of Glyptotermes pubescens of family Kalotermitidae has been found each year since 1967 in a single stump in the North Cut Center.

Procedures

Surveys of nests and tunnels were made in each of the three centers. The nest survey included the area within the 80 m radius of the Centers, while the tunnel survey included only the area within the 30 m radius. New nests were sought and old nests were examined for degree of activity or abandonment. Trees and vertical stumps and trunks were examined for tunnels or tunnel remnants. Tunnels were recorded as occupied or not occupied and by which termite species.

Results

Nests. Three formerly active N. costalis nests were found to be abandoned in July 1969, one in the Radiation Center (#12) and two in the South Control Center (#8 and #10). Nest 12 had appeared to be very feeble in 1968 (as evidenced by very low emergence of soldiers when the surface was disturbed) and it was assumed to be in the process of abandonment at that time. Another nest in the South Control Center (#9) had, on the same basis, been pronounced abandoned in 1968. In 1969 it was again mediumly active and the tunnels leading from it were filled with termites. This is the only nest that has "recovered" to date.

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Two new and active nests were discovered in the South Control Center (#28 and #29), plus an apparently long-abandoned one (#30) which had become exposed with the decaying away of an old stump. The two active nests were small and within a meter of each other, the first case of such proximity of nests.

Figure 1 shows the sites of the nests and nest condition for the Radiation and South Control Centers in 1969.

Wiegert, who had first mapped the nests of these Centers, did not similarly map the nests of the North Cut Center. In the latter, in 1966, was found only one long-abandoned nest with a 30 m radius. After the 1967 census a large active N. costalis nest (N) was transported to the NNE 30 m radius in the North Cut Center and settled at the base of a large tree. The colony continued to be active and sent out tunnels over adjacent trees.

Tunnels. Approximately 10% of the trees in each Center exhibited tunnels or remnants of tunnels in 1966. This percentage has remained relatively constant, although the trees involved have changed.

Tables 1 and 2 summarize the annual tunnel data since 1966 for the two major termite species at El Verde. The most interesting finding was the fact that in the Radiation Center trees with occupied tunnels, which in 1966 and 1967 had constituted about 12 per cent of trees bearing tunnels of any description, in 1968 had risen to 24 and in 1969 to 55 percent. Figure 2 illustrates this rise and further shows that tunnel occupancy in the South Control and North Cut Centers did not show such precipitous rises. In 1969 all showed comparable levels of occupancy.

The increase in tunnel occupancy shown by the Radiation Center was due to the presence of the new N. costalis nest that was first observed there in 1968. Table 2 shows that the percentage of Parvitermes tunnel occupancy increased but little from 1966 to 1969.

Another notable finding in 1969 was the large increase in N. costalis tunnel occupancy in the South Control Center and the simultaneous, large decrease in P. discolor occupancy. The two new (in 1969) N. costalis nests in this Center doubtless played a part in increasing the number of occupied N. costalis tunnels. Previously both species had been well-represented in the Center. The North Cut Center has consistently had more evidence of P. discolor than of N. costalis.

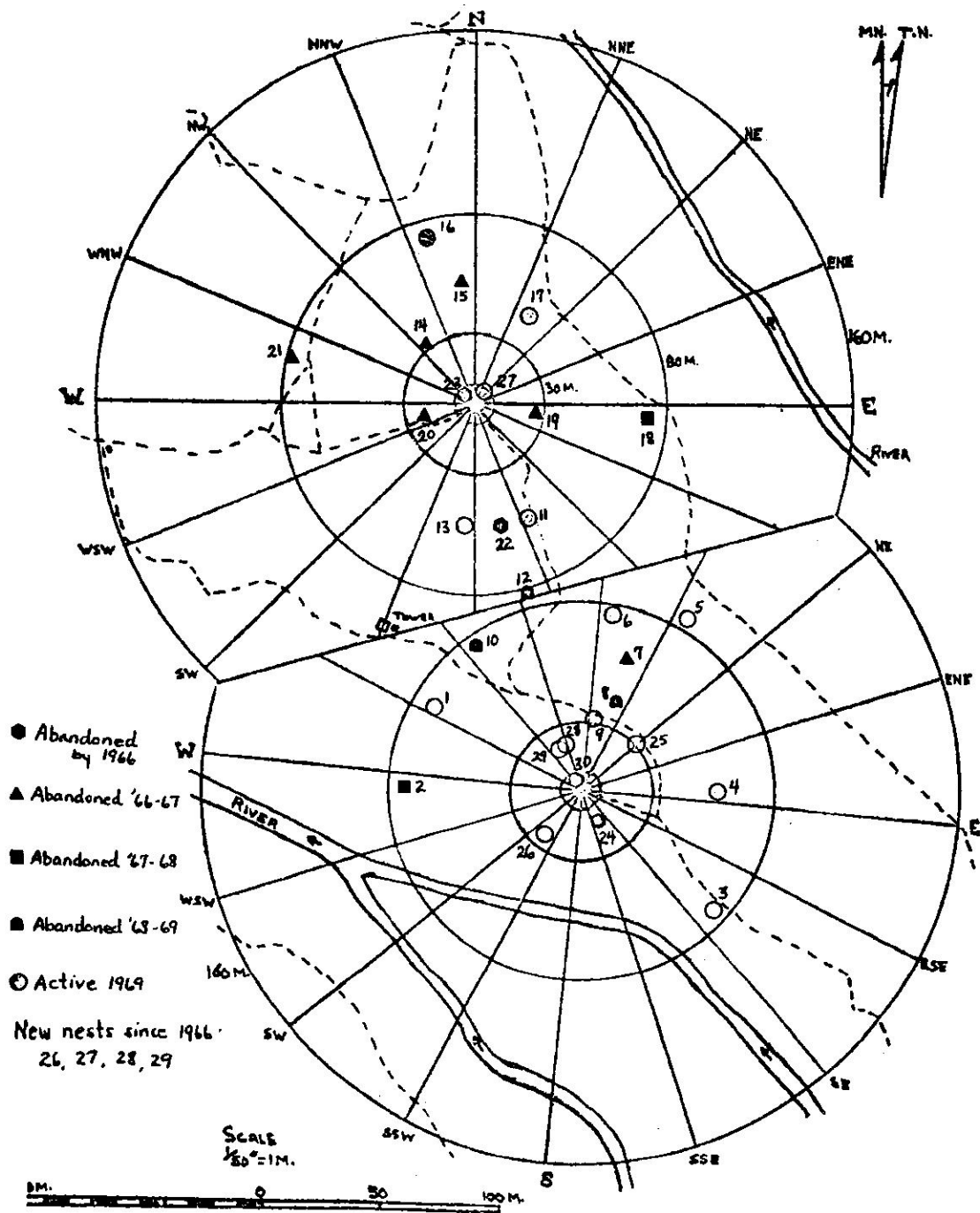


Fig. 1. Status of termite nests at El Verde in July, 1969.

