Final report on the course in Tropical Ecology Venezuela 2007

Instytut Nauk o Środowisku Uniwersytetu Jagiellońskiego (INoS UJ)

Instituto Venezolano de Investigaciones Científicas (IVIC)

Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez (MIZA)

Kraków – Caracas – Maracay

The course took place on July 4^{th} – 21^{st}, 2007, following the plan outlined below:

04.07: arrival to Caracas, transfer to IVIC;

05.07 – 07.07: IVIC – lectures and field trips;

08.07: transfer to the field station at Araya Peninsula;

09.07 – 10.07: Araya Peninsula field station – field work, trips, seminars, etc.;

11.07: transfer to Rancho Grande field station;

12.07 – 20.07: Rancho Grande – field work and evening lectures;

21.07: end of the course, transfer of participants to Merida/Caracas

One PhD and nine MSc students participated in the course lead by Ryszard Laskowski. The main course organizers from Venezuela were dr Angel Viloria (IVIC), dr Astolfo Mata (IVIC), prof. José Clavijo (MIZA) and dr John Latke (MIZA).

The following students particiated and succesfully completed the course:

1. Agnieszka Bednarska, PhD student, INoS
2. Paula Dobosz, MSc student (Biology)
3. Anna Powałka, MSc student (Biology with Geography)
4. Joanna Stokłosa, MSc student (Biology with Geography)
5. Radosław Łabno, MSc student (Nature Conservation)
6. Tomasz Profus, MSc student (Biology with Geography)
7. Dominika Galera, MSc student (Biology)
8. Mariusz Gajewski, MSc student (Nature Conservation)
9. Agnieszka Bem, undergraduate student (Biotechnology)
10. Ana Stanewa, MSc student (Biology with Geography)

An important part of the course was the work on “mini research projects” performed by the students during their stay in Rancho Grande field station. On the following pages students’ reports are presented. The reports were the final requirement for the course completion.

The Institute of Environmental Sciences, all course participants and myself (R. Laskowski) in particular express their deepest gratitude to our colleagues in Venezuela who helped to make the course a success. Special thanks are due to dr Angel Viloria, dr Astolfo Mata, prof. José Clavijo and dr John Latke. Help from Universidad Oriente is also greatly appraciated.
We would also like to thank all the teachers and staff of IVIC and Rancho Grande field station for their invaluable help which made our stay in Venezuela not only successful but also very nice and social.

Vertical distribution of mosquitoes' larvae (Diptera:Culicidae) in *Heliconia bihai* phytotelmata in the vicinity of Rancho Grande field station, Henri Pittier National Park, Northwestern Venezuela

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ABSTRACT

One of the most wide-spread insects all over the world are Diptera, commonly known as true flies. Typical true flies are mosquitoes (Culicidae), whose larvae spend their life in standing water found in different types of still-water microcontainers and phytotelmata. Phytotelmata are structures formed by non-aquatic plants that impound water.

One of the common plant species in the Neotropics whose modified leaves accumulate rainwater and thus serve as substratum for associated fauna is *Heliconia bihai*. *Heliconia bihai* was chosen to study the abundance and diversity of the invertebrate fauna, especially mosquitoes larvae (Diptera: Culicidae), supported by the accumulated fluid, and their vertical distribution in the range between 800 and 1600 m above see level in Venezuelan cloud forest at Rancho Grande. The highest number of mosquitoes (60 individuals) and at the same time the highest number of all identified group of organisms (12 groups) recovered from *Heliconia bihai* was found at 904 m a.s.l. Both the number of mosquitoes and the number of groups of organisms decreased significantly with the altitude increase ($p < 0.0001$).

INTRODUCTION

True flies (Diptera) is an order of phylum Insecta - one of the most wide-spread insects all over the world except Antarctica; the number of species it 240.000. Most of them are little organisms with holometabolic metamorphose in the water. Mosquitoes (Culicidae) are typical true flies, whose larvae live in still-water microcontainers and phytotelmata. They are so minuscule, that they have to be associated with their hatch place (e.g. with phytotelmata plant) and thus associated with local environmental conditions (temperature, elevation, humidity). Most of the mosquito species inhabit tropical humid forests where it is warm and humid, and there is plenty of plants with inquilines biota.

A very common phytotelmata plant in the mountain cloud forest is *Heliconia bihai var. aurea* (Heliconiaceae) (**Fig. 1**) which occupy the attached range between 0 and 1600 m a.s.l. That wide range of *Heliconia bihai* in tropical forests enabled us to study the vertical distribution of mosquitoes and other invertebrates associated with this plant. Phytotelmata exist wherever the long-term stagnic water occur in concave parts of plants, e.g. bromeliad tanks, tree holes, bamboo internodes and axil waters (Kitching, 2000). Phytotelmata (inquilines biota, fitolelmas) are commonly called "container habitats" because they are like small containers, this mean water ecosystems in micro scale. *Heliconia bihai var. aurea* is a very good recognizable plant in the mountain cloud forests. Their glowing flowers (red and yellow with green edge), high stalk (1-3 m) and giant banana-shaped leaves, make them unmistaken and visible from a long distance. *Heliconia bihai* evolved as a defense against flower-feeding and seed-feeding insects (Seifert, 1982). *Helicon bihai* is one of type phytotelmata: axil waters are collected by bracts (leaves encloseing flowers). *Heliconia's* axil waters are rather small (maximum about 35 cm^3) but their size depends on their stage, flower age, as well as local conditions like precipitation, temperature and, in particular, the level of light (e.g. forest gaps, orientation of slopes where *Heliconias* grow). *Heliconias* have relatively simple fauna and majority of that fauna compose larvae and pupas of true flies (Dipteras), mainly mosquitoes (Culicidae), hoverflies (Syrphidae), fruit flies (Drosophilidae), soldier flies (Stratiomyidae) and flies (Muscidae). *Heliconia* inhabitants are also chironomids (Chironomidae), beetles (Coleoptera), particularly scirtid beetles (Scirtidae), also adult and larvae of Hydrophilidae, as well as nematodes (Nematoda), Oligochaeta (e.g. Tubificidae family) and many rotifers (Rotifera). Permanently water-filled *Heliconia's* bracts may be inhabited by vertebrate species adapted to water, e.g. amphibians tadpoles (Amphibia: Anura). In *Heliconia's* axil waters, in general, there is lack of abundant crustaceans (Crustacea) because large number of mosquitoes have a negative influence on them (Maguire, 1970). *Heliconia* bracts collect rainwater and produce their own fluids. Herein we discuss the number of mosquitoes, as well as of the groups of organisms higher taxonomic ranks (insects, rotifers, oligochaeta) living in the water-filled bracts of *Helicania bihai* located in Venezuelan cloud forest at elevation ranging from about 800 to 1600 m a.s.l. and we compare our results with those previously reported for *Helicaonia* species living in Neotropical forests.

MATERIALS AND METHODS

Study area

Our study area included central-south part of Parque Nacional Henri Pittier (H.P. National Park) establish in 1937 (Hilty, 2003). H.P. National Park is located in Aragua state, 120 km west of Caracas, between Lake Valencia and Caribbean Sea, and contain Caribbean Mountains (Cordilliera de la Costa) reaching 2434 m a.s.l., a part of the mountain chain of North Andes, and is limited from north by southern coast of the Caribbean Sea. There is very clear floral zonation along the park's elevation gradient: mangrove and coastal xeric shrubs (0 - 300 m a.s.l.), dry forest (300 - 700/800 m a.s.l.), the humid mountain cloud forests (700 - 1500 m a.s.l.) and the high elevation cloud forests/ sub-paramo (1500/1600 - 2434 m a.s.l). Our study covered the range of altitude between 823 and 1602 m a.s.l., what means that two vertical floral zones the mountain cloud forest and the high-mountain cloud forest were included. The average annual temperature and precipitation vary between the zones but of the elevation 1100 m a.s.l where Rancho Grande field station is located it is around 22 ºC and 1880 mm during all year. The H. P. National Park is principally famous for a wide variety of birds (580 species) and is covered by evergreen trees, tree-ferns, palms with numerous epiphytes, and water-keeping smaller plants (Golka, 2005). Most of our samples were found nearby Rancho Grande Central University of Venezuela field station in Rio Periquito Valley, on the south and southwestern hill-sides of Lacumbre (1550 m a.s.l) in the massive of Pico Guacamaya (1830 m a.s.l) and in the region pass (1630 m a.s.l) in Fila Alta, 9 km east of Rancho Grande (**Fig. 2**).

Sample collection

All *Helicania bihai* (**Fig. 1**) selected for sample collection were numbered and fluid from all bracts (modified leaves accumulating water) of selected plant was pumped out into a separate bottle at each sampling site. To pump out the water we constructed the equipment ourselves as it is shown on **Fig. 2**. The fluid amount, its pH and temperature as well as the air temperature were measured. The bracts from where the fluid was pumped were counted. Additionally, to recover all of organisms we washed (once) bracts with clean water and again pumped out this water to the bottle. The samples of the fluid from each plant were preserved and transported to the laboratory in Rancho Grande field station. Then the samples were preserved with alcohol and transported to Poland where the mosquitoes and other invertebrates were identified and counted.

All information about samples (ID number of *Heliconia*, the amount of fluid recovered from bracts, pH and temperature of the fluid, as well as the GPS position of plant collection) are presented in **Table 1**. Based on the original GPS positions, we classified the samples acordin to the altitude of their collection into eight groups, at 100 m intervals (**Table 2**) to obtain at least three replicates for each altitude group. Accuracy of the value of rectangular coordinates reading on the GPS came to 4-31 m and the values of elevation came to 5-50 m. Samples were collected during eight-days field work on July 2007, in the rainy season (April to November; Golka, 2005).

Statistical analysis

The distributions of the data were checked for normality (Shapiro-Wilk's W test). Because the data were not normally distributed, we log-transformed them to render the data close to a normal distribution. The multiple regression analysis was used to find out which variables affected the measured endpoints. The measured endpoints were: number of mosquitoes and number of groups of organisms, and the independent variables were: fluid volume, fluid temperature, air temperature and pH. The variables with the highest p value were removed consecutively from the model as long as there were any variables with $p>0,05$.

The measured variables were also compared between particular 100-m ranges of altitude using ANOVA.

All analyses were done using Statgraphics Centurion XV

RESULTS

During eight days of field work on July 2007 we collected the samples from 31 *Heliconias* from the altitude range 823 - 1602 m a.s.l. Within this altitude range there was clear temperature gradient: the temperature decreased at ca. 0,4 ^{o}C/100 m with increasing altitude (**Fig. 4**). Within collected samples we were able to identify 12 different groups of organisms from different systematic levels: family, order, subclass, class and phylum. The number of mosquitoes differed a lot between samples, and the lowest number of mosquitoes (only two larvae) were found in the *Heliconia* from 1595 m a.s.l. and the highest number (60 mosquitoes larvae) in a plant from 904 m. a.s.l. (**Table 1**). Also the number of mosquitoes calculated per ml of fluid differ between samples and was the lowest in *Heliconia* found at 1110 m a.s.l. and the highest in *Heliconia* found at 835 m a.s.l.. We did not find any *Heliconias* at the altitude range between 1351 and 1449 m a.s.l. In all altitude groups but 1300 m there were at least three replicates (**Table 2**). The volume of fluid collected from *Heliconias* at different altitude ranged from 6 to 240 ml.

Both the number of mosquitoes and the number of higher-rank groups of organisms recovered from *Heliconias* decreased with the altitude increase ($p<0.0001$). The model included the number of mosquitoes ($p<0.0001$) and the volume of fluid ($p<0.0001$) explained above 87% of the total variability (r^2 =87.4%; adjusted r^2 =86.5% , **Fig. 5**). The regression models relating number of groups of organisms to altitude was also well fit ($p<0.0001$; r^2=56.0% , **Fig. 6**).

Based on the results of ANOVA, in general, we found that the samples collected at lower altitudes did not differ much between each other but were significantly different from the samples collected at higher altitudes (**Fig. 7**).

DISCUSSION

Different species of insects can colonize *Heliconia* phytotelmata depending on the different bract condition resulted from differences in ageing. Insect species that are better adapted to water low in detritus are found in the youngest bracts, whereas insect species that use, or are tolerant of, high levels of detritus are found as the older bracts (Seifert, 1982). It was shown that bract age of *Heliconia aurea* collected in Rancho Grande had an effect on survival and spatial partitioning of the bracts by different mosquito species. For example *Trichoprosopon digitatum* was found in the youngest bracts, while *Culex bihaicolus* was found in the oldest bracts (Seifert, 1980). Unfortunately, because of the lack of experience in identification of the bracts' age, we could not define the age of *Heliconias* in our study and we pumped out the fluid from all available bracts irrespective of their age and size. We only noticed that some *Heliconias* had reddish floral bracts with yellow edging (*H. aurea*) and others bracts were also reddish but with rather green than yellow edging (*H. bihai aurea*) or with dark-green upper edge (*H. bihai gran papa*; found mainly nearby Rancho Grande field station building). However it is hard to state if those differences resulted from differences in the *Heliconias* age or differences between various subspecies.

Heliconia bracts collect rainwater and can produce their own fluids. In our study the volume of fluid obtained from *Heliconias* differed a lot between samples and decreased significantly with the altitude increase. Also the temperature of fluid and its pH values differed between samples, but only for pH values weak but significant positive relationship with the altitude was found ($p=0.001$, $r=0.55$, data not shown). Anyway, the range of pH in our samples (7.43 – 9.06) may indicate that we collected rather newly accumulated rainwater from the latest rainfall than standing water, since organic decay acidifies standing water.

The aquatic and semi aquatic insects living inside the water-filled bracts were *Heliconia*-specific. In all samples we found species from Culicidae and Drosophilidae family as well as Rotifera phylum. Additionally, in at least 90% of samples we identified representatives of Syrphidae, Chironomidae family, Oligochaeta subclass and Nematoda phylum (**Table 1**).

We did not find any *Heliconia bihai* at the elevation of ca. 1400 m a.s.l. The reason was that at this elevation there was no many sunlit locations (as road cuts or gaps in the forests left by tree falls or landslides) prefered by *Heliconias* (Kricher, 1999). Most of our 31 samples we found down the road between Rancho Grande and Ocumare de la Costa, along the footpaths in H.P. National Park and along stream banks, where light was abundant. Our study covered the range of altitude between 823 and 1602 m a.s.l., what means that two vertical floral zones: mountain cloud forest and high-mountain cloud forest were included. The differences in number of mosquitoes larvae and also in number of groups of organisms between this two zones can be partly seen from the ANOVA results: the significantly less mosquitoes and group of organisms were found at higher altitudes (1500-1600 and 1300-1600 m a.s.l., respectively) which more or less agree with high-mountain cloud forest floral zone, than at lower altitudes which correspond to mountain cloud forest (**Fig. 7**).

Through the examination of the vertical distribution of the mosquito larvae in *Heliconia* *bihai* we intended to find out the importance of altitude in determining the pattern of abundance of the mosquitoes within plants, and we indeed found this pattern. In the next step of our work, we will try to identify all organisms found in our samples to the species level to calculate different biodiversity indexes and check their relationship with altitude. Also more effort will be put to find more literature data and compare our results with those previously obtained for *Helicaonia* species living in Neotropical forests, especially in Venezuelan rainforests.

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TABLES AND FIGURES

Table 1. Groups of organisms and number of identified individuals from the fluid of *Heliconia bihai* samples collected in the vicinity of Rancho Grande field station.

ID number of sample	GPS position of sample collection	altitude above see level [m]	air/fluid temperature [ºC]	volume of fluid recovered from *Heliconia* [cm^3]	pH of the fluid	gropus of organisms and number of individuals identified in each group
1	10^{o}20'51,1" N 67^{o}41'16,1" W	1128	22,5/23,0	20	7,43	Culicidae (5), Muscidae (2), Drosophilidae (2), Syrphidae (2), Chironomidae (1), Coleoptera (2), Nematoda (1), Oligochaeta (2), Rotifera (∞)
2	10^{o}20'57,2" N 67^{o}41'05,2" W	1110	24,5/20,4	33	7,63	Culicidae (10), Muscidae (2), Drosophilidae (1), Syrphidae (2), Stratiomyidae (1), Chironomidae (1), Coleoptera (3), Oligochaeta (1), Rotifera (∞)
3	10^{o}20'57,2" N 67^{o}41'05,2" W	1110	24,5/20,4	35	8,67	Culicidae (11), Muscidae (1), Drosophilidae (2), Syrphidae (2), Chironomidae (1), Coleoptera (5), Lepidoptera (1), Nematoda (1), Oligochaeta (2), Rotifera (∞)
4	10^{o}20'57,2" N 67^{o}41'05,2" W	1110	24,5/20,3	37	8,60	Culicidae (12), Drosophilidae (1), Syrphidae (2), Stratiomyidae (1), Chironomidae (1), Coleoptera (2), Nematoda (1), Oligochaeta (2), Rotifera (∞)
5	10^{o}21'09,1" N 67^{o}41'05,1" W	1189	22,4/23,6	17	9,06	Culicidae (12), Drosophilidae (1), Syrphidae (1), Stratiomyidae (1), Chironomidae (2), Nematoda (1), Oligochaeta (1), Rotifera (∞)
6	10^{o}21'09,1" N 67^{o}41'05,1" W	1192	22,4/21,6	58	8,78	Culicidae (15), Drosophilidae (1), Syrphidae (1), Stratiomyidae (1), Chironomidae (1), Coleoptera (3), Nematoda (3), Oligochaeta (2), Rotifera (∞)
7	10^{o}21'09,1" N 67^{o}41'05,1" W	1194	22,1/20,5	130	8,02	Culicidae (22), Muscidae (1), Drosophilidae (3), Syrphidae (5), Stratiomyidae (1), Chironomidae (4), Coleoptera (2), Nematoda (3), Oligochaeta (1), Rotifera (∞)
8	10^{o}21'15,7" N 67^{o}41'11,6" W	1476	21,4/24,6	8	8,84	Culicidae (3), Drosophilidae (1), Syrphidae (1), Nematoda (1), Rotifera (∞)
9	10^{o}21'15,5" N 67^{o}41'11,7" W	1471	21,4/21,5	21	8,87	Culicidae (4), Drosophilidae (2), Syrphidae (1), Chironomidae (2), Nematoda (1), Oligochaeta (2), Rotifera (∞)
10	10^{o}21'15,5" N 67^{o}41'11,7" W	1471	21,5/21,6	31	8,55	Culicidae (5), Drosophilidae (1), Chironomidae (1), Nematoda (1), Oligochaeta (1), Rotifera (∞)
11	10^{o}21'12,0" N 67^{o}41'14,5" W	1285	22,0/24,4	9	9,01	Culicidae (4), Drosophilidae (2), Syrphidae (1), Chironomidae (2), Coleoptera (2), Nematoda (2), Oligochaeta (1), Rotifera (∞)

ID number of sample	GPS position of sample collection	altitude above see level [m]	air/fluid temperature [ºC]	volume of fluid recovered from *Heliconia* [cm^3]	pH of the fluid	gropus of organisms and number of individuals identified in each group
12	10^{o}21'12,0" N 67^{o}41'14,5" W	1285	22,0/22,5	11	9,01	Culicidae (5), Muscidae (1), Drosophilidae (1), Syrphidae (2), Chironomidae (2), Nematoda (1), Oligochaeta (1), Rotifera (∞)
13	10^{o}20'55,2" N 67^{o}41'03,1" W	1090	20,7/21,3	13	8,50	Culicidae (10), Muscidae (2), Drosophilidae (3), Syrphidae (3), Stratiomyidae (2), Chironomidae (2), Coleoptera (4), Nematoda (3), Oligochaeta (5), Rotifera (∞)
14	10^{o}20'48,0" N 67^{o}41'17,0" W	1145	19,6/20,4	12	8,64	Culicidae (6), Drosophilidae (1), Syrphidae (1), Chironomidae (1), Coleoptera (1), Nematoda (1), Oligochaeta (1), Rotifera (∞)
15	10^{o}20'51,1" N 67^{o}41'16,2" W	1128	21,8/22,3	10	8,72	Culicidae (8), Muscidae (1), Drosophilidae (1), Syrphidae (2), Stratiomyidae (1), Chironomidae (1), Coleoptera (2), Nematoda (1), Oligochaeta (2), Rotifera (∞)
16	10^{o}20'51,1" N 67^{o}41'16,2" W	1128	21,5/22,3	10	8,74	Culicidae (6), Muscidae (1), Drosophilidae (1), Syrphidae (1), Stratiomyidae (1), Chironomidae (1), Nematoda (1), Rotifera (∞)
17	10^{o}20'51,4" N 67^{o}41'16,1" W	1110	22,2/22,2	150	8,38	Culicidae (14), Muscidae (2), Drosophilidae (3), Syrphidae (3), Stratiomyidae (2), Chironomidae (3), Coleoptera (5), Nematoda (2), Oligochaeta (1), Rotifera (∞)
18	10^{o}20'41" N 67^{o}42'47" W	834	25,1/24,6	83	8,35	Culicidae (28), Muscidae (2), Drosophilidae (4), Syrphidae (4), Stratiomyidae (2), Chironomidae (3), Coleoptera (2), Nematoda (2), Oligochaeta (6), Rotifera (∞)
19	10^{o}20'41" N 67^{o}42'47" W	835	25,1/24,4	30	8,42	Culicidae (25), Drosophilidae (3), Syrphidae (3), Stratiomyidae (1), Chironomidae (3), Coleoptera (4), Nematoda (3), Oligochaeta (4), Rotifera (∞)
20	10^{o}20'57" N 67^{o}42'11" W	904	24,0/24,5	175	8,38	Culicidae (60), Muscidae (3), Drosophilidae (6), Syrphidae (6), Stratiomyidae (4), Chironomidae (7), Coleoptera (8), Nematoda (6), Oligochaeta (11), Rotifera (∞), Gastropoda (1), Amphibia (6)
21	10^{o}21'03" N 67^{o}41'29" W	1000	22,4/22,8	240	8,55	Culicidae (37), Muscidae (2), Drosophilidae (4), Syrphidae (3), Stratiomyidae (2), Chironomidae (2), Coleoptera (5), Nematoda (2), Oligochaeta (7), Rotifera (∞)
22	10^{o}21'03" N 67^{o}41'29" W	1000	22,4/22,4	175	8,62	Culicidae (30), Muscidae (1), Drosophilidae (4), Syrphidae (1), Stratiomyidae (1), Chironomidae (3), Coleoptera (2), Nematoda (2), Oligochaeta (3), Rotifera (∞)

ID number of sample	GPS position of sample collection	altitude above see level [m]	air/fluid temperature [ºC]	volume of fluid recovered from *Heliconia* [cm^3]	pH of the fluid	gropus of organisms and number of individuals identified in each group
23	10°20'55" N 67°41'30" W	1013	22,5/23,4	31	8,91	Culicidae (15), Drosophilidae (3), Syrphidae (3), Chironomidae (1), Coleoptera (1), Nematoda (2), Oligochaeta (2), Rotifera (∞)
24	10°20'55" N 67°41'30" W	1013	22,5/22,4	60	8,84	Culicidae (13), Muscidae (1), Drosophilidae (2), Syrphidae (2), Stratiomyidae (1), Chironomidae (3), Coleoptera (4), Nematoda (4), Oligochaeta (2), Rotifera (∞)
25	10°20'57" N 67°42'07" W	906	22,4/23,5	120	8,55	Culicidae (32), Muscidae (1), Drosophilidae (3), Syrphidae (5), Stratiomyidae (3), Chironomidae (3), Nematoda (6), Oligochaeta (5), Rotifera (∞)
26	10°20'57" N 67°42'07" W	903	22,4/22,8	325	8,60	Culicidae (35), Muscidae (2), Drosophilidae (7), Syrphidae (5), Stratiomyidae (3), Chironomidae (3), Coleoptera (7), Nematoda (5), Oligochaeta (8), Rotifera (∞)
27	10°20'57" N 67°42'07" W	903	22,4/22,5	280	8,61	Culicidae (39), Muscidae (1), Drosophilidae (3), Syrphidae (3), Chironomidae (3), Coleoptera (4), Nematoda (4), Oligochaeta (4), Rotifera (∞)
28	10°20'40" N 67°42'49" W	823	23,8/24,8	137	8,55	Culicidae (31), Muscidae (2), Drosophilidae (4), Syrphidae (5), Stratiomyidae (1), Chironomidae (3), Coleoptera (3), Nematoda (3), Oligochaeta (3), Rotifera (∞)
29	10°21'33,0" N 67°35'04,0" W	1602	21,0/21,5	13	8,91	Culicidae (3), Drosophilidae (1), Syrphidae (2), Chironomidae (1), Nematoda (1), Oligochaeta (2), Rotifera (∞)
30	10°21'33,1" N 67°35'04,0" W	1595	19,4/22,7	22	8,92	Culicidae (5), Drosophilidae (1), Syrphidae (1), Chironomidae (1), Coleoptera (2), Nematoda (1), Oligochaeta (1), Rotifera (∞)
31	10°21'33,1" N 67°35'04,0" W	1595	19,4/21,0	6	8,99	Culicidae (2), Drosophilidae (1), Oligochaeta (1), Rotifera (∞)

Table 2. Sample classification towards the altitude of their collection: number of samples in each group and minimum and maximum altitude within 100-m altitude ranges.

Altitude [m]	Number of samples (*Heliconias*)	Minimum altitude [m]	Maximum altitude [m]
800	3	823	835
900	4	903	906
1000	4	1000	1013
1100	8	1090	1145
1200	3	1189	1194
1300	2	1285	1285
1500	3	1471	1475
1600	3	1595	1602

Figure 1. *Heliconia bihai*; distinctive, colorful red bracts and huge, elongate leaves closely related to bananas.

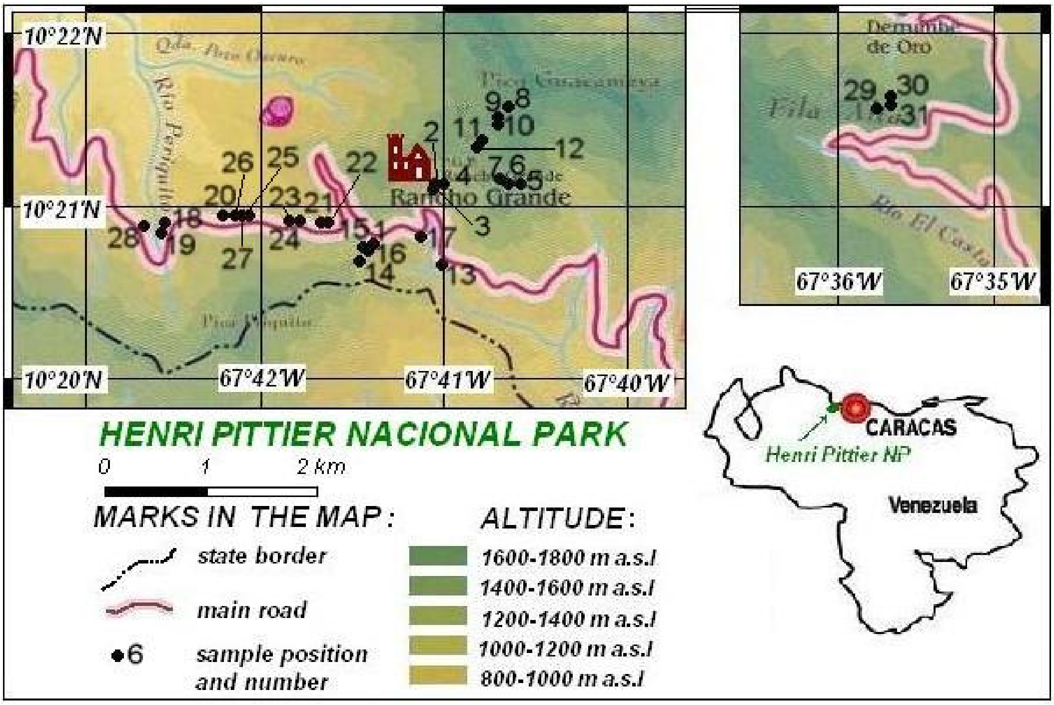

Figure 2. A map showing the sampling sites near Rancho Grande field station in Venezuelan rainforest. The black points with numbers (1-31) denote sampling sites.

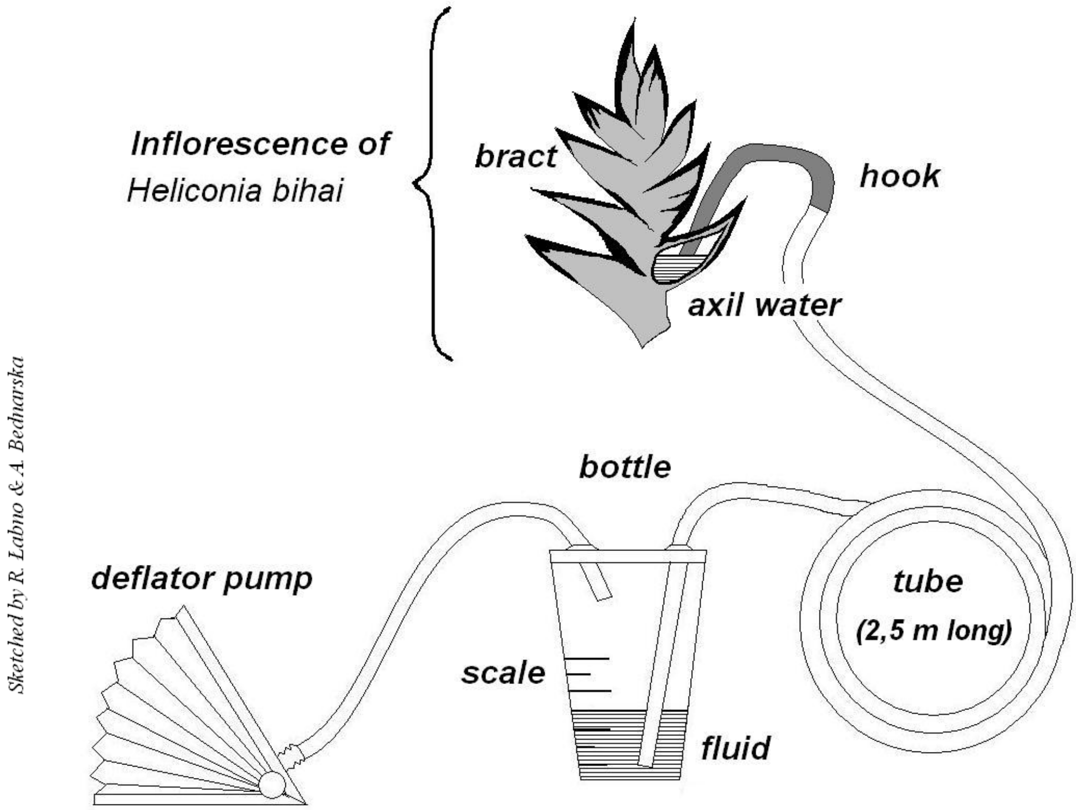

Figure 3. The equipment we used to pump out the fluid from *Heliconia bihai* samples.