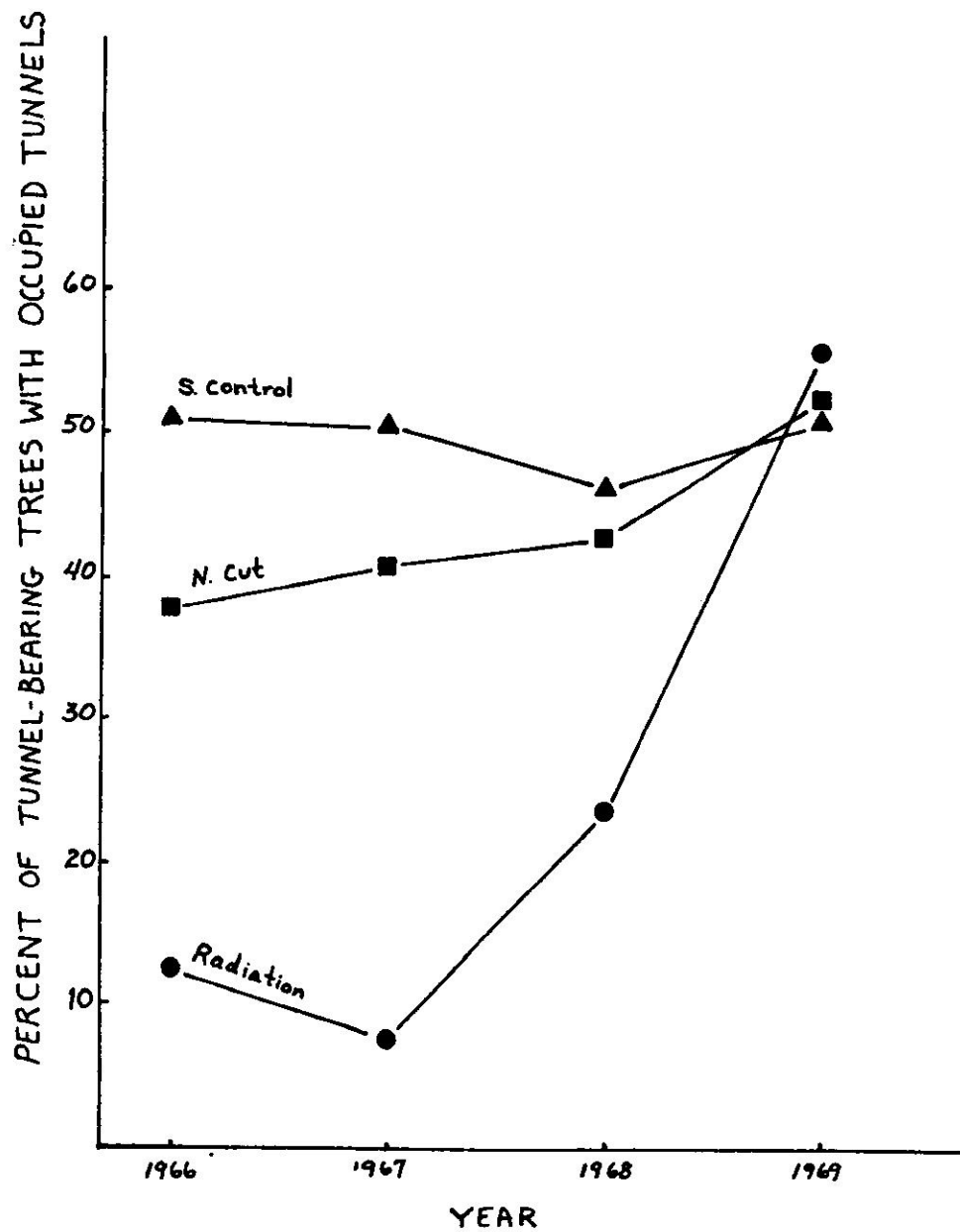


Fig. 2. Comparison of tunnel occupancy in the three centers at El Verfe for the years 1966-1969.

Table 1. Tunnel Survey Data for Nasutitermes costalis
in the Three Centers

Year	Radiation		South Control			North Cut		
	Trees with Tunnels	Tunnels Occupied No. %	Trees with Tunnels	Tunnels Occupied No. %	Trees with Tunnels	Tunnels Occupied No. %		
1966	90	3 3.3	92	22 23.9	29*	0 0.0		
1967	102	1 1.0	108	21 19.4	78	7 9.2		
1968	93	15 16.1	136	23 16.9	82	9 11.0		
1969	89	39 43.8	120	44 31.7	82	10 12.2		

*Survey to 20 m radius only.

Table 2. Tunnel Survey Data for Parvitermes costalis
in the Three Centers

Year	Radiation		South Control			North Cut		
	Trees with Tunnels	Tunnels Occupied No. %	Trees with Tunnels	Tunnels Occupied No. %	Trees with Tunnels	Tunnels Occupied No. %		
1966	90	8 8.9	92	25 27.2	29*	11 38.0		
1967	102	7 7.0	108	34 31.5	78	25 32.1		
1968	93	7 7.5	136	40 29.4	82	26 31.7		
1969	89	10 11.2	120	17 14.2	82	33 40.2		

*Survey to 20 m radius only.

Discussion

In the Radiation Center only 4 of the 11 original (1966) active nests were still active in 1969. In the South Control Center 7 of the original, active nests were still active. One new nest was first observed in the South Control Center in July 1967, one in the Radiation Center in July 1968, and two (possibly representing a single colony because of their proximity) were first observed in the South Control Center in July 1969. Too little is known at present regarding the circumstances surrounding the foundation of a new colony or at least the initiation of a new nest to permit more than guesses regarding the delay in recolonization of the Radiation Center.

The greater incidence of nest abandonment in the Radiation Center over that in the South Control Center has been attributed to the sterilization of the reproductives and the accompanying diminution of the population (McMahan, PRNC Rain Forest Annual Reports 1968, 1969). As evidence for this hypothesis was cited the lack of nymphal forms in tunnels or participating in nest repair. Recent literature (Krishna and Weesner, 1969. Biology of Termites. Academic Press) has caused a re-evaluation of this interpretation.

The termite genus Nasutitermes is reported to have two lines of definitive workers, large and small, neither of which gives rise to the other. Nymphs, which are immature individuals with wing pads in the alate (reproductive) line, do not normally participate in foraging, nest construction, etc., and should not be expected to be encountered at these tasks.

The group that appeared to be lacking in the irradiated nests, then, was not necessarily the nymphs, but the small worker group, previously mistakenly called "nymphs". The basis for their apparent absence is not understood. Since small workers often give rise to the soldier caste, L.A. Burns has suggested (personal communication) that perhaps a stress such as radiation (resulting, perhaps, in loss of reproductives) triggers the excessive development of soldiers from small workers thus "using up" the latter. Perhaps this comes about through lack of a pheromonal interaction with reproductives which permits this excess of soldier formation beyond the ratio limits usually characteristic of the species.

In possible support of this view is a termite colony from outside the experimental areas, studied in July 1969. It was a nest in relatively poor repair and only moderately active, and it was chosen as one possibly in the early stages of abandonment. When it was found to contain two empty royal chambers, one in an older nest section and encrusted with fungal growth, and the other in the more recent and active part of the nest. No royal pair, nor brood, were detected in any part of the nest. A large number of white soldiers were observed

(the stage just preceding the adult soldier stage) indicating a possible soldier flare. Samples of the inhabitants of this nest were taken in order to compare the ratios of all types of individuals with those for a normal nest containing a king and queen. If it shows a deficiency of small workers but an excess of soldiers and white soldiers it may support the hypothesis that small workers may tend to decline in favor of soldier increase in stressed nests on their way, perhaps, to abandonment. This comparison has not yet been made.

If any case, the Radiation Center is now comparable in termite tunnel occupancy to that of the other two Centers. Careful searches of the Centers out to 80 m should be conducted to document the probable re-invasion of colonies into the areas where nests have been abandoned.

POLYETHISM IN WORKERS OF NASUTITERMES COSTALIS (HOLMGREN)

Elizabeth A. McMahan¹

Polyethism within the termite worker caste, similar to that reported for honey bees (Lindauer 1952, Ribbands 1952), bumble bees (Free 1955), Halictid bees (Batra 1964) and ants (Weir 1958a,b) has been suspected for years (Heigh 1922, Grassé and Noirot 1950, Kalshoven 1958). Its experimental investigation, however, has been recent (Pasteels 1965, Howse 1968). In the Hymenoptera, worker job preferences have usually been associated with worker age, as well as with colony need. This association has also been indicated for termites. Pasteels observed that in Nasutitermes lujae (family Termitidae) (family Termitidae) older stage individuals of both the small and the large worker lines more readily ventured outside the nest and hence tended to be foragers. Howse, working with a species without a definitive caste, Zootermopsis nevadensis (family Hodotermitidae), reported that as the individual termite progressed from young larva to nymph it tended to spend more time at colony maintenance and less in trophallaxis.

The present paper reports a behavioral difference within the worker caste of Nasutitermes costalis (Holmgren) similar to that reported by Pasteels. Also a member of family Termitidae, this species has both large and small definitive workers which presumably represent separate lines, neither giving rise to the other. In caste makeup and development, N. costalis is assumed to be similar to N. arborum described by Noirot (1955), to N. lujae described by Pasteels (1965), and to other members of the genus. Workers in both the large and the small lines are presumed to continue to molt and thereby to represent at least three different age stages of large workers and at least two age stages of small workers. In other species these stages have been differentiated on the basis of pigmentation, certain morphological characteristics, and sex (Noirot 1955, Weesner 1965, Pasteels 1965). Although no biometric studies were carried out with N. costalis, the workers could be readily separated into the stages indicated, chiefly on the basis of size and head pigmentation.

The study was carried out in August 1967 in a montane rain forest in northeastern Puerto Rico at an altitude of about 450 m. It grew out of an investigation of the effects on natural populations of termites of gamma radiation from a 10,000-curie ¹³⁷Cs source exposed in the rain forest for 93 days in the spring of 1965. It was part of a large study sponsored by the United States Atomic Energy Commission through the Puerto Rico Nuclear Center (Odum, 1970 In press).