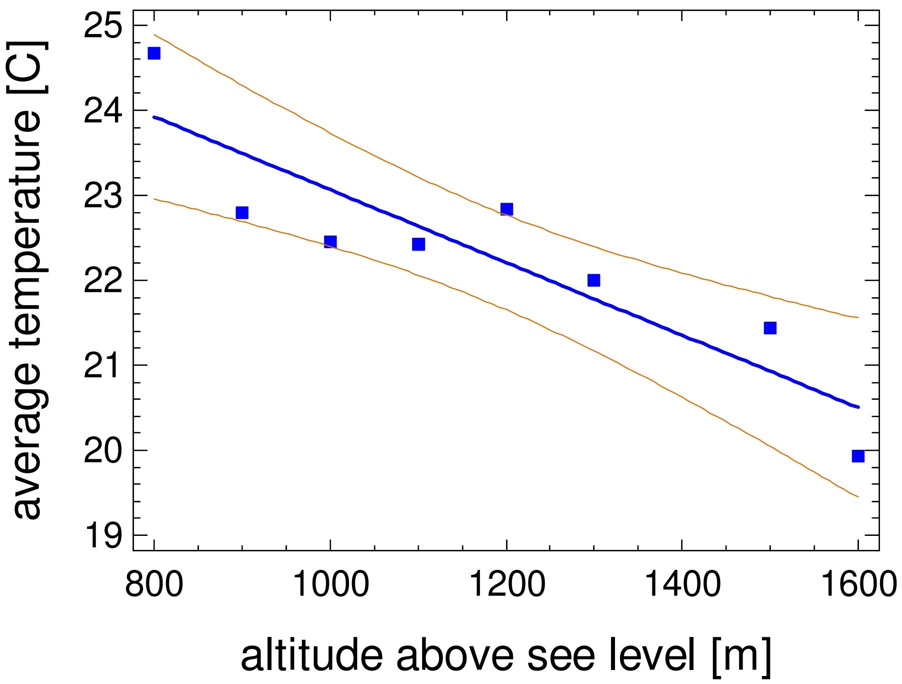

Figure 4. Relationship between average air temperature and altitude above see level, $p=0.003$, $r = -0.9$, $r^2 = 0.8$; the temperature decreased ca. 0,4 ^{o}C/100 m with altitude increase.

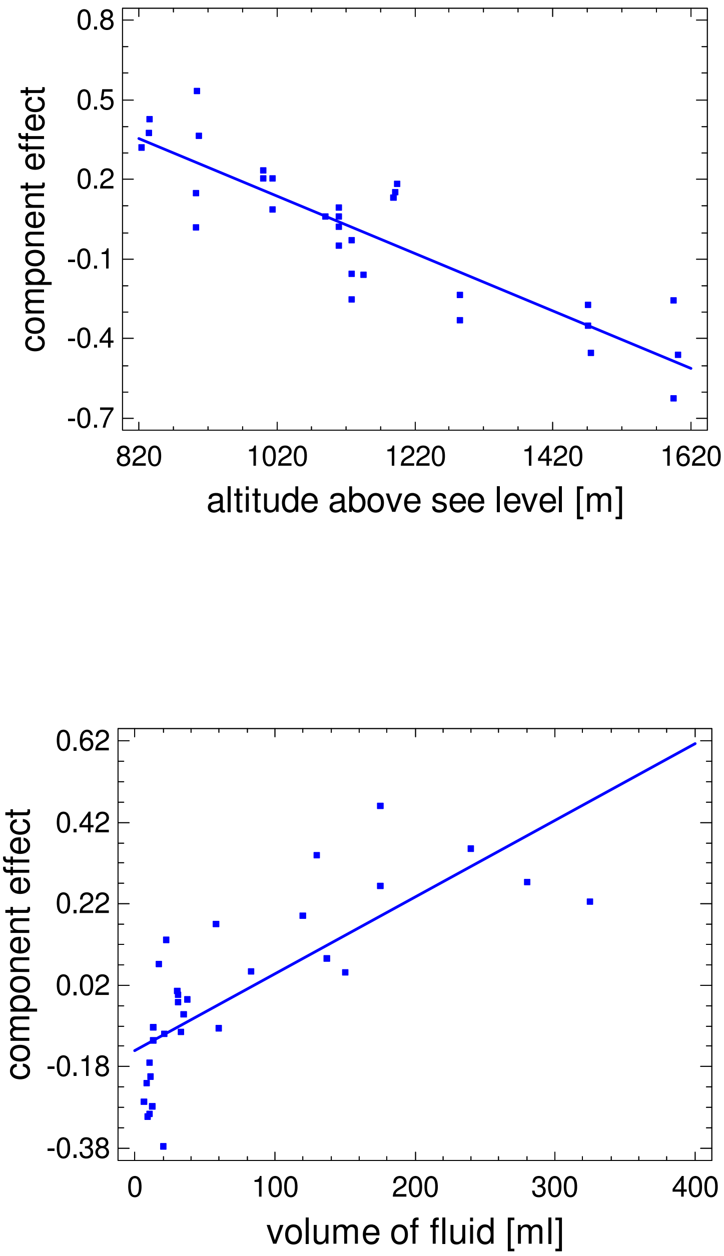

Figure 5. Results of multiple regression analysis: effect of altitude above see level (upper plot) and fluid volume (lower plot) collected from *Heliconia bihai* on number of mosquitoes larvae. The model is significant at $p<0.0001$; $r^2 = 0.87$. Component effect indicated how number of mosquitoes changes over the observed range of an independent variable if all other variables in the model are kept constant.

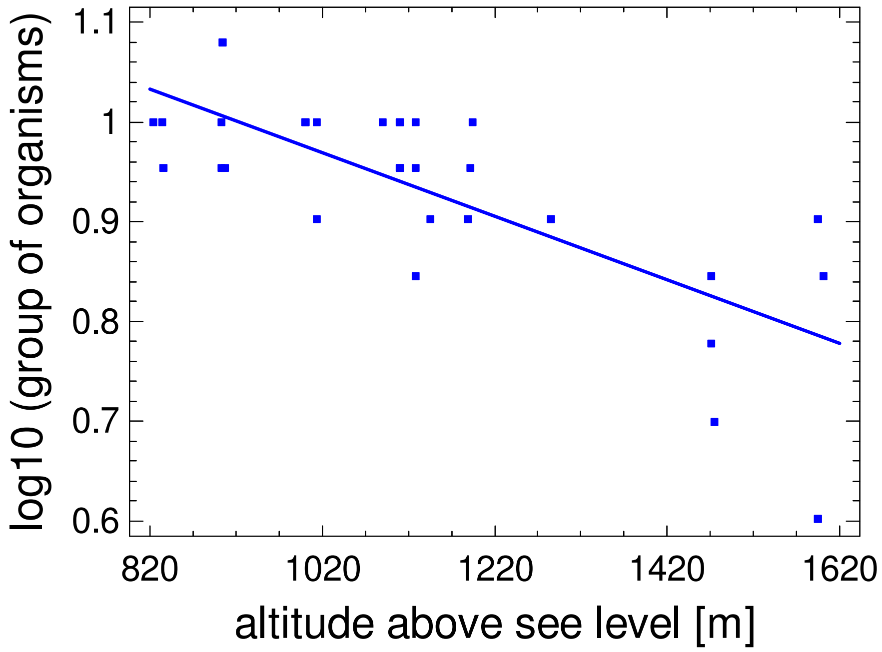

Figure 6. Effect of altitude above see level on number of group of organisms in *Heliconia bihai*; $p<0.0001$, $r^2=0.56$.

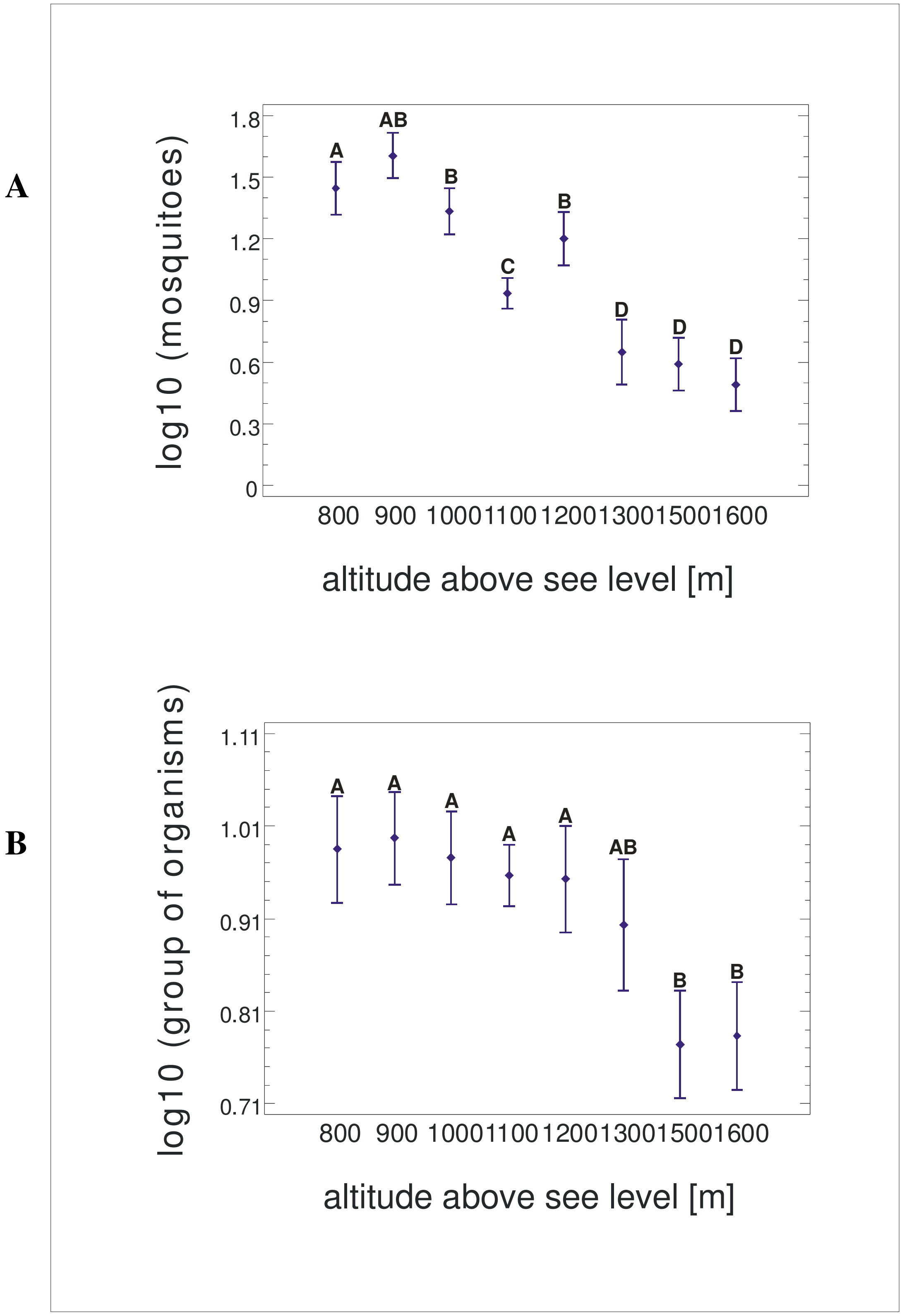

Figure 7. Results of analysis of variance: effect of 100-m altitude ranges on $\log_{10}$ mosquitoes (A) and $\log_{10}$ groups of organisms (B) collected from *Heliconia bihai* samples.

Fruit-eating and insect-eating birds in the tropical montane cloud forest of the Henri Pittier National Park

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ABSTRACT

Field bservations of foraging behavior of tropical insect-eating and fruit-eating birds were made in the cloud forest of northern Venezuela. Of the 14 species observed, 5 captured light-attracted insects, 6 foraged on fruits offered on a bird feeder, and 3 species fed on both insects and fruits. After additional observations in the park a total of 36 species of birds were recorded, 25% of which represent the Tanagers group.
Key words: foraging behavior, tropical birds, insect-eating, fruit-eating, Venezuela.

Henry Pittier National Park (Rancho Grande) was created in 1937 as the first national park of Venezuela. It occupies a total area of 107 800 ha between the states Aragua and Carabobo in the middle part of the Coastal Cordillera. From the highest point in the park, the 2430 m Pico Cenizo, the terrain goes down to the Carribean coast (Lentino and Goodwin 1991). The broad range of altitude and the exposure of the terrain result in differentiated precipitation, temperature and insolation, so important for the local richness of biodiversity.

Ecosystems of the park include: xerophytic shrubbery, dry forest, semideciduous selva, low and high cloud forest. Ecosystem diversity, correlated with species diversity of plants and animals, provide a lot of different food sources for the organisms living there. Because of the abundant food available throughout a year many animals specialize in feeding on only one (as the *Euphonias* that tend to feed heavily on mistletoe berries (family *Loranthaceae*)) or two types of food (e.g., fruit and nectar). Others are more generalistic feeders and eat the most widespread food (Plotkin 1997).

Amongst the rich tropical fauna birds are especially interesting to study. Lentino and Goodwin (1991) published a list of 545 species of birds recorded in the Henri Pittier National Park, which was almost 42% of all 1368 species of Venezuela. Today this numbers are higher because of the new species recorded in the last few years.

The Rancho Grande Biological Station, located at 1110 m above the sea level, among the thick vegetation of a montane cloud forest, offers a great opportunity for field studies and observations without a necessity to go deep into the forest (Photo 1). In 1954 Schaefer and Phelps presented results of their study in the work „Birds of Rancho Grande", and 29 years later Collins and Watson (1983) described their observations of bird predation on neotropical moths.

This work presents a short study, done during the field course in tropical ecology organized jointly by the Jagiellonian University in Cracow (Poland), Instituto Venezolano de Investiciones Científicas and Universidad Central de Venezuela - Facultad de Agronomía in July 2007. The objective was the observation and identification of insect-eating and fruit-eating birds in the vicinity of the biological station, and their foraging behavior. The data collected during the study will be also used to prepare a short guide to the most common birds species in the park, which will be used by future participants of the course.

Photo 1. The landscape tarrace of the Estación Biológica Rancho Grande, a wonderful place for bird observations.

METHODS

Bird observations were made on the landscape terrace of the Rancho Grande Biological Station during six following days between July 13th and 18th, 2007. During the first three days fresh banana (*Musa sp.*), mango (*Mangifera indica*), and avocado (*Persea americana*) fruits were offered on a bird feeder made from a metal grid (40 x 40 cm), where birds could come and forage. The grid was located ca. 110 cm above the ground of the tarrace on a metal pole. Observations were made from a distance of 6-7 meters to avoid frightening the birds. All birds visiting the feeder and in its vicinity (nearby trees and the building of the Station) were recorded (Table 1) together with time of appearance, sex (if possible) and fruit species preferred. When a bird ate no fruit, it was recorded as observed only (O). The numbers in Table 1 demonstrate how many times individuals from a particular species came and ate (or not) each kind of fruit (frequency of appearance on the grid and in its vicinity).

During the next three days insectivorous birds species were observed some 30 m away from the fruit feeder. A mercury light (400 W) was used to attract nocturnal insects, especially moths, which would then settle on a white sheet (150 x 220 cm) spread between two poles. The lamp was turned on throughout a night, and turned off with the sunrise. The same parameters as for the fruit-eating birds were recorded. The birds that came to glean moths from the porch and railing of the station and to the immediate vicinity of the light and collecting sheet (area A) were recorded. Also birds foraging in the nearby vegetation (area C) and occasionally sallied to take insects in the air (area D) or insects, especially moths, resting on the outer walls of the building (area B) taken into account (Collins and Watson 1983).

For this study, the light-attracted insects sitting on the collecting sheet and than captured from it by birds were divided into three categories: small (S) up to 1,5 cm, medium (M) – 1,5-3 cm, and large (L) – bigger than 3 cm. Only families (not species) of insects were identified (see results).

Observations of both fruit-eating and insect-eating birds were made continuously from about 06.00 to about 07.30/08.00 h.

The birds foraging on these light-attracted insects are summarized in Table 2.

No relationship was observed between time of appearance on the porch (hour of foraging) and bird species, so this question was not analyzed in this work.

Throughout the six days of the study and during two additional days observations of all bird species encountered were made in the forest nearby the station. One nocturnal observation was made too. The data is collected in Table 3.

During the observations Tasco binoculars (8x21mm) and digital camera Canon PowerShot Pro 1 were used.

Pictures taken were used for species identification and for documentation of the study.

Photo 2. The collecting sheet with light-attracted insects left from the previous night. Right down, a golden-crowned flycatcher escaping with a prey to the nearby tree can be seen.

RESULTS AND DISCUSSION

A total of 14 species of birds were noted in the two observation places. Some of the birds are frugivorous, others are insectivorous, but there were few species which fed on both fruits and insects.

Nine species of birds were observed in the vicinity of the feeder, from which 7 ate fruits, and two were only observed (Table 1). Three of this species, the blue-gray tanager, the white-lined tanager and the russet-backed oropendola, were fairly frequent. The speckled tanager was ocasionally observed during the first and the thrird day of the study. Also the swallow tanager was regularly seen, but in the canopy of nearby trees, and not feeding on the grid. A pair of silver-backed tanagers was noted only in the first morning, willingly eating banana fruit. The slate-throated witestart was seen only one time on the grid, but frequently in the forest around the biological station, capturing small insects in the air.

It was noted that most of the observed birds definitely prefered banana fruit, supposedly because of its sweet and soft pulp. Banana was eaten with a frequency of 30 times, while mango and avocado 8 and 5 times respectively. Banana was prefered by all species seen on the feeder, especially by the blue-gray, the white-lined and the silver-backed tanagers. Five of the seven species foraging on the feeder belong to the Tanagers family. It is well known that most of the Tanagers feed on fruits, which they mash before (Plotkin 1997).

Only three species of birds consumed mango fruit, and only two ate avocado. The white-lined tanager was the only species eating all three kinds of fruit.

Table 1. Birds eating fruits offered on the feeder observed at Rancho Grande.

Species	Fruit: Banana	Mango	Avocado	Observed only	Presence[2]
Blue-gray Tanager * *(Thraupis episcopus)*	8[1]		3	9	1
Swallow Tanager *(Tersina viridis occidentalis)*				4	2
Russet-backed Oropendola * *(Psarocolius angustifrons)*	3	3		3	1
White-lined Tanager * *(Tachyphonus rufus)*	6	3	2	5	1
Silver-backed Tanager *(Ramphocelus carbo)*	8			2	3 */seen only the I day/*
Speckled Tanager *(Tangara guttata)*	2			1	2 */not seen the II day/*
Palm Tanager *(Thraupis palmarum)*	2	2			
Slate-throated Whitestart *(Myioborus mimatus)*	1			1	3
Pale-breasted thrush				1	
Total	30	8	5	26	

[1] Frequency of appearing.

[2] 1 = frequently observed; 2 = regularly to ocasionally observed; 3 = infrequently to rarely observed.

* Birds feeding on both light-attracted insects and fruits offered on the feeder.

Eight species of birds feeding on the ligth-attracted insects were observed (Table 2). Some of them, like the white-lined tanager, the pale-edged flycatcher and the golden-crowned flycatcher, were most frequent. These species were seen foraging more often directly on the collecting sheet or in the vicinity of the light bulb, alone or in pairs. The russet-backed oropendola was frequent visitor of the porch too, but normally it came in small and noisy flocks of 4-8 individuals, sometimes trying to steal some insect just catched by other species of bird (for example the pale-breasted thrush). The two species of flycatchers were the only ones sallying and hovering in the air for insects, especially for small ones from the *Arctiidae* family, generally considered to be unpalatable to vertebrates (Collins and Watson 1983).

The blue-gray tanager was not seen foraging on the porch. It catched insects in the foliage of the nearby trees, and especially on the outer walls of the building. This is a typical behavior for this bird, perhaps because it is the most synanthropic bird of Venezuela and it is used to live in urban areas and forest edges (Hilty 2003).

Only single individuals of two species, the swallow tanager and the tropical pewee, were observed in the vicinity of the porch. They were not seen foraging during the early-morning observations, but in other time of the day sallying and gleaning insects from the foliage in the canopy of nearby trees was frequently observed. Groups of 10-15 swallow tanagers, mainly females, were normally seen foraging on small insects in the canopy of a *Ficus*-like tree.

Because of the speed at which the birds captured and ate insects it was very difficult to identify food items. For this reason the light-attracted insects were divided into three size categories: small (S), medium (M) and large (L; see methods), including moths, beetles, flies and others (Photo 2).

Photo 3. Russet-backed oropendola eating fruit from the feeder during the early-morning observations.

To the group S we accepted mainly tiger moths (*Arctiidae*) and flies, to the group M inchworms (*Geometridae*), owlet moths (*Noctuidae*) and medium-sized beatles, and to the group L hawk-moths (*Sphingidae*), large geometrids and noctuids, giant silkmoths (*Saturnidae*), and large beatles (*Coleoptera*).

Most of the birds recorded distinctly preferred small- and medium-sized insects (Table 2). Small insects were captured 32 times, medium ones 24 times, and large only 7 times. Birds which preferred smaller-sized moths, ate medium-sized too, but not large insects. On the other hand, that species which captured more medium-sized food were more willing to catch large insects too, like the white-lined tanager, and especially the russet-backed oropendola. This behavior can suggest some level of specialization in prey size. When visiting the collecting sheet or the ground below it, the oropendolas seemed to look after the nearest and biggest *Coleoptera* and *Sphingidae* (Photo 4). After capturing an insect, the russed-backed oropendolas escaped with the prey to a nearby tree. Like oropendola, the pale-breasted thrush and the white-lined tanager, de-winged large moths before eat them (Photo 4).

Among all the 14 species reported, there were 3 species eating both light-attracted insects and fruits offered on the bird feeder (Tables 1 and 2).

Table 2. Birds eating light-attracted insects observed at Rancho Grande.

	Foraging area[1]				
Species	A	B	C	D	Presence[2]
White-lined Tanager* *(Tachyphonus rufus)*	5[4]S[3], 8M, 1L, 2O		4S		1
Pale-edged Flycatcher *(Myiarchus cephalotes caribbaeus)*	3S, 2M			1S	1
Blue-gray Tanager* *(Thraupis episcopus)*		3M,3O	2S		1
Golden-crowned flycatcher *(Myiodynastes chrysocephalus)*	11S, 6M, 2O			1S	1
Swallow Tanager *(Tersina viridis occidentalis)*	1O				3
Tropical Pewee *(Contopus cinereus bogotensis)*			1O		3
Russet-backed Oropendola* *(Psarocolius angustifrons)*	3S, 5M, 5L				1
Pale-breasted thrush *(Turdus leucomelas albiventer)*	2L		2S		2

[1] Foraging area: A = porch and vicinity of light bulb; B = outer walls of building; C = nearby trees, undergrowth, and ground; D = aerial.
[2] 1 = frequently observed; 2 = regularly to ocasionally observed; 3 = infrequently to rarely observed.
[3] Insect's size: S = small, M = medium, L = large; O = only observation.
[4] Number of insects catched.
* Birds feeding on both light-attracted insects and fruits offered on the feeder.

Photo 4. A russet-backed oropendola capturing a big *Sphingidae* moth from the collecting sheet.

Throughout the whole study, a total of 36 species of birds were observed, belonging to 18 families, in the vicinity of the biological station (Table 3). More than one fourth of them (10 species) were from the Tanagers family (*Thraupidae*), which have both insects and fruits in their diet. Flycatchers and hummingbirds are represented by three species each group, and tityras, trogons and ground antbirds by two species per family. Especially frequent were the quite big and noisy flocks of white-tipped swifts, a species which lives in holes in the station's walls.

Two rare species of birds were seen: the handsome fruiteater and the northern helmed curassow. Four of the noted species are endemic for Venezuela: the groove-billed toucanet, the handsome fruiteater, the violet-chested hummingbird and the blood-eared parakeet, which is abundant in the forest of Rancho Grande. Only one diurnal predator was recorded – the white hawk.

During the nocturnal observation only one species was noted: the representant of *Otus* genera – the Foothill Owl.

Photo 5. A pale-breasted thrush de-winging large Sphingid moth before eating it.

Insects, fruits and seeds are the most widespread food in the tropical wet forest. There is a great abundance of these kinds of food not only seasonally, as in the temperate zone, but all the year round. Because of the more or less stable climatic conditions in the tropics, some plants produce fruits and nectar throughout the year. The same can be said about many insects. For this reason it can be said that the availability of food is constant in the tropics (at least in comparison to temperate ecosystems). A number of neotropical birds use this food availability, specializing and feeding on one or two types of food. Most of the birds recorded during this study are from the passerines group. Some of them utilize insects and other arthropods as their major food sources, while other specialize in vegetal food. These groups are among the most species-rich found anywhere (Plotkin 1997).

The Rancho Grande Biological Station offers optimal conditions to observe tropical birds feeding behavior. During this short study in the Henri Pittier National Park a number of fructivorous and insectivorous bird species were observed.

The foraging behavior of most of the species recorded during the early-morning observations can suggest some levels of food specialization. For the frugivores, especially for small mashers (Tanagers for example), fruits with soft and sweet pulp such as banana are preferred. This can explain the frequent presence of this group of birds in plantations or cultivated areas. In the natural environment mashers normally prefer juicy and sweet fruits, berries and nectar, which provide them more energy from saccharides (Plotkin 1997).

Table 3. Species of birds recorded near Rancho Grande Biological Station (13.07. - 20.07.2007) and their food preferences.

N	*Latin name*	English name	Fructivorous	Insectivorous
1.	*Thraupis episcopus*	Blue-gray Tanager	+	+
2.	*Ramphocelus carbo*	Silver-beaked Tanager	+	+
3.	*Tangara guttata*	Speckled Tanager	+	+
4.	*Tachyphonus rufus*	White-lined Tanager	+	+
5.	*Thraupis palmarum*	Palm Tanager	+	+
6.	*Tersina viridis*	Swallow Tanager	+	+
7.	*Tangara gyrola*	Bay-headed Tanager	+	+
8.	*Dacnis cayana*	Blue Dacnis	+	+
9.	*Coereba flaveola*	Bananaquit Dacnis	+	-
10.	*Euphonia xanthogaster*	Orange-bellied Euphonia	+	+
11.	*Tityra cayana*	Black-tailed Tityra	+	-
12.	*Tityra semifasciata*	Masked Tityra	+	+ (rarely)
13.	*Aeronautes montivagus*	White-tipped Swift	-	+
14.	*Myioborus miniatus*	Slate throated Whitestart	+	+
15.	*Ortalis motmot*	Little Chachalaca	+	-
16.	*Pauxi pauxi*	Northern Helmeted-Curassow	+ (mainly seeds)	-
17.	*Aulacorhynchus sulcatus*	Groove-billed Toucannet	+ (and seeds)	-
18.	*Phacellodomus inornatus*	Plain Thornbird	-	+
19.	*Psarocolius angustifrons*	Russet-backed Oropendola	+	+
20.	*Melanerpes rubricapillus*	Red-crowned Woodpecker	+	+
21.	*Adelomyia melanogenys*	Speckled Hummingbird	nektar	-
22.	Aglaiocercus kingi	Long-tailed Sylph	nektar	-
23.	*Sternoclyta cyanopectus*	Violet-chested Hummingbird	nektar	-
24.	*Trogon collaris*	Collared Trogon	+	+
25.	*Pipreola formosa*	Handsome Fruiteater	+	-
26.	*Pharomachrus sp.*	Quetzal	+	+
27.	*Syndactyla guttulata*	Guttulated Foliage-gleaner	-	+
28.	*Pyrrhura hoematotis*	Blood-eared Parakeet	+	-
29.	*Turdus leucomelas*	Pale-breasted Thrush	+	+
30.	*Myiarchus cephalotes*	Pale-edged Flycatcher	-	+
31.	*Myiodynastes chrysocephalus*	Golden-crowned Flycatcher	-	+
32.	*Contopus cinereus*	Tropical Pewee	-	+
33.	*Chamaeza campanisona*	Short-tailed Antthrush	-	+
34.	*Formicarius analis*	Black-faced Antthrush	-	+ (esp. ants)
35.	*Leucopternis albicollis*	White Hawk	-	- (reptiles)
36.	*Otus roraimae*	Foothill Owl	-	- (mammals)

The species of insectivorous birds that were observed, definitely preferred small- and medium-sized insect. During this study was not possible (and was not the objectivefo the study) to identify insect species eaten, but more frequently *Geometridae* and *Noctuidae* were consumed. Some medium- and large-sized Coleoptera, and large *Sphingidae* and *Noctuidae* moths were captured by oropendolas. The smallest moths, especially unpalatable *Arctiidae*, were catched by the noxious-tolerant golden-crowned and pale-edged flycatchers. These results accord with those obtained by Collins and Watson (1983). The two aspects of birds foraging behavior: the preferences to both palatability and size of prey could be studied in further observations.

Interestingly, most of the species seen eating light-attracted insects near the collecting sheet or fruits offered on the grid are used to live along forest edges, in light forests or even in settled areas (Hilty 2003). For example, Tanagers are found abundantly from lowland forests to high montane and cloud forests. They are particularly common around forest-edge habitats and are frequently seen on fruiting trees even in parks and gardens. This can possibly explicate the "no-fear" behavior of these birds in the vicinity of buildings and people.

More generalistic bird species (in the sense of ecological conditions) have broader food preferences. Living in various environments, such as cultivated regions with scattered trees, secondary growth and forest edges, plantations or urbanized areas, they are apter to feed on different kinds of food, instead of specializing in only one or two. Wiedenfeld (1991) suggests that forest species have smaller ranges than edge species. Maybe it is the larger terrytory used by the edge species that offers them a wider range of nourishment resources and, inversely, non-specialized food preferences allow these species to explore new ecosystems, including the antropogenic ones.

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Insects diversity in the Rancho Grande area depending on habitats and bait preference

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Fot. 1. Biological Station Rancho Grande surrounded by cloud forest.

Introduction

Insects are the most diverse and abundant group of animals in tropical forests. Previous studies have found that more than 50,000 insect species live on a single square mile of rainforest (www.sciencedaily.com 2006). Researchers suppose that of the estimated 5-10 million insect species living on Earth, only about 2 million have been identified.

Tropical mountain cloud forest is an evergreen moist forest characterized by a high incidence of a low-level cloud cover, usually at the canopy level. The tropical cloud forest of the Parque Nacional Henri Pittier is one of the best known forests in Venezuela and probably in whole South America. It is internationally known because of the high fauna and flora diversity. For instance, the cloud forest surrounding Rancho Grande Biological Station harbors 150 different species of trees in 0.25 ha (Hubert 1986). The diversity of insects in the park is also very high and has been estimated at over one million (Osuna 2000) but our knowledge about them is still not sufficient and limited to species descriptions and distribution records.

The aim of our project was to compare insects diversity in different habitats of the Rancho Grande area: cloud forest, forest edge and open area. We also checked which trophic forms of insects predominate in each habitat. Additionally, preferences for different baits were studied in the insects.