N. costalis nests of carton were abundant in the area and were almost invariably on the ground. Normal nests, except when in the process of expansion, had intact surfaces, at least over occupied portions.

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Part of the radiation study involved comparison of speed of nest repair in irradiated and non-irradiated colonies. When a small hole was punched in a nest surface the immediate reaction of the termites in the vicinity of the breach was a precipitous flight downward into the interior of the nest of all except the soldiers (nasutes) which, in contrast, rushed to the opening. Some of the soldiers poured out over the nest surface, running erratically about with snouts lifted. Gradually their activity subsided, and after three or four minutes, if no further disturbance was forthcoming, they retreated into the opening but stood ringing it, heads pointing outward. No workers of any type were seen near the hole for about five minutes from the time the breach was first made. Then one by one workers would appear and begin to repair the hole by daubing fecal cement (carton) on the broken margins.

It was soon noted that the termites which returned to begin the task of closing the hole appeared almost always to be large workers. This observation led to an attempt to obtain quantitative evidence for this apparent behavioral difference between the members of the two worker lines.

Series 1: Preliminary Tests of Nest Repair

Procedure

The first tests were carried out on 9 different nests between August 19 and August 23, 1967. Five had been within 40-80 meters of the radiation source and four had been at distances greater than 80 m. Nests closer than 40 m had all been abandoned by August 1967. None were in the process of expansion when sampled.

Sampling, from an opening (1 cm in diameter) punched in the nest surface, was made with an aspirator having an intake tube 5 mm in diameter and occurred within 10 minutes of the breaching disturbance. After a number of repairing workers had congregated at the hole a sample was taken by placing the aspirator intake tube at the opening and aspirating quickly all termites possible. Average aspiration time for a single sample was two seconds; after this time only soldiers were available, all workers having fled inward. Usually from 5 to 15 workers, plus a greater number of soldiers, were captured in each sample. About 10 separate samples, with intervals of about 10 minutes between were taken at each nest.

Each sampling disturbance set up the sequence of soldier-out-pouring, graduate subsidence of soldier activity, and eventual emergence of workers to begin repair.

All termites aspirated from a single nest were preserved in a separate vial of 70% alcohol and were later identified to type.

Results

Table 1 gives the sample composition by nest. The nests are separated according to their distance from the radiation source.

The data indicate that although average numbers of all types of termites captured at disturbed surfaces were slightly less for nests in the irradiated than for those in the non-irradiated area, percentages of soldiers, large workers, and small workers captured were similar for the two. In both cases large workers (mostly third stage) constituted about 90% of the workers captured. The low percentage of small workers (and of first and second stage large workers) at the breach implied a possible tendency for them not to take a prominent part in nest repair.

Observations of spontaneous expansion of nests, however, showed that small workers appeared to be as much involved in the construction work as large workers. Therefore the hypothesis was considered that large workers (mostly third stage) do most of the surface repair work following disturbances, while both large and small workers expanded the nest. Test series 2 tested this hypothesis.

Series 2: Expansion versus Repair

Procedure

These tests were conducted on August 26 and 27, 1967. They involved 3 of the previous nests plus additional nests. Two were sampled twice (on different days). All nests sampled were selected because they were in the process of spontaneous expansion, with workers working busily at openings they themselves had made in the nest surface. Expansion holes, like the punched holes, averaged about 1 cm in diameter.

Without prior disturbance, one quick (2 second) aspiration was made at an expansion holes at a given nest. The workers were counted immediately and preserved in a labelled vial, along with the simultaneously aspirated soldiers. An artificial hole was then punched on the same nest, at least 6 inches from the expansion site. As fast as workers appeared at this punched opening they were captured and preserved, one by one, until a total had been reached approximately equal to the number aspirated at the expansion hole. The aim was to compare worker composition under the two sampling conditions.

Table 1
Comparison of Sample Composition of Termites from Nests
in Irradiated and in Nonirradiated Areas (Series 1)

Nests from Irradiated Area							Nests from Nonirradiated Area						
Nest	^a W _{L3}	^b W _{L2}	^c W _{L1}	^d W _{S2}	^e W _{S1}	^f S	Nest	^a W _{L3}	^b W _{L2}	^c W _{L1}	^d W _{S2}	^e W _{S1}	^f S
11	136	0	0	2	1	227	4	85	6	1	9	3	205
12	93	2	0	4	9	152	6	126	0	2	10	6	187
16	60	10	2	8	8	175	10	192	8	0	17	2	301
17	70	5	1	4	4	265	26	125	3	4	10	11	390
18	133	0	0	0	0	223							
Total	492	17	3	18	22	1042	Total	528	17	7	46	22	1083
Mean	98.4	3.4	0.6	3.6	4.4	208.4	Mean	132	4.25	1.75	11.5	5.5	270.75

^aW_{L3} Third stage large worker
^bW_{L2} Second stage large worker
^cW_{L1} First stage large worker
^dW_{S2} Second stage small worker
^eW_{S1} First stage small worker
^fS Soldier

Table 2. Comparison of Sample Composition of Termites Taken from Expansion
Holes and from Repair Holes without Delay (Series 2)

Sample Vials	No. of Nests	From Expansion Holes						Sample Vials	No. of Nests	From Repair Holes					
		^a W _{L3}	^b W _{L2}	^c W _{L1}	^d W _{S2}	^e W _{S1}	^f S			^a W _{L3}	^b W _{L2}	^c W _{L1}	^d W _{S2}	^e W _{S1}	^f S
1E	9	27	15	8	20	26	139	1E	9	93	5	1	6	1	260
2E	10	48	19	8	19	29	156	2E	10	117	2	0	9	4	301
3E	3	11	8	10	12	28	61	3R	3	66	4	1	3	1	149
4E	3	8	4	7	6	18	81	4R	3	42	0	0	2	0	113
Total		94	46	33	57	101	437	Total		318	11	2	20	6	823
			173			158					331			26	

^aTermite types as in Table 1.

Results

Table 2 presents the data. Four sets of collecting vials were used in the study and their data are given separately to show consistency of results. Vials 1E and 1R represent, of course, one group of 9 nests; vials 2E and 2R represent another group of 10 nests, etc.

While nearly half the "expander" workers were of the small type, only about 7% were of this type among the "repairers". There were, in fact, smaller percentages of all types of workers at the repair holes except for third stage large workers, which increased from 28% to 89%. Soldier capture was also greater at repair holes, a predictable result because of the continued disturbance there. At expansion sites, first stage small workers exceeded in numbers even third stage large workers.

The expansion site data and the similarity of worker composition of the repair hole samples to that of the samples of Series 1 appeared to support the hypothesis that small workers tended to expand nests much more readily than to repair them. A more likely hypothesis, however, concerned the alacrity with which they emerged to commence nest repair following a surface disturbance.

After one of the nests in Series 2 had been sampled it was examined again more than an hour later. The artificial hole was still being repaired, and now small workers were seen to be much in evidence. It appeared that they repaired nests as diligently as they expanded them but were merely shyer than large workers about returning to a disturbed area. A third test series explored this hypothesis.

Series 3: Delayed Repair

Procedure

This series (August 27 and 28, 1967) studied the effect of waiting for 50 minutes or more after a breaching disturbance before making one quick (2 second) aspiration of the termites that had come to make repairs. Ten nests were sampled.

Results

The total sample composition (Table 3) was similar to that of the nest expansion sample (Table 2). The data show that large and small workers participated in repair to a comparable degree when there had been no surface disturbance for 50 minutes or more. Like the expansion data, they show that first stage small workers (WS1) were as prominent as third stage large workers (WL3) in nest construction.

Table 3. Sample Composition of Termites Taken from Repair Holes 50 Minutes or More after a Disturbance.

No. of Nests	Termite Types					
	^a WL3	WL2	WL1	WS2	WS1	S
10	26	11	10	7	26	161
		47		33		

^aTermite types as in Table 1.

Table 4. Comparison of Proportion of Worker Types in a Mature Colony with that of Workers Participating in Disturbed and in Undisturbed Nest Construction.

	Worker Types					Total
	^a WL3	WL2	WL1	WS2	WS1	
Nest population	719	575	535	137	415	2381
	30.2%	24.1%	22.5%	4.8%	17.4%	
Recently disturbed construction	1338	45	12	84	50	1529
	87.5%	2.9%	0.8%	5.5%	3.3%	
Non-disturbed construction	120	57	43	64	127	411
	29.2%	13.9%	10.5%	15.6%	30.9%	

^a Termite types as in Table 1.

Comparisons of Worker Types Engaged in Construction
with those in a Mature N. costalis Colony

Differences in proportions of worker types captured at both disturbed and undisturbed construction sites indicated that behavioral differences existed. In order to evaluate properly the relative participation of each type, however, knowledge of its respective number in each of the colonies sampled would be desirable. These numbers are not known. However, the entire population of another N. costalis nest from the same general area was analyzed for colony composition. All the termites from the nest were pooled in a large container of 70% alcohol, and five independent samples, totaling 2381 workers (plus soldiers, alates, and immature stages) were analyzed. Table 4 shows the average percentage of each type of worker present in the nest. Standard deviations for these data (using the arc sin method for transformation of the percentage figures) did not exceed 0.2 percent in any case. The colony had been large and active, with a royal pair and brood, so its population was assumed to be typically constituted.

Table 4 compares the percentages of each of the worker types found under the following three conditions: a) in the nest population as a whole, b) participating in nest construction (repair) under conditions of recent disturbance, and c) participating in nest construction (expansion and delayed repair) under conditions of non-disturbance.

Chi-square evaluations of the data, taking the frequency distributions of two of the conditions at a time, gave highly significant probabilities (.0001) that the three sets of data are not homogeneous. In other words, the data indicate that significant differences in behavior relating to nest repair were elicited from different termite types under the conditions of the tests.

Discussion

The data support those of Pasteels (1965) who studied trail-laying in N. lujae. He found that older stages in both the large and small worker lines were more venturesome than younger stages. The nest construction data for N. costalis similarly showed that third stage large workers (WL3) far exceeded all other workers in the alacrity with which they arrived at a surface breach to begin repairs. Although much fewer in number, second stage small workers (WS2) were next most venturesome. First stage large workers (WL1) were especially reluctant to carry out repairs.

The data also indicated a possible tendency toward "job preference" that may be distinct from a difference in readiness to confront alarming situations. While WL3 workers at undisturbed construction sites were present in about the same percentage as their assumed number in the

colony (30%), the other large worker stages (W_{L2} and W_{L1}) were present to only about half their "expected" percentage. Both small worker stages (W_{S2} and W_{S1}), on the other hand, nearly doubled their "expected" participation. This greater tendency of small workers, in the absence of disturbing conditions, to participate in nest construction may be an example of a true division of labor among termite workers.

Summary

A field study of colonies of Nasutitermes costalis in a montane rain forest in Puerto Rico indicated that third stage large workers returned much more readily to nest surfaces to make repairs following a breaching disturbance than other worker types. Where no recent disturbance had been (or was an hour past) small workers, constituting (presumably) only about 24% of all workers in a nest, composed over 46% of the workers collected at the construction site. These findings are evidence of polyethism within the N. costalis worker caste.

Acknowledgments

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Bibliography

- Batra (S.W.T.), 1964. Behavior of the social bee, Lasioglossum zephyrum, within the nest (Hymenoptera: Halictidae). Insectes Sociaux 11, p. 159-186.
- Free (J.B.), 1955. The division of labour within bumblebee colonies. Insectes Sociaux 2, p. 195-211.
- Grassé (P.-P), and Noirot (Ch.) 1950. -Documents sur la Biologie de l'Odontotermes magdalenae n. sp. Ann. Sc. Nat. Zool., Paris 11^{es}. 11, p. 117-148.
- Hegh (E.), 1922. -Les Termites. Imprimerie Industrielle et Financiere. Bruxelles.
- Howse (P.E.), -On the division of labour in the primitive termite Zootermopsis nevadensis (Hagen). Ins. Soc. 15, p. 45-50.
- Kalshoven (L.G.E.), 1958. -Observations on the black termites, Hospitalitermes spp. of Java and Sumatra. Insectes Sociaux 5, p. 9-30.
- Lindauer (M.), 1952. Ein Beitrag zur Frage der Arbeitsteilung im Bienestaat. Z. vergl. Physiol. 34, p. 299-345.
- Noirot (C.), 1955. Recherches sur le polymorphisme des Termites supérieurs (Termitidae), Ann. Sci. Nat. Zool. Biol. Animale 11 17, 399-595.
- Odum.(H.T.), ed., 1970. A Tropical Rain Forest. U.S. Atomic Energy Commission. In press.
- Pasteels (J.M.), 1965. -Polyethism chez les ouvriers de Nasutitermes lujae (Termitidae Isopteres). Biol. Gabonica 1, p. 191-205.
- Ribbands (C.R.), 1952. Division of labour in the honeybee community. Proc. roy. Soc. B. 140: 32-43.
- Wessner (F.M.), 1965. -The Termites of the United States, A Handbook. Nat. Pest Control Assoc. Elizabeth, N.J.
- Weir (J.S.), 1958a. -Polyethism in workers of the ant Myrmica. Insectes Sociaux 5, p. 97-128. 1958b. Polyethism in workers of the ant Myrmica. Part II. Ins. Soc. 5, p. 315-339.

OCCURRENCE AND DISTRIBUTION OF AQUATIC FUNGI IN THE
EL VERDE FOREST: A PRELIMINARY REPORT¹

Roland Seymour²

Introduction

Except for a report by Rossy-Valderrama (1956), the aquatic fungal flora (Phycomycetes) of Puerto Rico is unknown. This report, while describing three new taxa and extending the distribution records of fifteen existing species, dealt exclusively with terricolous forms.

Opportunity was afforded between November 10 and November 18, 1969 of obtaining from the El Verde forest, collections of soil and water which might yield aquatic fungi. The results of this preliminary survey were indeed promising since out of a total of 265 collections, 226 isolates were obtained, representing thus far 8 genera and 22 species. Since specific determinations have not yet been possible in all the resulting collections, a complete taxonomic account will appear in a later report.

Materials and Methods

Techniques for collecting and isolating Phycomyceteous fungi are well established (Johnson, 1956; Sparrow, 1960; Scott, 1961; Seymour, 1970). Samples of wet and dry soil were collected in 35 ml. polypropylene bottles and returned to the laboratory within three hours of collection. Approximately 10 g. wet weight of soil was placed in a sterile Petri plate and covered with distilled water to a depth of about 1 cm. After the particulate matter had settled, various baits (boiled halves of hemp seed, pieces of snake skin, shrimp skin, cockroach wing, and pine pollen) were added to each culture. The gross cultures were incubated at 23-25°C. Each piece of bait was examined periodically with the aid of a compound microscope after the third day of incubation.

Water samples were baited in the same manner, except that each 35 ml. sample was dilute with 10-15 ml. of distilled water before baiting.

¹Continuing study; financial support from the National Science Foundation (Grant GB-13404) is acknowledged with gratitude.

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Collections of algae, insect exuviae and submerged plant debris (leaves, stems, fruits, seeds) were returned to the laboratory in sterile plastic bags and examined microscopically. After examination the material was placed in individual Petri plates, covered with distilled water, and allowed to incubate for 3-5 days. The material was periodically examined after the incubation period.

Since the primary objective of the survey was to obtain living isolates of the Saprolegniaceae, no attempt was made to isolate or culture other forms encountered. The Saprolegniaceae species were isolated and propagated in unifungal culture on hemp seed and returned to the investigator's laboratory for future work.

Results and Discussion

Collections were made of wet soils from the edge of the Rio Sonadora and from various streams and temporary pools throughout the forest. Soil samples were also taken from diverse ecological habitats along paths, open areas, around roots of vegetation, under moss and vegetation, and under forest litter. Fungi were found in 87 of the 110 collections made.

In addition, 35 soil samples were collected, air-dried, packaged and returned to the investigator's laboratory for subsequent examination. All samples have yielded species of aquatic fungi.

Water samples containing sediments and debris were taken from the Rio Sonadora and from various streams and pools. A survey for fungal substrates was also made at these locations. A few bits of driftwood and submerged leaves were found, examined directly for hyphomyceteous fungi, then incubated as previously described. This material yielded no species of aquatic Hyphomycetes.

The river, streams, and pools were also carefully searched for algae and insect exuviae. Several species were detected following incubation of this material.

Inasmuch as no studies of aquatic fungi of the El Verde forest have been made prior to the present one, no general conclusion may yet be formed. There are, however, a few points of interest in connection with this relatively small number of collections, although the latter cannot be considered to offer more than a fragmentary picture of the regional flora. Little evidence has been found of a large or unknown tropical flora. On the other hand, a number of genera are particularly well represented and call for intensive investigation in tropical laboratories. The lack of certain genera (*Allomyces*, for example) common to tropical regions may perhaps be attributed to the wetness of the soil. Future collections will undoubtedly yield many species suspected to occur in this region, but which are as yet uncovered.