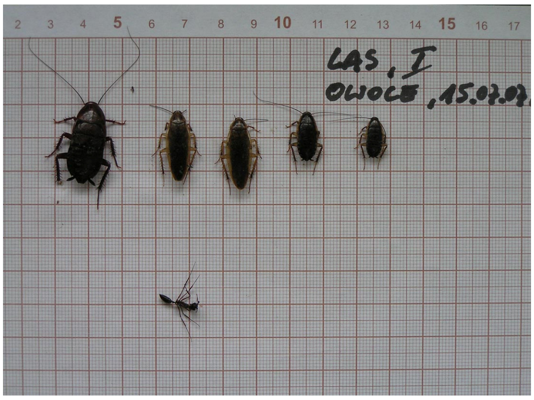
Study area

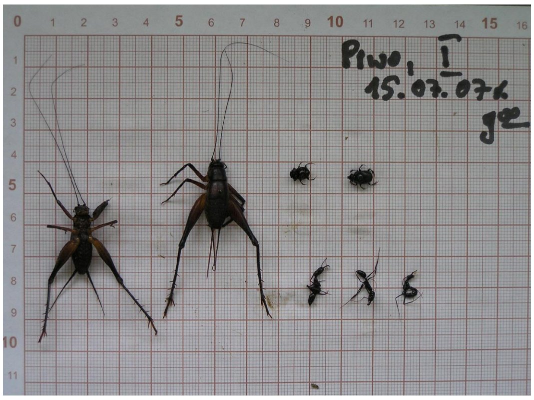
The investigations were carried out at Rancho Grande area which is located in Henri Pittier Nacional Park, State of Aragua, Venezuela (10° 21'N, 65° 41'W; Clavijo 1997). Henri Pittier N. P. covers 1078 km^2 area and is located in the central part of Cordillera de la Costa mountain range that extends from east to west along the Venezuelan Caribbean coast. Several distinct habitats are present along the park altitudinal gradient (from the sea level to 2400 m). These include mangroves, savannas, dry forest, humid forest and cloud forest. Changes in vegetation composition and variation in climatic zones sustain extremely high faunal diversity.

Rancho Grande is a biological station located in a mid-elevation Venezuelan cloud forest at 1,100 m. Most of the precipitation in the cloud forest occurs as mists persisting from early morning until late day, resulting in reduction of the direct sunlight and thus of evapotranspiration. Trees in these regions are generally shorter and more heavily stemmed than in lower altitude forests in the same region. The moisture promotes development of an abundance of vascular epiphytes (Haber 2000). Soils are rich but boggy, with a preponderance of peat and humus. A lot of the precipitation within cloud forests is in the form of fog drip, where fog condenses on tree leaves and then drips onto the ground below.

Materials and metods

The investigations were carried out from 12.07.2007 to 20.07.2007. Three different habitats near Rancho Grande station were chosen (all at the same altitude): cloud forest, a transition zone between forest and open area, and a regularly mowed and maintained grassland. Insects were caught with modified Barber-type pitfall traps. Twelve traps were disposed at the studied area, four in each of the selected habitats. The traps were dug down to the ground level with 1 m gap between them. Plastic cups with 92 mm diameter and 120 mm deep were used. In each habitat three cups contained different baits: (1) fruits, (2) beer, (3) dung, and the fourth cap was kept empty as a blank test. The traps were emptied twice per day with replacing the baits. Caught insects were identified to families.

a) 

b) 

Fot. 2. Examples of insects caught in the cloud forest in traps with fruits (a) and beer (b).

Results

Habitat preference

181 insects, belonging to 8 families, were caught during 6 days (Tab. 1). The most abundant families were Scarabaeidae (63%), Blattellidae (12%), Formicidae (11%) and Gryllidae (7%). The remaining families were represented by a few specimens: Forficulidae (3%), Julidae (2%), Curculionidae (1%) and Noctuidae (1%).

Table 1. Number of individuals belonging to different insect families caught in different habitats of the Rancho Grande area.

	Tropical cloud forest	**Forest edge**	**Grassland**	**Total (Σ)**
Scarabaeidae	71	33	12	**116**
Blattellidae	13	8	1	**22**
Formicidae	19	1	-	**20**
Gryllidae	5	7	-	**12**
Forficulidae	5	-	-	**5**
Julidae	3	-	-	**3**
Curculionidae	-	1	1	**2**
Noctuidae	-	-	1	**1**
Total (Σ)	**116**	**50**	**15**	**181**

The highest number of insects was caught in the cloud forest (64%), intermediate on the forest edge (28%), and a very few on the grassland (8%) (Fig. 1). Eight families were identified, out of which 6 were found in the cloud forest, 5 at the forest edge and only 4 at the grassland. Two of them were common in all habitats (Scarabaeidae and Blattellidae), whereas four were found both in the forest and forest edge.

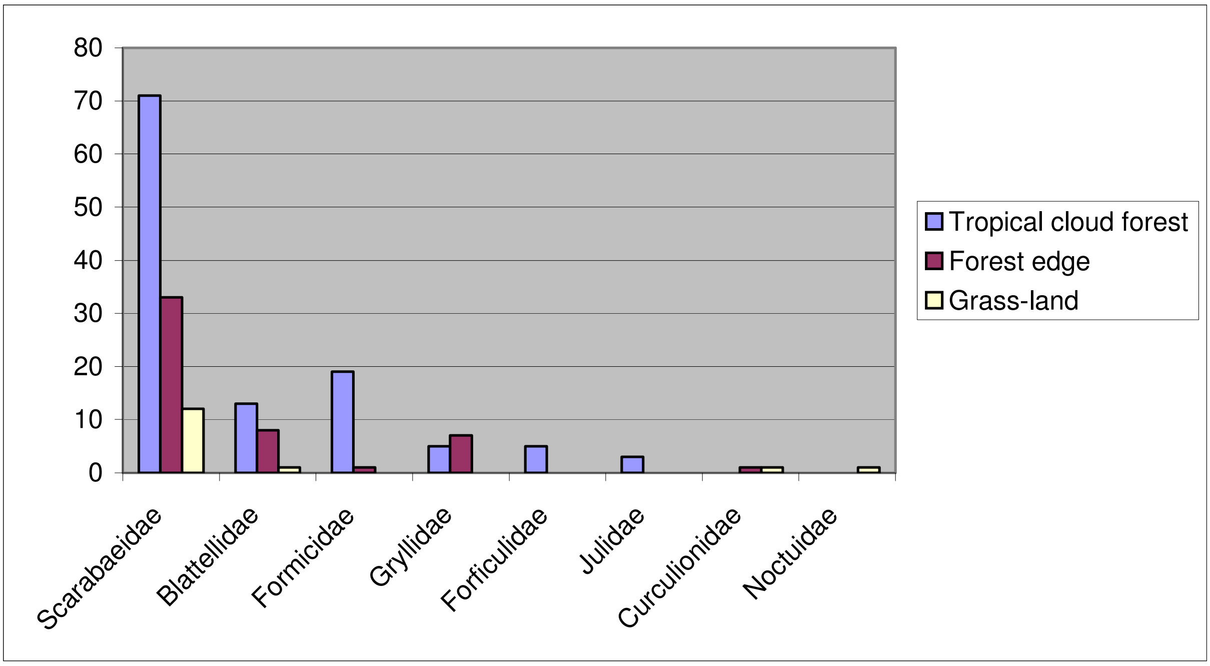


Fig. 1. Numbers of insects belonging to particular families at the Rancho Grande area depending on the habitat.

The insects collected during the study include three ecological groups: detritivores, omnivores and herbivores. The most numerous group were detritivores (64%). It was represented by caprophagous insects from Scarabaeidae family. Omnivores was the most diverse group, with Blattellidae, Formicidae, Gryllidae and Forficulidae families (32%). Herbivorous insects were represented by Julidae and Curculionidae (4%).

Fot.3 Black Witch (*Ascalapha odorata*) - a moth from Noctuidae family, feeding on overripe rainforest fruit, especially bananas. Caught in a trap with fruits on grasslands.

Bait preference

The highest numbers of insects were caught in traps with dung, where the most abundant family was Scarabeidae (Tab. 2). However, the diversity of families in these traps was the lowest (only four families).

Table 2. Bait preference of among insects caught in the Rancho Grande area.

	Dung	**Beer**	**Fruits**	**Empty test**	**Total (Σ)**
Scarabaeidae	89	20	6	1	**116**
Blattellidae	2	3	16	1	**22**
Formicidae	2	9	7	2	**20**
Gryllidae	-	12	-	-	**12**
Forficulidae	3	-	2	-	**5**
Julidae	-	-	3	-	**3**
Curculionidae	-	2	-		**2**
Noctuidae	-	-	1	-	**1**
Total (Σ)	**100**	**44**	**31**	**4**	**181**

The second bait in terms of the number of insects caught was the one with beer. These were the only traps where representatives of Gryllidae and Curculionidae families were found. The most numerous family was Scarabaeidae, similarly like in dung traps. Five families were recognized in these traps. In cups with fruits insects from Blattellidae family were mostly found. Insects that were caught only in traps with this bait were representatives of Julidae and Noctuidae. The number of families in those traps (six) was the highest. Only 4 insects were caught in the empty trap.

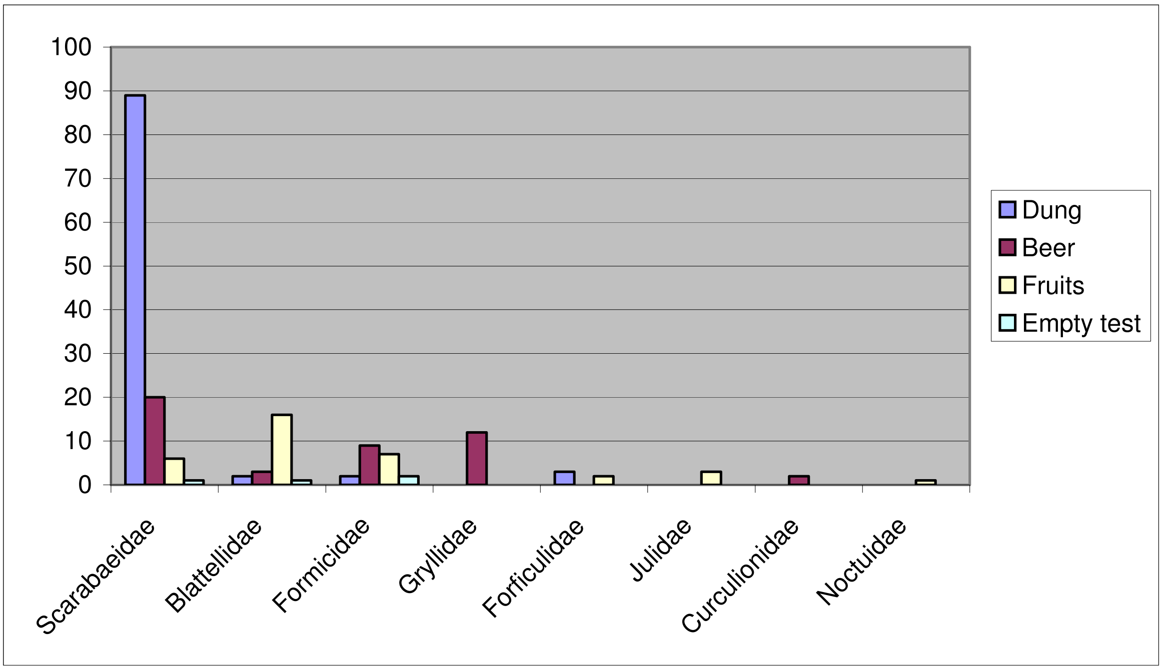


Fig. 2. Diversity of insect families depending on the bait type.

Conclusions

Measuring species diversity of a biocenosis requires two categories of data: number of species and their relative numbers (Krebs 1996). In the study presented herein we considered taxonomic diversity on the family level only. Nevertheless, despite relatively low taxonomic resolution, the study clearly showed substantial variety between the habitats, both in the number of families and in the number of individuals (Fig. 3). The highest number of organisms (116) was caught in cloud forest where the highest families diversity was also noted. Less then half of that (50) was caught on the forest edge. At the open area less then

10% from the total number of organisms were found (15 individuals), with the lowest number of families. The differences that have been noted between the three types of habitat are probably the result of anthropogenic activity, affecting unfavorably both the diversity and the number of insects. According to McNeely (1995) deforestation is one of the main causes of the reduction of biodiversity. Monoculture grassland area, artificially created as the result of human work, has considerably deteriorated living conditions for insects in this area. Previous studies reported that the diversity of flora and fauna species increases with diversity and complexity of physical environment (Krebs 1996). Consequently, higher numbers and diversity of species are present in cloud forest, witch creates more variable ecologic niche than the grassland. In transitional zone between forest and open area we did not observe increase of species diversity nor concentration of insects characteristic for ecotone environments.

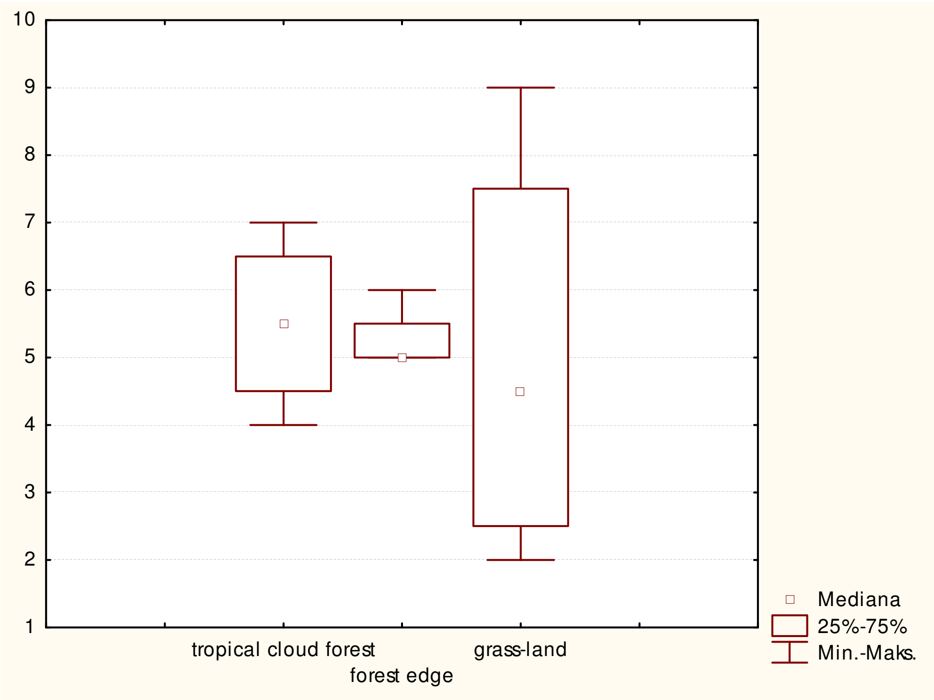


Fig. 3. Comparison of three types of habitats: cloud forest, forest edge and grassland with taking into consideration of median and percentile: 25 and 75.