Summary

Of the 265 collections of soil, water, and organic debris made in the El Verde forest, 226 isolates of aquatic fungi representing, thus far, 8 genera and 22 species were obtained.

Literature Cited

- Johnson, T.W., Jr. 1956. The genus Achlya: morphology and taxonomy. 180 pp., 22 pls. Univ. Mich. Press: Ann Arbor.
- Rossy-Valderrama, C.R. 1956. Some water molds from Puerto Rico. J. Elisha Mitchell Sci. Soc., 72: 129-137, figs. 1-29.
- Scott, W.W. 1961. A monograph of the genus Aphanomyces. Tech. Bull. 151, Va. Agr. Exp. Sta., 95 pp., 9 pls. Va. Polytech. Inst.: Blacksburg.
- Seymour, R.L. 1970. The genus Saprolegnia. 123 pp., 18 pls. J. Cramer, Lehre, Germany.
- Sparrow, F.K., Jr. 1960. Aquatic Phycomycetes. 2nd Ed., 1187 pp., 1 pl., 91 figs. Univ. Mich. Press: Ann Arbor.

RADIOTRACER STUDIES OF NUTRIENT CYCLING

Nellie Stark*

Abstract

Studies on the depth of efficient ^{32}P uptake by second growth and climax vegetation show that Cecropia peltata, the second growth dominant tree species, is deep rooted and able to take up nutrients from 1.0 M depth. Guarea trichilioides, Dacryodes excelsa, Heliconia sp., and Piper blattera are shallow rooted with feeder roots capable of taking ^{32}P from shallow depths. Young Sloanea berteriana and Manilkara nitida are deep rooted, absorbing ^{32}P from 0.5 and 1.0 M depth, while near-mature specimens develop a surface feeder root system capable of taking up nutrients from 0-6 cm depth. In studies of the pathway of nutrient cycling using young saplings with mycorrhiza, it was found that all tests with ^{59}Fe , ^{75}Se , and ^{65}Zn showed an initial increase in the radiation level in leaves and roots during the first month. Since rhizomorph was scarce during this rainy season, it is possible that heavy rains leached the isotope from the litter or humus-root mat. Collection of soil animals and fungi showed that these agents are important in nutrient cycling on Puerto Rican latosols. Increases in the radiation level of the humus was usually coincident with increases in radiation level in the roots, suggesting that the humus is a main source of nutrients for surface roots. No positive evidence for direct nutrient cycling on Puerto Rican latosols was found.

Introduction

Studies by Went and Stark (1968a, b) described the direct nutrient cycling hypothesis which states that on the poorest soils in the tropics, nutrients are removed from dead organic matter by mycorrhizal fungi and are transported through rhizomorph tissue to living roots, thus by-passing the soluble phase of nutrients in the soil. Direct nutrient cycling is recognized as only one of several possible nutrient cycling pathways. It is thought to operate most efficiently on white sand areas where the mineral soils are too poor in most biologically important elements to support the lush rain forest which grows there. In this type of rain forest, the bulk of biologically important elements is held in the organic phase of the ecosystem, and nutrient transfer appears to be from dead organic litter to living roots (direct), rather than from the soil solution to roots (indirect).

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If direct nutrient cycling is functioning, then much of the uptake of elements must be from the litter or upper 1-3 cm of soil. Observations suggest that direct nutrient cycling may occur in many other ecosystems in a modified form. Other pathways of nutrient cycling include breakdown by soil animals, bacteria, and free-living soil fungi, with uptake from the humus or soil solution.

To test the hypothesis, radioactive tracers have been used in greenhouse studies of potted plants at the Desert Research Institute in Reno, Nevada, with field extensions of this work at the Puerto Rico Nuclear Center.

The Puerto Rico studies were primarily field tests of the greenhouse methods, with modifications designed for the specific field problems. One test defined the depth from which nutrients are taken up from the soil by the roots of second growth and climax vegetation at El Verde. The other tested methods to describe the pathways of nutrient cycling which predominate on the latosols at El Verde.

The objectives of this work were to test the use of tracers in tying soil organisms into nutrient cycling; to test the depth of nutrient uptake by second growth and climax vegetation; and to describe the pathways of nutrient cycling in climax vegetation on latosols.

Methods

Depth of Nutrient Uptake

Two plots were established in the climax forest at El Verde. One had a large number of young individuals of the climax forest species, and the other had nearly mature individuals whose leaves could be reached from a tower. A third plot was established in second - growth where leaves could be easily reached.

Six to eight similar individuals of several species were marked in each plot wherever possible. Two plants of each species received ^{32}P at 0-3 cm, two at 0.5 M, and two at 1 M. Two additional plants received no isotope (controls). The rockiness of the soil prevented the introduction of ^{32}P at depths greater than 1 M. The plants were as near in height and vigor as possible, although in some cases, it was impossible to find 8 individuals of one species within the fenced area for treatment. The species used are listed in Table 1 by plots with the depth of treatment.

Table 1. Depths, and highest counts for most effective ^{32}P uptake by second growth and climax plants on Puerto Rican latosols, El Verde.

<u>Species</u>	<u>Depth (M)</u>	<u>Highest cpm/g*</u>
Young Climax Plants		
<u>Euterpe globosa</u>	0.03	2,600
	1.0	2,680
<u>Guarea trichilioides</u>	0.03	3,024
	1.0	416
<u>Manilkara nitida</u>	0.03	0
	1.0	449
<u>Sloanea berteriana</u>	0.03	0
	0.5	596
	1.0	1,999
Near Mature Climax Plants		
<u>Dacryodes excelsa</u>	0.03	587
	1.0	0
	1.5	0
<u>Manilkara nitida</u>	0.03	1,574
	1.0	499
<u>Sloanea berteriana</u>	0.03	309
	1.0	0
Second Growth Plants		
<u>Cecropia peltata</u>	0.03	0
	1.0	1,389
<u>Heliconia sp.</u>	0.03	2,753
	0.5	1,769
	1.0	0
<u>Piper blattera</u>	0.03	6,517
	1.0	1,321

*Corrected for background

The initial level of radiation in the vegetation was sampled on all test plants using a cork borer and a rubber cork for backing. The cork borer was the same diameter as the planchet used in the beta counter so that the geometry of the samples was kept constant.

A soil auger was used to dig holes of known depth for the introduction of the isotope. A piece of Tygon tubing 2 cm i.d. was inserted in the hole. The dosage of ^{32}P , 46 Ci, was pre-measured in 10 ml of water in plastic bottles, so that one bottle represented a single dose. The isotope solution was poured into a funnel inserted in the top of the Tygon tubing and another 10 ml of plain water followed the isotope to rinse the tubing. The tubing was allowed to drain for 15 minutes before being removed from the hole. The 0.03 M treatment was applied by sprinkling the solution over an area of 1 dm² after removing the litter. The large trees received 140 Ci of ^{32}P applied at several places at the same depth in the soil.

Samples of new and old leaf tissue were removed daily from the trees and adjacent vegetation using the cork borer technique and counted on a beta counter for each of 20 days after the application of the isotope. The average counts per minute were expressed as cpm/g. In a few cases, it was possible to obtain enough fungal and root tissue and soil for ^{32}P detection. Roots of the species tested were examined microscopically for types of mycorrhiza.

Nutrient Pathways

The second set of tests used ^{75}Se , ^{65}Zn , and ^{59}Fe to label living plant material. Seedlings and fresh leaves were placed with their roots or petioles in 2 liters of 100 Ci of the aerated isotope solution in a plastic tray with the rest of the seedling or leaf supported in an upright position by the openings of chicken wire screens (technique developed by Dr. C. Jordan). Aeration of the isotope solution facilitated nutrient uptake. The material was exposed to the isotope solution for 24 hours.

In one test, the root mat with humus from the forest floor was cut into segments 6 x 3 dm and rolled up. The rolled root mat was wrapped in paper towels and tied with string. The roll was lowered into the aerated isotope solution (^{75}Se or ^{59}Fe) on chicken wire and left for 24 hours. At the end of the feeding period, the chicken wire was raised and the leaf bases, mat, or seedlings were allowed to drain for several hours. When the liquid had dried from the plant surfaces, the material was removed from the chicken wire using plastic gloves and was placed in marked plastic bags. The inexpensive apparatus used in feeding was disposed of at the completion of the feeding.

The leaves, root mat, or cut-up seedlings were taken to the field and placed over the root systems of seedlings and saplings (to 1 M high) which were known to have mycorrhiza. Data were not taken by tree species, but the plots contained Rourea glabra, Sloanea berteriana, Manilkara nitida, Euterpe globosa and Dacryodes excelsa. For 5 months, samples of roots, soil, hyphae or rhizomorph insects, leaves and litter were collected from each plot twice monthly for counting on a 400-channel gamma counter. Samples were taken once a month beginning in September. The results were expressed as cpm/g (dry weight) of tissue.

Soil animals from 1 dm³ were collected once a month using a Tullgreen funnel.

Results and Discussion

Depth of Nutrient Uptake

The plants studied were not actively producing new leaves in early April, except for Heliconia and Cecropia. By late April, most of the trees had begun new growth, so that counts taken in late April on young leaf tissue showed good levels of ³²P, whereas counts in early April which had to be made on older leaf tissue showed no ³²P. This isotope was used because phosphorus is readily taken up by roots if the plants are physiologically active and growing. Table 1 shows that Guarea trichilioides, Heliconia sp., Piper blattera, mature Manilkara nitida and Dacryodes excelsa have shallow roots capable of taking up ³²P which is in the upper 0.03-0.06 M of soil. Soil taken from 0.1 M beneath the 1 dm² areas where the isotope had been applied showed no ³²P. The ³²P must have been bonded on soil colloids so that it was not leached downward by rain. The total rainfall for the 20 days of study was 9.1 cm in the young climax forest, 6.4 cm in the near mature forest, and 20.5 cm in the second growth plot. High counts of ³²P were found in soil at 0.03-0.05 M and in roots and rhizomorph tissue in all plots.

Young Manilkara nitida and Sloanea berteriana absorbed ³²P most efficiently from intermediate depths, 0.5 to 1.0 M (Table 1). Euterpe globosa and Piper blattera were efficient in ³²P uptake at 0.03 and 1.0 M suggesting a root system with access to a large soil volume. Cecropia peltata appeared to be deep rooted, taking up ³²P from 1.0 M only.

The depth of uptake shown by these data corresponds generally with what is known about the root systems. Cecropia peltata appears to have deep, penetrating roots, and mycorrhiza have been found on this species. It is possible that indirect nutrient cycling occurs

in Cecropia, some species of which are known to concentrate biologically essential elements on poor soils (Stark, in press). Dacryodes excelsa typically forms a surface root mat and has abundant mycorrhiza, suggesting that direct nutrient cycling could occur. Fungal tissue from one meter away from the treated zones on the soil surface showed high levels of ^{32}P 10 days after treatment. This suggests that the fungal tissue was conducting ^{32}P , although it was impossible to establish that the fungal tissue in question was in living contact with root cells.

Sloanea and Manilkara saplings were most efficient at taking up ^{32}P from the 0.5 and 1.0 M levels in the soil. Near mature Sloanea and Manilkara were most efficient at ^{32}P uptake from 0.03 M, suggesting that this species changes in rooting habit and mode of nutrient uptake with age. Excavation shows that young Sloanea and Manilkara trees have deep roots going into the soil. As the trees mature, they develop a surface feeder root system which appears to obtain nutrients from the litter and upper few centimeters of soil.

Roots of all test plants from 0.03 M showed very high counts for ^{32}P . Because of the irregular geometry of roots, fungus, and soils, quantitative data cannot be given for these parts of the ecosystem. However, detection of ^{32}P was easy, indicating that ^{32}P was present in all three materials. Adjacent weeds and other trees took up ^{32}P from 0.03 M only, indicating that there is an extensive intermixing of roots, especially at the surface.

The techniques used for introducing the isotope can be improved by adding smaller dosages of isotope at different points around the plant being studied. In one case, an isotope introduced at one point near a Didymopanax morototoni never showed up in the leaves. The isotope may well have been introduced where there were no roots, or roots of another plant. Although the method involves some problems, it is most useful in studying rooting habit and ^{32}P uptake if extensive sampling is possible and if the plants are growing. The data from this study confirm the theory that Cecropia is deep rooted and taps a large volume of soil (Stark, in press).

Nutrient Pathway

Figure 1 shows that ^{59}Fe moved quickly into the roots and leaves from labelled litter, but almost no ^{59}Fe occurred in the soil until a month after the labelled litter was applied. The isotope moved into the humus and 0-3 cm soil about a month after the labelled litter was placed on the soil. The end of April until early June was a rainy period when the movement of isotope by leaching was possible. Since the period was very rainy, fungus growth was slow and it was not

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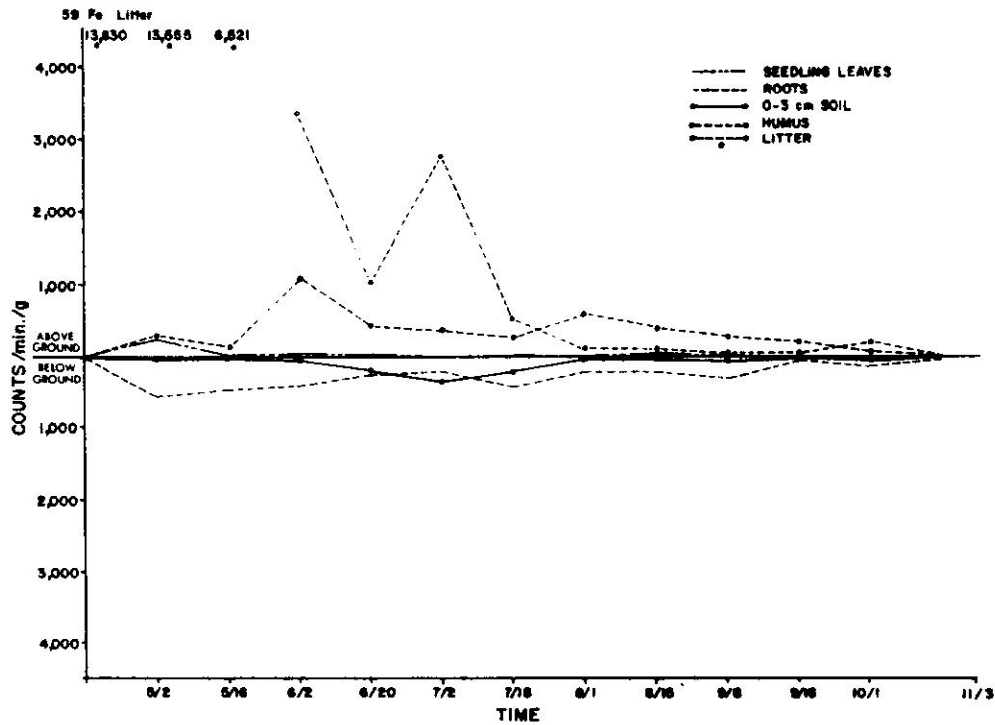


Fig. 1. Monthly or bi-monthly radiation levels in seedling leaves, litter, humus, roots and 0-3 cm soil in plots treated with ^{59}Fe labelled litter at El Verde, Puerto Rico. No ^{59}Fe was found at 10 cm in the soil.

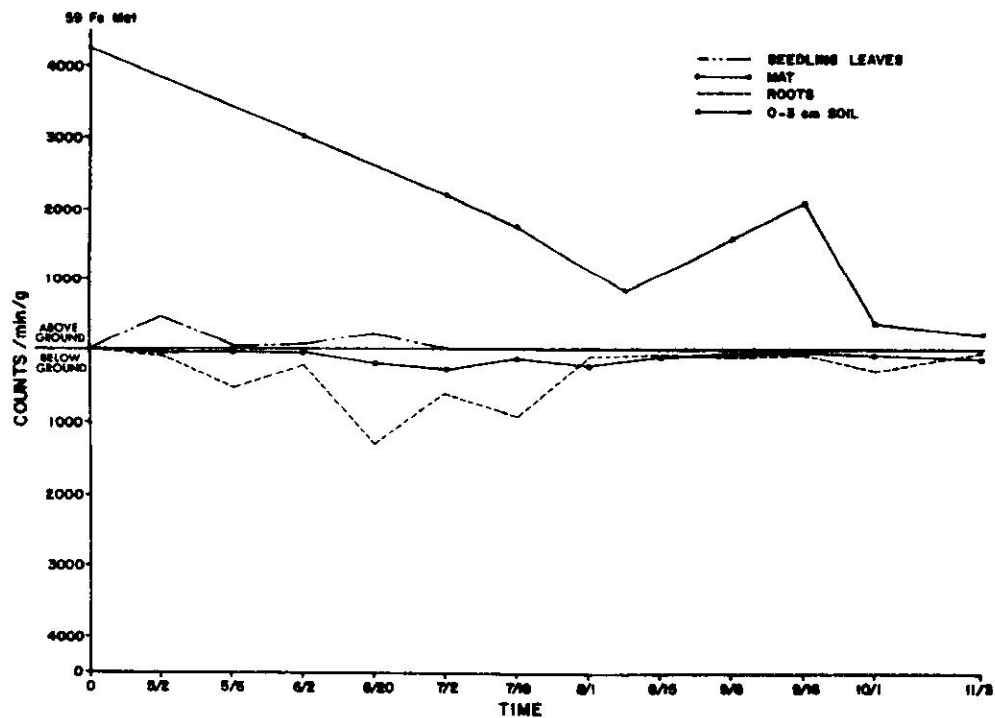


Fig. 2.- Monthly or bi-monthly radiation levels in seedling leaves, mat, roots, and 0-3 cm soil in plots treated with ^{59}Fe labelled humus-root mat at El Verde, Puerto Rico. No ^{59}Fe was found at 10 cm in the soil. Mat = humus.