The study affirmed food selectivity of insects and allowed to estimate relative abundance of insects belonging to different trophic groups in this area. Among the traps with baits, the highest number of insect families was stated in traps with fruits, intermediate in traps with beer, and the lowest in dung traps. The two latter ones were dominated by Scarabaeidae (almost 90%), which also constituted the highest percentage of individuals caught in traps with beer (ca. 40%). Many tropical Scarabaeidae are scavengers that recycle dung, carrion, or decaying plant material (Hogue 1993). The second family in terms of numbers caught in trap with beer was Gryllidae (ca. 20%). Representatives of this family were found only in this type of traps. Gryllidae are omnivores and scavengers feeding on organic materials, as well as decaying plant material, fungi, and some seedling plants. Specimens belonging to this family were noted only in forest and at the forest edge. Predominant group in traps with fruits included Blattellidae (over 50%) which were represented only in small percentage in other traps. Most of the species from Blattellidae family live in soil and dead leaves on the forest floor. They are omnivores that eat almost any organic matter that can be found. They were observed mainly in forest and at the forest edge. Specimens from Curculionidae family were found only in beer traps (only 4%). These include only plant feeders, most species are associated with a narrow range of hosts, in many cases living only on a single plant species (Hogue 1993). They were found only at forest edge and grasslands. We also found a lot of Formicidae but it is hard to assign them to specific trophic group because we did not identify them to the species level. It is well known that ants can eat many different kinds of food depending on species specialization. Forficulidae family, found only in the forest in traps with dung and fruits, can feed on other insects, plants and ripe fruit. Only 4 insects were caught in the empty traps showing that in individual traps the fortuity was very low and the type of collected insects indeed depended on the bait.

Omnivores and scavengers living in the forest floor predominated in our study (>90%), whereas herbivorous were less common (<10%). However, it is important to note that the baits and the type of traps used in the study were directed to catch epigeic insects. Basing on the obtained results it can be concluded that one of the basic factors limiting occurrence of insects at the open area around the biological station is the lack of food for the insect groups described above.

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Field observations of insectivorous and frugivorous birds at the Estacion Biologica de Rancho Grande in Henri Pittier National Park

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Abstract

Field observations of insectivorous and frugivorous birds were made in Rancho Grande, northern Venezuela, from 13 to 20 July 2007. During observations 35 species of birds were noted. Some bird species were present in great numbers: Blue-gray Tanager, White-lined Tanager and Russet-backed Oropendola. Observations have shown that frugivorous birds were choosing mostly banana, and insectivorous ones small or medium moths. Insects were gleaned directly from sheet as easy prey.

Introduction

Henri Pittier National Park is the oldest park in Venezuela, established in 1937. Originally called Rancho Grande, the park has been known as Henri Pittier National Park since 1953. The park covers 107 800 hectares and occupies the State of Aragua's portion of the Coastal Mountain Range. The highest point within the park exceeds 2 430 meters (Pico Cenizo), and the park extends down to the sea level. Its ecosystems vary with altitude. Topography of the park is characterized by steep slopes covered with tropical forest and cloud forest at higher elevations. Along the northern coast there are cactus and thorn bushes.

The park has high diversity of plant and animal life but is famous mainly for its birdlife. Because the park lies on a significant migratory route (Portachuelo Pass), the bird population is particularly rich (Hilty, 2003): as many as 545 species of birds have been recorded (Lentino & Goodwin, 1993). More than 42% of Venezuela's bird species have been identified in Henri Pittier National Park and it is one of the highest recorded species densities in the world.

The Estacion Biologica de Rancho Grande is located at an elevation of 1110 m a.s.l. among thick vegetation of cloud forest and is a perfect location for biological and ecological observations and experiments without a necessity of entering deep into the forest. In 1954 Schäfer and Phelps published results of their observations in the article *Las aves del Parque Nacional Henri Pittier (Rancho Grande) y sus funciones ecológicas - Birds of Rancho Grande and their ecological functions* (Schäfer & Phelps, 1954). Almost 20 years later Collins and Watson (1983) published their observations made in Rancho Grande on bird predation on Neotropical moths.

Our observations had a character of small investigation carried out as part of the course in tropical ecology organized by the Jagiellonian University (Cracow, Poland), Instituto Venezolano de Investigaciones Científicas and Universidad Central de Venezuela - Facultad de Agronomía. The main aim was to record food preferences and customs of insectivorous and frugivorous birds and then compare them with the work of Collins and Watson.

Methods

Field observations were made at the Estacion Biologica de Rancho Grande in Henri Pittier National Park. They were carried out in eight days from 13 to 20 July 2007. At the first and the last day we observed all birds in surroundings of Rancho Grande. From 14 to 16 July we observed frugivorous birds. On a feeding tray we put a number of different kinds of fruits: avocado, banana, mango, papaya and pineapple. The feeding tray was situated on the second floor at the terrace. It was about 110 cm high and 40 x 40 cm area (Picture 1). During investigations we recorded birds which ate fruits, those that showed interest in fruits but did not eat them, and also birds which were in the nearby branches.

From 17 to 19 July we observed insectivorous birds. We hung a white sheet of 150 x 220 cm near a mercury lamp. The light was switched on at about 20:00 and left on all the night till 6:00 in the morning. During the night numerous insects, especially moths, were attracted by the light and many of them still covered the sheet at dawn (Picture 2).

Our observations of insectivorous and frugivorous birds was done from 6:00 to 7:30. The arrival time of each bird was jotted down everyday. One additional observation was carried out at night. During observations binoculars and camera were used to identify bird species.

Results

During observations at Rancho Grande 35 species of birds were recorded (table 1). Birds were noted not only in the neighbourhood of the feeding tray and collecting sheet but also in the forest and the whole area of Rancho Grande station. Some of the birds were observed only in the neighbourhood of the feeding tray or on and around the sheet but most of them were noted in both research areas. Some bird species were present in great numbers, for example: Blue-gray Tanager, White-lined Tanager and Russet-backed Oropendola.

In the immediate vicinity of the feeding tray 9 species of birds were recorded (table 2). Over a half of the observed individuals (42 out of 76) were eating fruits (area A in table 2). Most birds (25 out of 76) were observed on the feeding tray without eating fruits (area B in table 2). Two species were observed only in the area C (nearby trees): Swallow Tanager and Groove-billed Toucannet. Our investigations have shown that birds prefer banana over other fruits when they have a choice (table 3).

Close to the collecting sheet 9 species were noted. Almost a half of the observed birds (57 out of 124) were gleaning insects directly from the sheet (area A in table 4). A quarter of them were eating insects from the terrace and the walls in direct surroundings of the sheet (area B in table 4). Birds which were resting on trees (area C in table 4) and those which sometimes caught moths in the air (area D in table 4) were also recorded. On the grounds of our observations we affirmed that birds preferred small or medium moths (table 5).

Discussion

Although 35 species of birds were noted during the whole stay at Rancho Grande, just 4 species were observed only in the neighbourhood of the feeding tray. These species were: Groove-billed Toucannet, Palm Tanager, Silver-beaked Tanager and Speckled Tanager. These species were not seen in the immediate vicinity of the collecting sheet, which means that they were interested only in fruits. We also noted 4 species which were present only in the neighbourhood of the collecting sheet: Blood-eared Parakeet, Golden-crowned Flycatcher, Pale-edged Flycatcher and Tropical Pewee. This observations suggests that all of them are strictly insectivorous.

Most of the birds observed in both research areas (the sheet and the feeder) ate both insects and fruits. These generalist species included: Blue-gray Tanager, Pale-breasted Thrush, Russet-backed Oropendola, Swallow Tanager and White-lined Tanager. However, the majority of identified birds where present neither in the neighbourhood of the feeding tray nor in the area of the collecting sheet.

Our observations suggest that most of the birds which were seen in the area of the feeding tray preferred bananas to other fruits. However, bananas where offered to the birds every day while other fruits not. Some fruits, for example papaya and pineapple, where given by other students during the day so the chances to see birds eating all kinds of fruits in the morning were not equal. This, unfortunately, could influence the results of our study.

Insectivorous birds learned to exploit moths resting on the sheet in the morning. Our results showed that most birds were choosing small or medium moths. It was clearly connected with the size of a bird, because larger birds were choosing the largest moths. Small moths usually were swallowed whole, but the large ones were de-winged before swallowing.

During observations it was affirmed that some species appeared as the first ones every morning and were later followed by other species. Sometimes we saw that some birds were chased away by others, but this topic needs further investigations.

ACKNOWLEDGMENTS

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Tables

Table 1 List of observed birds at Rancho Grande in Henri Pittier National Park.

English name	Latin name
Bananaquit Dacnis	*Coereba flaveola lutea*
Bay-headed Tanager	*Tangara g. gyrola*
Black-faced Antthrush	*Formicarius analis saturatus*
Black-tailed Tityra	*Tityra c. cayana*
Blood-eared Parakeet	*Pyrrhura h. hoematotis*
Blue Dacnis	*Dacnis c. cayana*
Blue-gray Tanager	*Thraupis episcopus*
Collared Trogon	*Trogon collaris exoptatus*
Foothill Owl	*Otus r. roraimae*
Golden-crowned Flycatcher	*Myiodynastes chrysocephalus cinerascens*
Groove-billed Toucannet	*Aulacorhynchus s. sulcatus*
Guttulated Foliage-gleaner	*Syndactyla g. guttulata*
Handsome Fruiteater	*Pipreola f. formosa*
Little Chachalaca	*Ortalis m. motmot*
Long-tailed Sylph	*Aglaiocercus kingi margarethae*
Masked Tityra	*Tityra semifasciata columbiana*
Northern Helmeted-Curassow	*Pauxi p. pauxi*
Orange-bellied Euphonia	*Euphonia xanthogaster*
Pale-breasted Thrush	*Turdus leucomelas albiventer*
Pale-edged Flycatcher	*Myiarchus cephalotes caribbaeus*
Palm Tanager	*Thraupis palmarum*
Plain Thornbird	*Phacellodomus i. inornatus*
Red-crowned Woodpecker	*Melanerpes r. rubricapillus*
Russet-backed Oropendola	*Psarocolius angustifrons oleagineus*
Short-tailed Antthrush	*Chamaeza campanisona venezuelana*
Silver-beaked Tanager	*Ramphocelus carbo*
Slate throated Whitestart	*Myioborus miniatus ballux*
Speckled Hummingbird	*Adelomyia melanogenys aeneosticta*
Speckled Tanager	*Tangara guttata chrysophrys*
Swallow Tanager	*Tersina viridis occidentalis*
Tropical Pewee	*Contopus cinereus bogotensis*
Violet-chested Hummingbird	*Sternoclyta cyanopectus*
White Hawk	*Leucopternis a. albicollis*
White-lined Tanager	*Tachyphonus rufus*
White-tipped Swift	*Aeronautes m. montivagus*

Table 2 Birds observed in the neighbourhood of feeding tray.

Species	Foraging area: A	B	C	Presence
Blue-gray Tanager *Thraupis episcopus*	8	6	3	17
Groove-billed Toucannet *Aulacorhynchus s. sulcatus*	0	0	1	1
Pale-breasted Thrush *Turdus leucomelas albiventer*	0	1	0	1
Palm Tanager *Thraupis palmarum*	4	0	0	4
Russet-backed Oropendola *Psarocolius angustifrons oleagineus*	6	3	0	9
Silver-beaked Tanager *Ramphocelus carbo*	10	4	1	15
Speckled Tanager *Tangara guttata chrysophrys*	4	5	0	9
Swallow Tanager *Tersina viridis occidentalis*	0	0	4	4
White-lined Tanager *Tachyphonus rufus*	10	6	0	16
Total	42	25	9	76

Table 3 Food preferences of frugivorous birds.

Species	Kind of fruit: avocado	banana	mango	papaya	pineapple
Blue-gray Tanager *Thraupis episcopus*	+	+			
Groove-billed Toucannet *Aulacorhynchus s. sulcatus*					
Pale-breasted Thrush *Turdus leucomelas albiventer*					
Palm Tanager *Thraupis palmarum*		+	+		
Russet-backed Oropendola *Psarocolius angustifrons oleagineus*	+	+	+		
Silver-beaked Tanager *Ramphocelus carbo*		+			
Speckled Tanager *Tangara guttata chrysophrys*		+			
Swallow Tanager *Tersina viridis occidentalis*					
White-lined Tanager *Tachyphonus rufus*	+	+	+		

Table 4 Birds observed in the neighbourhood of collecting sheet.

Species	Foraging area: A	B	C	D	Presence
Blood-eared Parakeet *Pyrrhura h. hoematotis*	0	0	2	0	2
Blue-gray Tanager *Thraupis episcopus*	0	8	7	0	15
Golden-crowned Flycatcher *Myiodynastes chrysocephalus cinerascens*	17	0	4	9	30
Pale-breasted Thrush *Turdus leucomelas albiventer*	0	6	2	0	8
Pale-edged Flycatcher *Myiarchus cephalotes caribbaeus*	21	0	2	4	27
Russet-backed Oropendola *Psarocolius angustifrons oleagineus*	19	3	0	0	22
Swallow Tanager *Tersina viridis occidentalis*	0	0	1	0	1
Tropical Pewee *Contopus cinereus bogotensis*	0	0	1	0	1
White-lined Tanager *Tachyphonus rufus*	0	13	5	0	18
Total	57	30	24	13	124

Table 5 Preferred sizes of moths by insectivorous birds.

Species	Size of moth: small (1-2 cm)	medium (2-4 cm)	large (>4 cm)
Blood-eared Parakeet *Pyrrhura h. hoematotis*			
Blue-gray Tanager *Thraupis episcopus*	+		
Golden-crowned Flycatcher *Myiodynastes chrysocephalus cinerascens*	+	+	
Pale-breasted Thrush *Turdus leucomelas albiventer*			+
Pale-edged Flycatcher *Myiarchus cephalotes caribbaeus*	+	+	
Russet-backed Oropendola *Psarocolius angustifrons oleagineus*	+	+	+
Swallow Tanager *Tersina viridis occidentalis*			
Tropical Pewee *Contopus cinereus bogotensis*			
White-lined Tanager *Tachyphonus rufus*	+	+	+

Pictures

Picture 1. Russet-backed Oropendola on the feeding tray.

Picture 2. The collecting sheet.

INFLUENCE OF BIO-ACTIVE CHEMICAL SUBSTANCES ON STREAMS OF *ECITON BURCHELLI* AND *ACROMYRMEX OCTOSPINOSUS* ANTS IN RANCHO GRANDE FIELD STATION, VENEZUELA

Agnieszka E. Bem

Abstract

The studies were carried out to find out whether ants with different life styles, *Acromyrmex octospinosus* (leaf-cutter ant) and *Eciton burchelli* (army ant), differ in their ability to recognize different chemical substances. This was studied by counting the number of ants crossing a chemical barrier in the same period of time (35 s). The results showed significant differences between the species in their reaction to the same chemical substance as well as different reactions to different substances.

Introduction

The aim of this research was to compare behaviour evoked by different chemical substances on two different species of ants - *Eciton burchelli* (army ant) and *Acromyrmex octospinosus* (leaf-cutter ant). The behaviour was classified as positive, negative or neutral, and the intensity of effect was also measured.

Observed ants are characterized by different life styles and nutrition. Leaf-cutter ants can be called “stationary ants” because they live in characteristic nests for which they take care. Usually, they follow the same paths everyday. In contrast, *Eciton burchelli* are “nomadic ants” because their change their paths every day and have no permanent nest. During the night, army ants create a specific “live-structure” called *bivouac*. Leaf cutter ants are known as fungus growing ants, while army ants are strict predators.

Due to these different life styles, different reactions to the same chemical substances were expected in the two species. I assumed that *A. octospinosus*, being specialized in growing fungi on composted plant material, should be able to recognize chemical substances originating from plants more precisely that the predatory *E. burchelli*.

Materials and methods

The research took place in Rancho Grande field station in Henri Pitter National Park, the central north Venezuela. In the beginning (Experiment I), solutions of 33 chemical substances were prepared (Table 1), and after testing them on four different streams of different ant species, only 7 were used in further research (Experiment II). The substances chosen for Experiment II were those which evoked different reactions in different species or in the same species but among different streams (only for *A. octospinosus*). Because ascorbic acid combined with vitamin P gave negative reaction (except *A. octospinosus* (B)) and ascorbic acid alone gave the neutral one, the impact of vitamin P as an enhancer of negative factor on ants was also studied.