possible to find rhizomorph tissue for counting. Because no ^{59}Fe occurred in the soil for over a month, the isotope must have entered the leaves from the organic litter, but because rhizomorph tissue was not present in quantities suitable for counting, there is no proof that direct nutrient cycling was taking place. The data show that ^{59}Fe moved into the roots and leaves, but they do not show what pathway was taken, i.e., free-living fungi and bacteria, soil animals, or mycorrhiza. Heavy rains may have leached enough ^{59}Fe from the dead litter so that the roots had access to surface water with ^{59}Fe . The level of ^{59}Fe in roots and leaves decreased after the first month, while the ^{59}Fe content of the soil increased slightly through mid-July.

The humus increased in ^{59}Fe for several months as the isotope was released from the litter, and then gradually decreased. The increase in ^{59}Fe in the humus during July and August is probably the results of a concentration of the isotope from the decomposition of carbohydrates (i.e. loss of dry matter and conversion of litter to humus). The litter showed a reduction while the humus increased slightly in activity suggesting the conversion of litter to humus. Because the leaves did not increase in ^{59}Fe after the first month, we can conclude that little ^{59}Fe was transported from the roots to the leaves, and that the roots were not transporting ^{59}Fe from the litter, soil, or humus in significant quantities. The leaves were not growing during this time and new growth did not appear, except for a small amount in mid-June, which may explain the failure of the leaves to take up more ^{59}Fe . It is significant that ^{59}Fe never appeared at 15 cm in the soil in October as did the ^{75}Se in the companion tests, indicating that this soil is efficient at bonding and holding ^{59}Fe against leaching. The isotope was not moved into old leaves in significant amounts.

The pattern of ^{59}Fe movement from the labelled humus-root mat was very similar to that from labelled litter except that the young leaves and roots showed a more marked increase in the isotope in mid-June in the mat tests (Figure 2). This increase coincides with periods of heavy rains, although the rain may not explain the root behavior. Since the movement of ^{59}Fe out of the humus-root mat and out of litter was not obviously different, we can assume that this isotope moves from both media to roots via similar pathways. The data suggest that roots can obtain ^{59}Fe more readily from the humus mat than from litter, which defines a pathway of nutrient cycling involving free-living fungi, bacteria, and animals rather than mycorrhiza.

Young leaves showed a high level of ^{75}Se in early May when new leaves were appearing, but older leaves never showed significant levels of ^{75}Se in the litter plot (Figure 3). The young leaves

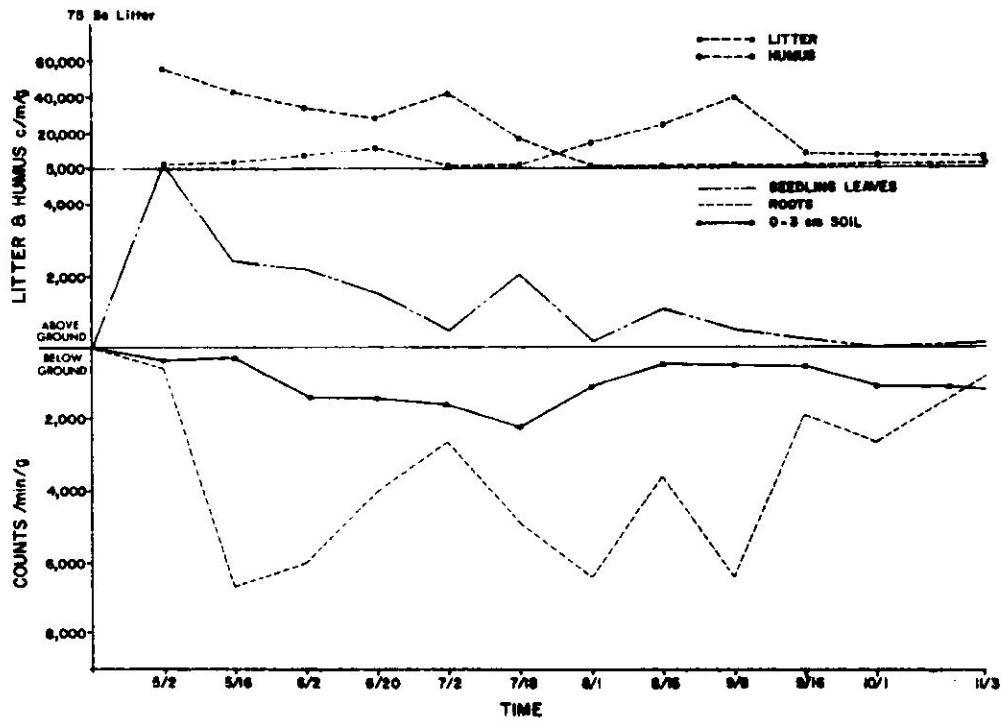


Fig. 3 - Monthly or bi-monthly radiation levels and 0-3 cm soil in plots treated with ^{75}Se labelled litter at El Verde, Puerto Rico. Upper scale represents the changes in the radiation levels of the litter and humus.

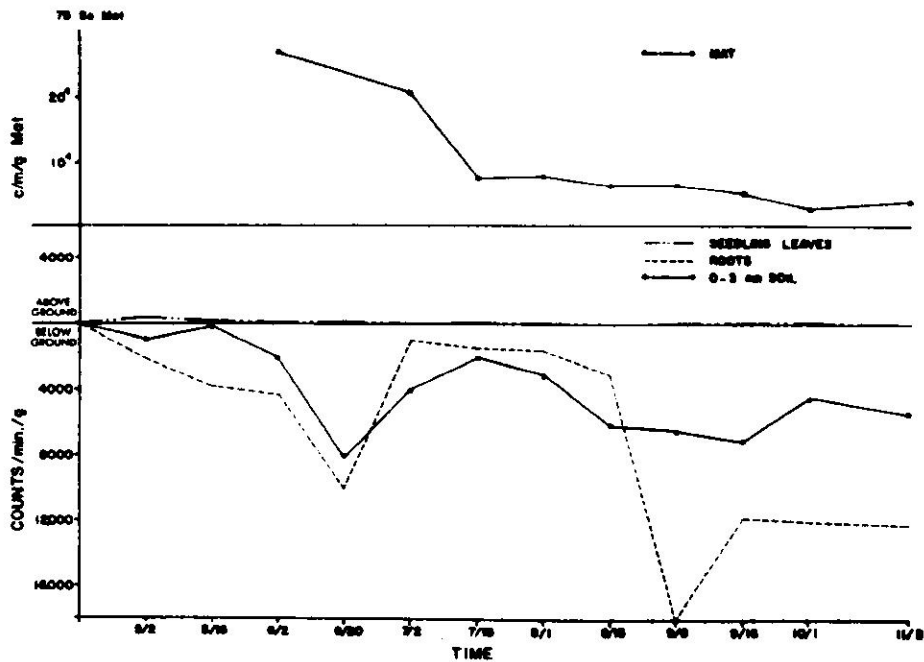


Fig. 4 - Monthly or bi-monthly radiation levels in seedling leaves, mat, roots, and 0-3 cm soil in plots treated with ^{75}Se labelled humus-root mat at El Verde, Puerto Rico. The top scale represents the radiation level of the humus-root mat.

showed a second increase in ^{75}Se in mid-July and mid-August, not in mid-June as with ^{59}Fe (mat). There is no good explanation for this phenomenon.

The roots of the seedlings increased in ^{75}Se during the first month, and then decreased markedly. From July 2 on, they increased in the level of ^{75}Se again. This fluctuation could result from either sampling error or some phenomenon of root metabolism. It is possible that the isotope was released from litter and taken up in pulses since similar fluctuations occurred in the ^{59}Fe root studies. The level of ^{75}Se gradually increased at 0-3 cm in the soil, but no significant amount of isotope reached 15 cm depth in the soil. The humus increased in the amount of label while the litter tended to decrease in radioactivity, as would be predicted. Unfortunately, it is difficult to get a uniform labelling in litter so that minor fluctuations may be due to initial differences in level of labelling, in spite of subsampling.

Soil animals collected in early June and August showed high levels of ^{75}Se suggesting that they were very important in breaking down litter in this habitat. Soil fungi were high in ^{75}Se , although there was no way of ascertaining whether these fungi were free-living or mycorrhizal.

The results from the ^{75}Se tests suggest that this isotope moved from the litter to soil fungi and soil animals in the upper soil layer (0-3 cm) and that uptake occurred in this layer (0-3 cm and humus). Nothing can be said about uptake of this isotope by deep roots because the isotope did not move into the deeper soil which is a heavy clay with restricted drainage. High levels of ^{75}Se in the roots during August-Nov. must have come from the humus or soil since the litter had almost no ^{75}Se . This pattern of restricted movement of isotopes was found for tritium in these soils (Kline and Jorian 1968).

When ^{75}Se was applied in the humus-root mat, almost no isotope appeared in either the young or old leaves (Fig. 4).

The roots and 0-3 cm soil increased in ^{75}Se at much the same rate, with more radioactivity entering the soil in the first two months than occurred with labelled litter. This suggests a leaching of ^{75}Se from the humus-root mat by rain. Previously, it was thought that much uptake of nutrients occurred in this humus layer, although these data only partly support that view for the months of April through November. Since the roots and soil became heavily labelled at the same time (mid-June and early September), this suggests that uptake of ^{75}Se was from the 0-3 cm soil or the mat directly and not from litter in this case. Since the mat was already partly decayed, the release of materials into the soil was more rapid than with litter. The study is complicated by the fact that the seedlings and saplings were small and heavily shaded

so that they were not able to put on rapid growth. Even when ^{75}Se was applied in the humus-root mat, no isotope appeared at 15 cm. In September, the roots in the ^{75}Se from the mat and uptake by the roots (Fig. 4).

None of the control leaves, roots, litter or soil showed significant levels of ^{59}Fe , ^{75}Se , ^{65}Zn .

The ^{65}Zn tests were designed to get around the problem of slow growth. The plants used in this test were 8 species of local tree seedlings planted in poor sand with a surface organic layer. The seedlings were under 3 dm high and were grown in plastic-lined galvanized tanks in semi-shade. These plants showed the same initial increase in ^{65}Zn in the leaves during the first month after application of the labelled litter as did the ^{75}Se and ^{59}Fe tests (Fig. 5). The roots of the seedlings and the 0-3 cm soil both increased slightly in ^{65}Zn for two months, and then decreased. By August 1, there was considerable ^{65}Zn in the 0-3 cm soil but little in the roots. No humus appeared in the first few months, so that it could not be measured. The litter gradually decreased in ^{65}Zn .

The isotope ^{65}Zn was applied to plant surfaces as well as taken up internally. I found that leaves, roots, and soil in plots treated with externally applied ^{65}Zn were higher in this element than were those in plots where ^{65}Zn was applied internally. This pattern persisted throughout the tests. Apparently ^{65}Zn can be leached from plant surfaces and is then available for uptake, while leaching of internal ^{65}Zn appeared to be insignificant.

Since zinc is needed in small amounts by plants, and since it is poorly transported, it is not unusual that it did not occur in the leaves in great quantity. Roots normally concentrate zinc, and it is surprising that ^{65}Zn did not become more abundant in the roots than it did. The roots showed a slight increase in ^{65}Zn on September 8 following the high levels of this isotope in the soil. Sampling in small containers tends to disturb the roots and may well do serious harm to nutrient cycling pathways. The seedlings did not put on as much new growth as was expected.

This technique of studying nutrient cycling requires that the scientist be continually resident in the field. Since this was not possible in the present study, some information was lost. Every modification of weather and every chance in plant metabolism will effect the ultimate results of this type of study. Some of the variability in this study resulting from sampling error can be overcome by wider sampling and more compositing of samples.

One major problem which should be studied is the effect of physiological activity of the plants on isotope uptake. Both of the

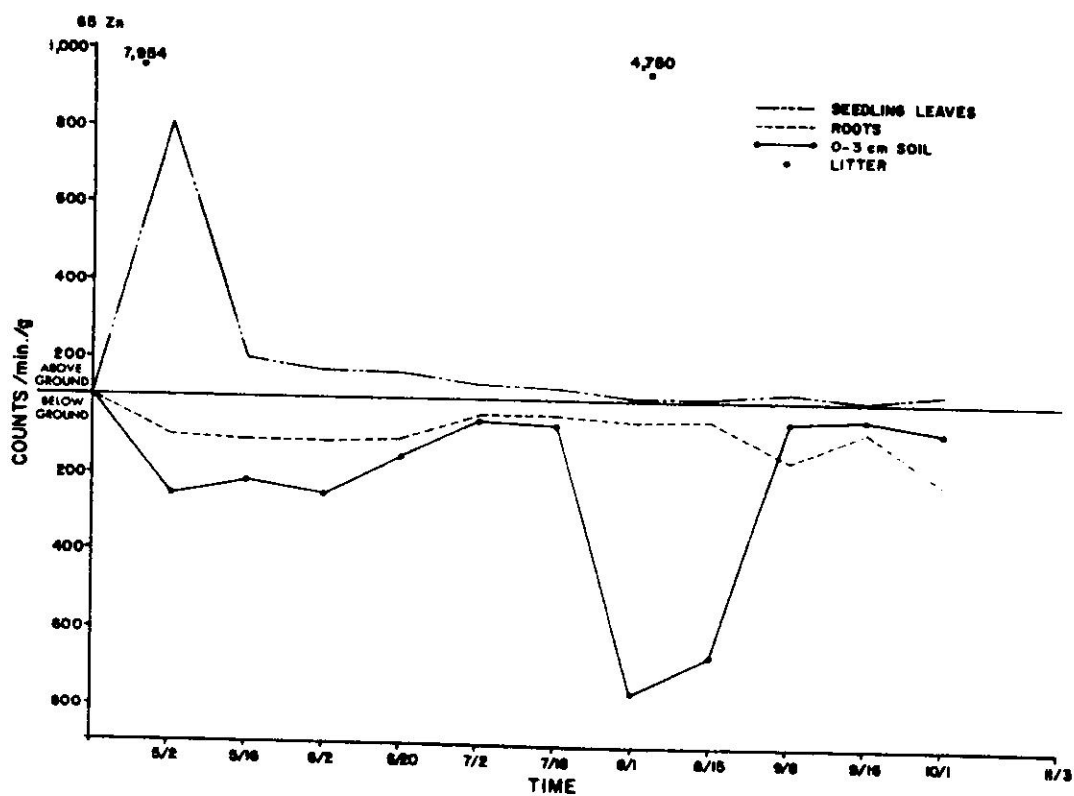


Fig. 5. Monthly or bi-monthly radiation levels in seedling leaves, litter, roots, and 0-3 cm soil for plots treated with ^{65}Zn labelled litter at El Verde, Puerto Rico.

studies reported here were limited by the failure of the plants to grow. Studies are needed which determine whether photosynthesis, root metabolism, or the rate of movement of the transpiration stream, or some other physiological process will best coincide with isotope uptake. The behavior of different physiological conditions of the tree would be helpful in defining which isotope to use and when. Unfortunately, most of the studies on isotope uptake have used rapidly growing greenhouse plants or temperate plants during the growing season. The periodicity of growth in the tropics is not well understood.

Acknowledgments

The author is grateful to the Atomic Energy Commission for making this study possible and for the cooperation and assistance of persons from the Puerto Rico Nuclear Center.

Literature Cited

- Kline, J., and C.F. Jordan. 1968. Tritium movement in the soils of the tropical rainforest. *Sci* 160: 550-551.
- Stark, N. 1969. Nutrient cycling in the Amazonian Rain Forest. In press.
- Went, F.W., and N. Stark 1968a. Mycorrhiza. *BioScience* 18 (11): 1035-1039.
- Went, F.W. and N. Stark. 1968. The biological and mechanical role of soil fungi. *Proc. of Nat. Acad. of Sci.* 60 (2): 497-504.

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