The chemical substances were medicines from a first aid kit. Water solution of each medicine was prepared (each time one pill was diluted in 5 ml of water). In the case of nicotine, the solution was made by pouring boiling water over a content of a cigarette. The solutions were used in the field trial by soaking in a solution a small piece of gauze which was applied perpendicularly to the ants' path. Then, ants behaviour was noted.

Table 1. List of chemical substances used in the experiments. Substances typed in boldface were used in Experiment II. (English name of the drug if very popular).

Symbol	*Main chemical substance contained in the drug*	*Symbol*	*Main chemical substance contained in the drug*
A1	neomycin, polymyxin B, gramicidin	**E2**	bacterial flora of humans
A2	**doxycycline**	**E3**	orofar (benzoxonium chloride, lidocaine hydrochloride)
A3	olopatadine hydrochloride	**E4**	**nitroglicerine**
A4	**neomycin, gramicidin**	**E5**	clioquinol
A5	neomycin	**F1**	salicylic acid
B1	budesonide	**F2**	92% ethanol
B2	**cetirizine**	**F3**	calcium
B3	hydrocortisone	**F4**	**ascorbic acid**
C1	aspirin (acetylsalicylic acid)	**F5**	carbon
C2	chloroquine	**F6**	isopropanole
C3	paracetamol	**F7**	NaCl
C4	**ascorbic acid, vitamin P**	**F8**	**nicotine**
D1	diosmectite	**F9**	ethanoic acid
D2	furaginum	**F10**	methanole
D3	nitrofurantoin	**F11**	colchicine
D4	**gastritis**	**F12**	KCl
E1	xylometazoline		

In Experiment I the reaction of ants was classified as negative (ants try to avoid the chemical barrier), neutral (ants follow their path normally, without noticing the chemical substance) and positive (ants gather around the chemical barrier). In Experiment II the numbers of ants crossing a chemical barrier in a certain period of time were counted. Period of time was set empirically at 35 seconds. First, the control reading was taken as the number of ants crossing an invisible line perpendicular to ants' path. Thus, control reading was expressed as the number of ants crossing in 35 s a point in the path not affected by any chemical. Only those paths were used in the study which were long enough and had no side paths. These two conditions were important because such a selection gave an opportunity to test substances on the same path many times, but each time a barrier was put before the previous one. This was necessary to study the first reaction of ants on a substance they did not encounter before (the probability of finding tested substances in the natural environment by ants was probably negligibly low). Only ants proceeding towards the nest or *bivouac* were counted. The reason for this was, again, to avoid using the ants that might have been already tested before. For both species the number of ants crossing the same chemical barrier was counted ten times.

For measuring time a stopwatch was used, and the ants crossing a barrier were counted by eyes.

Significance of effects was tested with analysis of variance (ANOVA) with ant species and chemical substance as factors. The means were separated with Tukey HSD post-hoc test.

Results and discussion

In most cases, the substances tested in the experiment affected behaviour of at least one of the studied species. Some chemicals clearly affected all species (Tab. 2).

Experiment I

As said before, the first part of the experiment focused just on behaviour of ants. All 33 substances were tested on paths of:

- *Eciton burchelli* (located on the educational path nearby Rancho Grande field station);
- *Acromyrmex octospinosus* (one path located on the balcony of Rancho Grande field station and the other one located behind the bus stop opposite to the gate to Rancho Grande field station);
- Myrmicinae (located on the educational path nearby Rancho Grande field station).

Results of Experiment I are reported in Table 2.

Table 2. Results of experiment I. *A. octospinosus* (A) was a group from the balcony of Rancho Grande field station, and *A. octospinosus* (B) was a group located behind the bus stop. “1” means positive reaction, “0” neutral and “-1” negative one.

Symbol	***Eciton burchelli***	***Acromyrmex octospinosus* (A)**	***Acromyrmex octospinosus* (B)***	**Myrmicinae**
A1	0	0	0	-1
A2	**-1**	**0**	**1**	**0**
A3	0	0	0	0
A4	**0**	**-1**	**-1**	**-1**
A5	0	-1	0	-1
B1	0	0	0	0
B2	**0**	**0**	**-1**	**-1**
B3	0	1	-1	0
C1	0	1	0	0
C2	0	0	0	-1
C3	0	0	0	0
C4	**-1**	**-1**	**0**	**-1**
D1	-1	0	0	-1
D2	0	0	0	0
D3	0	1	0	-1
D4	-1	0	0	-1
E1	0	0	0	-1
E2	0	0	0	-1
E3	0	0	0	-1
E4	**-1**	**1**	**-**	**-1**

E5	0	0	0	0
F1	-1	1	-1	-1
F2	-1	0	-1	-1
F3	0	0	0	0
F4	**0**	**0**	**0**	**0**
F5	0	-1	0	0
F6	0	1	-1	-1
F7	-1	0	-	0
F8	**0**	**0**	**-1**	**0**
F9	-1	-1	-1	-1
F10	-1	-1	-1	-1
F11	-1	1	-1	-1
F12	-1	0	0	0

Substances typed in boldface were chosen for Experiment II.

Among 33 chemical substances 12 did not affect ants behaviour (Tab. 2). They were: neomycin, olopatadine, budesonide, chloroquine, paracetamol, furaginum, xylometazoline, orofar, clioquinol, calcium, ascorbic acid and bacterial flora of humans. All four tested cases exhibited negative behaviour to ethanoic acid and methanole. There was no single substance causing positive behaviour in all species. In a few cases a positive reaction was found in *A. octospinosus.* Stream of *A. octospinosus* (A) gave positive reaction to hydrocoritisone, aspirin, nitrofurantoin, nitroglycerine, salicylic acid and isopropanole, while *A. octospinosus* (B) reacted positively only to doxycycline.

Experiment II

Tab. 3a. The numbers of *A. octospinosus* which crossed the barrier in 35 s.

A. octospinosus (B)									
	control	**doxy-cycline**	**ascorbic acid**	**certizine**	**Neomycin, gramicidin**	**ascorbic acid, vitamin P**	**nitrogli-cerine**	**gastritis**	**nicotine**
	26	8	13	28	13	6	2	0	2
	28	17	4	16	8	6	7	2	1
	15	16	18	14	9	18	1	0	1
	19	12	11	18	10	9	0	0	3
	27	13	10	22	13	4	6	0	1
	23	10	14	15	10	6	6	0	0
	24	16	16	5	11	10	0	0	5
	18	9	18	16	18	5	4	0	2
	11	14	15	20	14	10	5	0	4
	22	15	19	9	9	13	7	0	0
MEAN	21,3	13	13,8	16,3	11,5	8,7	3,8	0,2	1,9
SD	5,50	3,16	4,57	6,45	3,03	4,30	2,82	0,63	1,66

Tab. 3a. The numbers of *E. burchelli* which crossed the barrier in 35 s; the second control and substances which were compared with it are marked (*)

E. burchelli										
	control	**doxy-cycline**	**ascorbic acid**	**certizine**	**Neomycin, gramicidin**	**ascorbic acid, vitamin P**	**nitrog-licerine (*)**	**gastritis (*)**	**nicotine (*)**	**control (*)**
	12	6	4	11	10	9	17	3	13	50
	15	5	0	9	6	4	14	0	0	94
	26	7	3	3	7	3	7	5	0	75
	20	10	2	3	4	3	18	2	0	55
	9	7	3	5	11	2	20	0	1	85
	16	10	2	5	9	1	18	0	0	80
	20	5	2	4	8	0	13	0	6	72
	26	12	1	4	4	3	15	2	2	75
	30	5	3	2	6	2	25	0	5	70
	7	8	2	2	7	3	10	3	9	68
MEAN	18,1	7,5	2,2	4,8	7,2	3	15,7	1,5	3,6	72,4
SD	7,68	2,46	1,14	2,97	2,35	2,40	5,12	1,78	4,55	13,02

While working with *E. burchelli* two control readings were taken because it was observed that these army ants followed the same path only for a short period of time. Then, they abandoned their old path and set up a new one. Time which was demanded to work with these ants was too long to test all substances on the same path. Such a situation did not occur when testing *A. octospinosus* because it is really hard to make these ants to change their path.

According to the results of ANOVA, both the species and the chemical substance were significant factors ($p<0.0001$). Thus, the test showed that species differed in their reactions to the same chemical substances and that different chemicals caused different reactions (Fig. 1). All ants reacted negatively only to nitroderm ($p=0.2431$), gasitris ($p=0.15$) and nicotine ($p=0,20$). Doxycycline ($p=0.0063$), ascorbic acid ($p<0.0001$), certizine ($p=0.0312$), ascorbic acid and vitamin P ($p=0.0040$) evoked different reactions in the two species. The behaviour of *A. octospinosus* was affected to a lesser extent than of *E. burchelli*.

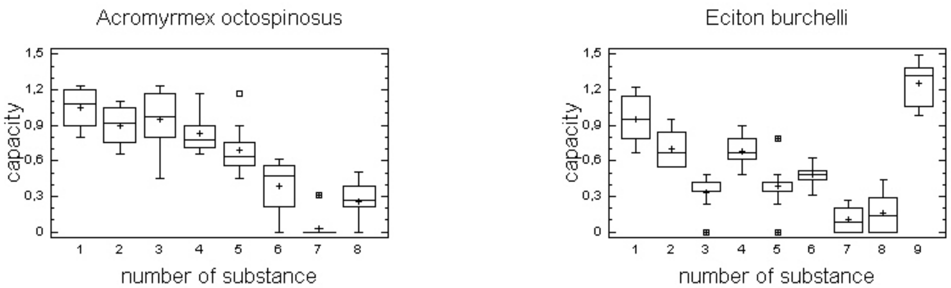


Fig. 1. Ants path capacity depending on chemical substances. Substances: 1. control, 2. doxycycline, 3. ascorbic acid, 4. certizine, 5. ascorbic acid and vitamin P, 6. nitroderm, 7. gasitris, 8. nicotine, 9. control (*) for nitroderm, gasitris and nicotine (only for *E. burchelli*)

The ascorbic acid (no matter whether combined with vitamin P or not) gave neutral reaction in *A. octospinosus* and negative one in *E. burchelli*. Ceritizine impacted negatively *E. burchelli*, and did not affect *A. octospinosus*. Doxycyline evoked neutral reaction in both species but the reaction of *E. burchelli* was more negative than neutral .

In all tested cases in the second part of the experiment, the presence of a chemical decreased the number of ants which crossed the barrier in 35 seconds (called "path capacity" thereafter). Path capacity differed between substances and species.

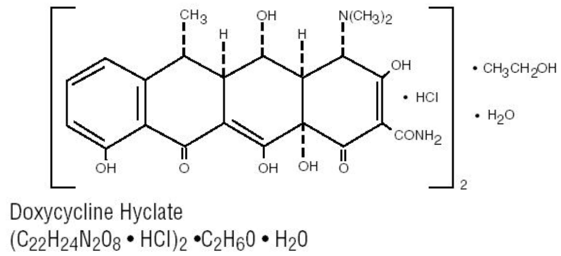
The differences observed in Experiement II between the species in their reaction to some chemical substances can be probably explained by differences in their general behaviour. First of all, *E. burchelli* are much more restless than *A. octospinosus*. Army ants change their paths frequently (it was observed that they change a path in a one hour period) and there is no problem for them to change their path a little when some barrier occurs. The reason for such a behaviour is the fact that *E. burchelli* are nomadic ants and they change their path each day anyway. They even do not build permanent nests. In contrast, the leafcutters have permanent nests and they follow the same paths for a very time. Two different leafcutters' nests were found near Rancho Grande field station: one, on which the experiment was performed, was the day-active one, and the other one, which was located nearby the educational path, was the night-active. The leafcutters hold their paths clean; the surroundings of the nest are totally clean with no plants around. For leafcutters it is really hard to change their path. They turn back or stop rather than trying to find a new path. Maybe, it is connected with the "luggage" their carry what requires well-maintained paths without any obstacles.

However, at the first instance the same behaviuor was observed in the two species when a barrier occured: the flow of ants stopped for a short time. Ants tried to investigate a new situation by moving their antenna and gathering nearby the barrier. Some of them went in the middle of the barrier and then turned back. Many times, it was observed that soldiers turned back and went away of the barrier while workers went through it. Finally, when a few first ants had crossed the barrier, the rest followed them slowly.

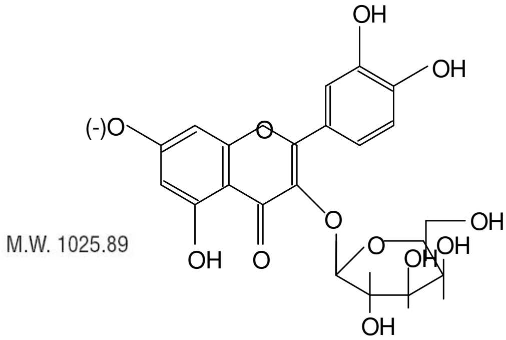
The most strange behaviuor of ants was observed during their contact with gastrolitis. This medicine consists of 30 mg NaCl, 75 mg KCl, 125 mg $NaHCO_3$, 1.625 g glucose and 25 mg extract from camomile (mainly salicid acid). Ants avoided gastrolitis strongly. This behaviour seems strange because the main chemical ingredient of gastrolitis is glucose, which is liked by ants. The reason for such a behaviour remains unknown and it should be tested separately for all ingredients of gastrolitis.

Summarizing, different substances used in the study influenced ants beahaviour to a different extent across the species, and some of them evoked different types of reaction on different species. Chemical structure of used substances as well as anatomic structure of ants' antenna may shed some light on the reasons for different reaction to different chemicals. The chemical structures of the substances used in Experiment II are shown below (Fig. 2).

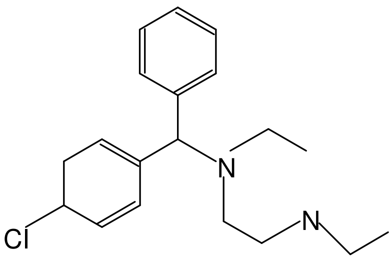
-doxycycline,



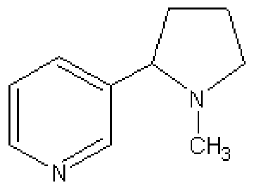
- vitamin P,



- certizine,



- nicotine,

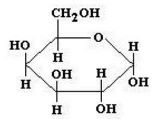


- gasitris,

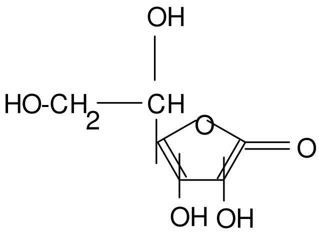
NaCl

KCl

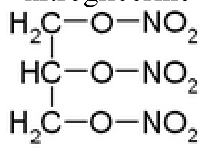
$NaHCO_3$



- ascorbic acid,



- nitroglicerine



- neomycin,

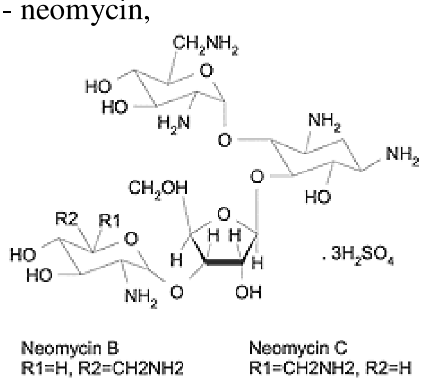


Fig. 2. Chemical structure of the substances used in Experiment II.

The sense of smell is really important in ants' life. It is used in communication between them as well as in recognizing topography [1]. My study shows that ants are able to recognize different chemical substances and react to them in a specific way. Many experiments with different chemicals were already done [1]. Unfortunately, as for now, the reasons for these different reactions remain unknown. Skaife [1] suggests that this may be the natural mechanism of the avoidance of natural toxins, such as nicotine. To find out which ingredients are responsible for which type reaction, further studied are necessary – this time with the use of specific groups of pure chemical compounds. For example, it might be tested whether ants are able to recognize specific reactive groups, such as OH^-, $COOH^-$, NO_2^-, NH_2^-, etc.

Acknowledgments

I am grateful to Miguel Riera for identifying ant species used in the study.

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Behavioural response of *Atta* ants to the different chemical compounds and the difference between *Atta* and *Ecitoninae*.

Paula Dobosz

Introduction

In 1820 a French biologist Geoffroy Saint-Hilairenoted, impressed by the devastation made by leaf-cutting ants, noted: "Either Brazil's killing the *Atta* or the *Atta* are killing Brazil!" [16]. In fact, in one night, leaf-cutting ants can completely strip an average-sized tree. The trails of leaf-cutting ants can cover great distances – even over 250 m long [16]. There was no significant difference in total leaf transport between the different environments. However the activity patterns differ between environments with a decrease in leaf transport during the hottest hours of the day in the open environment on sunny days [2]. Probably there may be a critical transportation speed also, and if the speed is not met, an individual will relinquish its leaf, although more specify investigations have to be performed [1].

Leaf cutting ants (known also as parasol ants) are among the most advanced of all the social insects [5]. *Attini* are native only to the New World and are found chiefly in Central and South America, although a few species occur in the southern parts of USA [8]. Leaf-cutters are an integral part of the ecosystem and are the dominant herbivores of the New World tropics. They consume far more vegetation than any other group of animals of comparable taxonomic diversity. Leaf-cutters prune the vegetation, which stimulates new plant growth. The broken down material decomposes and enriches the soil [3]. They are also one of the major deep excavators of soil and stimulators of root growth [5]. *Atta* has evolved to constantly change food plant, preventing a colony from completely stripping of leaves and thereby killing trees, thus avoiding negative biological feedback [9].

Ants have a special paired gland on the thorax, called the metapleural gland. Figure 1 shows the position of the metapleural gland on a major worker of *Atta cephalotes*. The metapleural gland has been shown to produce substances that can act as antibiotics. This gland is particularly well developed in the fungus-growing ants. Figure 2 shows a scanning electron micrograph of the right hand metapleural gland in *Acromyrmex octospinosus*. There is a large bulbous area of the cuticle (the bulla), which covers the main part of the gland. The substances produced by the gland flow out over the ant's surface through numerous pores. [9]

Similar research has shown that the gland produces at least 22 different chemical compounds, many of which are effective in killing a wide range of bacteria and fungi. [9]

The facts as given above inspirated the question about possible differences between *Atta* ants and other species in terms of recognising chemical compounds? Assuming that *Atta* ants recognise non-poisonous leaves, do they have the ability to distinguish the toxic and safe chemical compounds? Are they able to learn different kinds of compounds? To answer these question, we exposed *Atta* ants to a range of different chemical compounds and observed their behavioural responses.

Aims of research

Main aim of the research was to prove hipothetical differences in ants behavioural reaction to the variety of chemical substances, as well as to prove the different behavioural acting between herbivorous *Atta* ants and carnivorous *Ecitoninae* ants. Additionaly, the phenomena of habituation and learning were examinated either.

Materials and Methods

The research was performed as a field work, in neighbourhood of field station Rancho Grande, inside Henri Pittier National Park, located in northern part of Venezuela.

In this research three genera of ants were examinated: *Atta, Ecitoninae* and *Myrmicinae.* In *Atta*'s case, as a main subject of research, two nests were taken under observations; for *Ecitoninae* and *Myrmicinae* – one nest for each genus.

Classification of used animals: genus *Atta* belongs to the class *Insecta*, order *Hymenoptera*, family *Formicidae* [16]. There are more than 400 species of leaf-cutting ants in the world [16]. Ants in the tribe of *Attini* consist of about 190 species – most of them known as fungus growing ants or gardening ants [8]. The genus *Atta* is the pinnacle of *Attine* evolution with 15 species representing the premier leaf-cutting ants [8].

Commonly known as "leaf-cutter ants", *Atta* ants comprise one of the two genera of advanced attines within the tribe *Attini*, along with *Acromyrmex*. *Atta* is one of the most spectacular of the attines, and colonies can comprise in excess of one million individuals. *Atta* exhibits a high degree of polymorphism, five castes being present in established colonies. The high degree of polymorphism in this genus is considered a proof of high degree of social advancement. Every caste has a specific function, and some remarkably advanced phenomena have been observed in *Atta* species [9, 15]. The workers are strongly polymorphic and, relative to their size, they comprise four physical castes specialised for various roles: gardeners-nurses, within-nest generalists, foragers-excavators and defeders [6, 8].

Army ants in the Neotropical tribe *Ecitonini* are major predators of arthropods [7, 10]. Some conspicious and widespread army ant species, such as the tropical *Eciton burchelli*, have received extensive study, and their impact on arthropod communities have been relatively well documented [4, 12]. *Eciton burchelli* is a common swarm-raiding army ant whose prey includes many arachnids and other insects [11]. This species is most often seen because they form robust columns on the surface even during the day [11, 12]. Little is known about the impact of army ant predation on fungus-growing ants; only a few cases have been reported [4, 7, 13].

Examinated ants were exposed to 34 different chemical compounds, divided into 6 groups becouse of their chemical structure or character. The last chemical substance with number 34 was a clear water, taken to the examination as a control. Ants reaction to the water was taken as a neutral and compared with all other behavioural reactions. During all days of experiment, the same solution of all chemical compounds was kept. Detailed list of used chemical compounds is given in Table 1.

Furthermore, in this research a lot of laboratory equipment was used, such as laboratory glass, gauze, cotton-wool.

Main method used to collect data from the field work was an observation of ants behaviour and their responses to the chemical compounds. Possible ants behavioural responses are given in Table 3. For data analysis two statistic tests were used: ANOVA and Spearman's rank correlation, both with a computer programme named STATISTICA version 7.0.

Research was carried out within 6 days, but it was divided into two integral parts. In first one, durning one day, 3 different genera of ants were examinated: *Atta*, *Ecitoninae* and *Myrmicinae*. In the second part, durning all 6 days, several probes were made, but only with two different genera of ants: *Atta* and *Ecitoninae*. However in the first part of the experiment all 34 chemical substances were used, in the second part only a few of them. Substances recognized by ants as neutral in the first part of the research were rejected and finally in the second part only several of chemical compounds were used. What is more, substances from the second part of research were different to each genus – this suggests some differences in ability to recognise the chemical substances or different attitude to the chemical compounds known as "poisonous". Detailed list of chemical compounds used in this part are given in Table 2a and Table 2b.

Results and discussion

In the first part of this experiment ants from genera Atta*,* *Ecitoninae* and *Myrmicinae* were exposed to 34 different chemical compounds to check for any difference in their behavioural response among the genera; number 34 was a control with a shape of clear water.

According to the results of statistic tests, the biggest differences were seen between behavioural responses of *Atta* ants and *Ecitoninae* ants. This result goes with expectations, as those genera have totally different lifestyles and food habits. A little smaller, but still quite significant difference was seen between *Ecitoninae* and *Myrmincinae* – with similar reason to previous explanation. The smallest difference was seen between *Atta* and *Myrmicinae* ants; this suggests similar lifestyle or just similar chemical preferences of this two genera.

As a result, among three examinated genera, no difference was seen in response to the opathanol, budasonid, paracetamolum, furaginum, high solution of calcium and ice acetic acid.

Analysis of collected data from the second part of experiment allowed to see some distinct trends in ants behaviour. In most cases first reaction was totally different than this presented in next probes or days. This could indicates the ability of ants to learn, or maybe just an ability to transfer the information about chemical character of the substance between individuals. This trend was observed among the *Atta* ants, as well as among the *Ecitoninae* ants, but to the different substances.

Results for Atta

Among *Atta* ants, at the end of the experiment a distinct stabilisation of ant behavioural reactions was also observed. Even if at the beginning ant reaction was unclear (for instance, some individuals recognised one particular substance as positive, and others – as neutral), after several probes/days more and more ants decided to react in only one way; and finally, in last days of experiment, all examinated individuals manifests exactly the same behavioural response. This situation was observed while ants were given 90% ethanol - in this case in first probes of examinating some individuals of *Atta* recognised this substance as toxic (negative response), some as neutral, and others – as positive. But only after few hours (in next probes) their reaction begun to stabilisate, and finally, in next day of examination, all individuals recognised ethanol as positive; this final reaction was maintained until the end of research.

Nevertheless, all other chemical compounds gave totally different responses within *Atta* ants. In most cases, still the first reaction was different than this one performed in second or third day, and usually kept with no matter how many probes were made. The most frequent pattern was when the chemical substance at the beginning caused neutral response beneath ants, and than changed into negative – ants recognised this substance as a poisonous and did not change their reaction until the end of field work. Such situation was observed while used following chemical compounds: neomycin, naproxen, gastrolit, orofar, high solution of calcium and nicotine.

In some cases *Atta* ants recognised a particular substance as neutral in first probe, and in next few probes their response was chaotic and difficult to classify, but in the end (at least third day of examination) their response was clarified as a neutral again. This reaction was also maintained to the end of examination. This situation was observed in following substances: hydrocortisonum, diosmectit, nifuraxazidum, potassium chloride.

Still among *Atta*, in some cases quite opposite situation appears; at the beginning one substance was avoided, but only a few probes forward (next or third day) their attitude changed to neutral. This happened with acetic-salice acid, and can be an example of habituation phenomenon.

Only two chemical substances gave a clear, deeply negative reaction from the early beginning to the end of examination – high solution of vitamin C and ice acetic acid. *Atta* ants reaction was constantly the same all the time.

Some substances generated chaos in *Atta* ants response all the time of field work. Such responses were noticed in methanol and colchicine. This could be caused by a extremely poisonous character of this chemical compounds, killing people in a very small solutions either. Probably all individuals that ate this substances died very quickly; therefore they wasn't able to communicate with other individuals from their nest to transfer the warning information, even though they felt how dangerous this substance could be. To clarify this hypothesis, more specific research must be taken.

Results for Ecitoninae

Ecitoninae ants did not response in such a great diversity as *Atta* ants. Most of the chemical compounds were negative for them (only reaction for doxicyclinum and nitroglycerin were difficult to describe) at the beginning, than changed into neutral (most cases: gastrolit, high solution of Saccharomyces boulardii, ethanol, natrium chloratum, methanol, colchicine and potasium chloride) or positive (ice acetic acid); than the reaction could be maintained (gastrolit, high solution of Saccharomyces boulardii, ethanol, natrium chloratum) or could be changed to the first attitude and than kept (diosmectit and ice acetic acid).

Very interesting response was observed in *Ecitoninae* ants attitude to naproxen. At the beginning ants recognised this substance as negative, but during the second day this reaction was changed to very difficult to describe – and this response was kept until the end of examination. In could mean that ants do not know this substance in their natural environment and simply don't have any particular pattern of reaction; examination time could be insufficient to observe the real attitude of ants to this chemical compound, and therefore more investigations are required.

Only one substance was very negative to the *Ecitoninae* ants during the time of examination, and this response did not change, neither the ants hesitated in their reaction; this was a salicid acid. Should this response indicates that this particular substance appears in natural environment of ants, so they can recognise it as well known – therefore, the act as they avoid salicid acid.

Summary

According to the information given above, there was a significant difference between *Atta* ants and *Ecitoninae* ants in their attitude to the chemical compounds. They recognise different substances as positive and negative – this may be caused by different lifestyle of this genera. Also, could indicates the possibility of transferring information between individuals – perhaps via chemical way, such as pheromones. Moreover *Atta* ants prove high diversity of reactions in compare of *Ecitoninae*. This fact could means that this ants are more sensitive to the chemical substances; it could be a very useful trait, evolutionary created, helpful with recognising different species of trees (to differ toxic from eatable ones; for leaf-cutting ants) or different species of fungus (for fungus-growing ants). To decide what is the reason for such results, more detailed research must be taken.

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Figures and Tables

Figure 1. The position of the metapleural gland on a major worker of *Atta cephalotes*. (Source: www.zi.ku.dk/personal/drnash/atta)

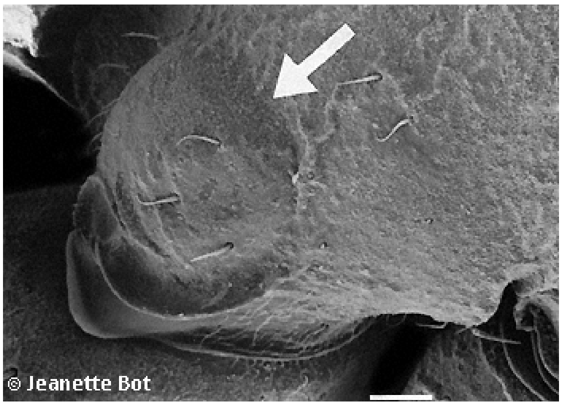

Figure 2. Scanning electron micrograph of the right hand metapleural gland in *Acromyrmex octospinosus*. (Source: www.zi.ku.dk/personal/drnash/atta)

Table 1. List of chemical compounds used in all parts of the research.

Chemical group	Name of substance
Antibiotics	Tribiotic (neomycin sulphate + polimyksinB sulphate + zincus bactracyne) Doxycyclinum Opatanol Dicortinef (neomycin + gramicidin + fludrocortizonum acetic) Neomycin
Anti-histaminates	Budasonid Cetirizini dichloride Hydrocortisonum
Anti-pyrogens	Acetic-salice acid Chloroquini phosphas Paracetamolum Naproxen
Gastro-intestinal medicines	Diosmectit Furaginum Nifuraxazidum Gastrolit (NaCl + KCl + Na_2CO_3 + glucosum + Siccus chamomilae estr.)
Other medicines	Xylometasolini hydrochloridum Trilac (Lactobacillus acidophilus + Lactobacillus delbrueckii + Bifidobacterium bifidum) Orofar (benzoxoni chloridum + lidocaini hydrochloridum) Nitroglycerin Sacchcaromyces boulardii
Other chemicals	Salice acid Ethanol High solution of calcium Vitamin C Carbon Isopropanol Nicotine Ice acetic acid Methanol Colchicine Potassium chloride Natrium chloratum Water

Table 2a. List of chemical compounds used in second part of the research – for *Atta* ants.

Chemical group	**Name of substance**
Antibiotics	Neomycin
Anti-histaminates	Hydrocortisonum
Anti-pyrogens	Acetic-salice acid Naproxen
Gastro-intestinal medicines	Diosmectit Nifuraxazidum
Other medicines	Orofar (benzoxoni chloridum + lidocaini hydrochloridum)
Other chemicals	Ethanol High solution of calcium Vitamin C Nicotine Ice acetic acid Methanol Colchicine Potassium chloride

Table 2b. List of chemical compounds used in second part of the research – for *Ecitoninae* ants.

Chemical group	**Name of substance**
Antibiotics	Doxycyclinum
Anti-pyrogens	Naproxen
Gastro-intestinal medicines	Diosmectit Gastrolit (NaCl + KCl + Na_2CO_3 + glucosum + Siccus chamomilae estr.)
Other medicines	Nitroglycerin Sacchcaromyces boulardii
Other chemicals	Salice acid Ethanol Ice acetic acid Methanol Colchicine Potassium chloride Natrium chloratum

Table 3. Discription of the possible ants behavioural responses.

Reaction	**Code**	**Observed behaviour**
Positive	1	If ants definitely likes the substance
Negative	2	If ants definitely escapes from the substance
Neutral	3	If the presence or absence of the substance makes no difference to the ants