

Foraging behaviour and territoriality of the strawberry poison frog (*Oophaga pumilio*) in dependence of the presence of ants

Konrad Staudt^{1,*}, Santiago Meneses Ospina², Dietrich Mebs³, Heike Pröhl¹

Abstract. The present study investigates foraging and territorial behaviour of the strawberry poison frog (*Oophaga pumilio*) in dependence of the presence of formicine and myrmicine ants, which constitute the main food source of the frogs. Species of the formicine ant genera *Brachymyrmex* and *Paratrechina* contain highly toxic alkaloids (pumiliotoxins), which the frogs incorporate and accumulate in their skin what may serve for predator deterrence. Twelve male frogs of two populations (primary and secondary forest) in Hitoy Cerere, Costa Rica, were observed each for a full day. Calling time, feeding attempts and time spent inside and outside the core area of their territories were recorded. Furthermore, twelve males of both populations were observed during the main foraging time to determine, whether the frogs search for prey in specific patches of their territories. The ants inside the core areas of twenty four frog territories were collected and classified to genus. Ants of the genera *Brachymyrmex* and *Paratrechina* were classified to species or morphospecies, respectively. The presence of formicine and myrmicine ants in territorial areas was compared to non-territorial sites. We found that formicine ants (*Brachymyrmex* and *Paratrechina*) were more present inside the territorial core areas than outside. The higher presence of these ants in the core areas was associated with longer foraging times. We verified that toxic alkaloids of the pumiliotoxin group are present in the dendrobatid frogs of Hitoy Cerere. The results of this study suggest that toxic diet may be linked to territoriality in this frog species.

Keywords: alkaloids, *Brachymyrmex*, *Dendrobates auratus*, foraging behaviour, histrionicotoxin, *Oophaga pumilio*, pumiliotoxin, territorial behaviour

Introduction

Territorial behaviour in animals is exerted, when benefits of defending an area outweigh the costs in fitness (Carpenter and McMillen, 1976). Fitness gain can be achieved through access to limited resources like shelter, breeding sites to raise offspring, the availability of mates or the abundance of food (reviewed by Davies, 1978; Pröhl, 2005a). Territoriality is widespread among all groups of vertebrates, and also reported for males and females in dendrobatid frogs (Wells, 2007). Males of the Neotropical strawberry poison frog (*Oophaga pumilio*) show extensive territorial behaviour, and are stenoecious to their locations from months to years (Bunnel, 1973;

Pröhl, 1997; Pröhl and Hödl, 1999). The core area of their territory (1-4 m²) is defended through calling and physical combat (Pröhl, 1997; Pröhl and Berke, 2001). The reasons for their territorial behaviour are not entirely understood. Shelter as well as prey is abundant in the rainforest, and defence of oviposition sites appears to be unlikely (Pröhl, 2005a). While tadpole-rearing sites seem to be a limited resource for females, males may initially establish their core area where female density is high (Pröhl and Berke, 2001). However, the core area might fulfil other purposes than just advertising for females, such as defending a high quality food source.

Toxic skin alkaloids seem to provide protection from potential predators of these aposematically coloured frogs (Brodie and Tumbarello, 1978; Fritz, Rand and De Pamphilis, 1981; Szelistowski, 1985; Daly, Myers and Whittaker, 1987; Daly, Spande and Garaffo, 2005). However, there are predators such as spiders (e.g., *Sericopelma rubronites*) and birds like the rufous motmot (*Baryphthengus mar-*

1 - Institute of Zoology, University of Veterinary Medicine, Bünteweg 17d, 30559 Hannover, Germany

2 - Departamento de Artrópodos, Instituto Nacional de Biodiversidad, 22-3100 Santo Domingo de Heredia, Costa Rica

3 - Center of Legal Medicine, University of Frankfurt, Kennedyallee 104, 60596 Frankfurt, Germany

* Corresponding author; e-mail: staudt@bio2.rwth-aachen.de

tii); both of which have been observed hunting the green-and-black poison frog (*Dendrobates auratus*) (Master, 1998; Summers, 1999). Furthermore, the Amazon ground snake (*Liophis epinephelus*) was observed preying on the most toxic poison frog, the golden poison frog (*Phyllobates terribilis*) (Myers, Daly and Malkin, 1978). Since most predators develop resistance but rarely complete immunity towards toxicity (i.e., Becky, Brodie and Brodie, 2002), it is likely that the more toxins the frogs acquire the less is the predation rate. Frogs obtain alkaloids from their food (Daly et al., 1994; Myers et al., 1995), which consists of small arthropods, mainly ants and mites (Donnelly, 1991). Recent studies showed that oribatid mites are the source of several alkaloids (pumiliotoxins, indolizidines and others; Takada et al., 2005; Saporito et al., 2007a; Saporito et al., 2007b), but a variety of alkaloids like those of the structural classes pyrrolizidines, pumiliotoxins, decahydroquinolines and others were found in ants (Saporito et al., 2007b). While myrmicine ants are suspected to contain histri- onicotoxins (HTX; Saporito et al., 2004; Saporito et al., 2007b), formicine ant species of the genera *Brachymyrmex* and *Paratrechina* have been found (next to oribatid mites; Takada et al., 2005) to contain pumiliotoxins (PTX 307A, PTX 323A; Saporito et al., 2004), which are highly toxic in comparison to histri- onicotoxins (Daly and Myers, 1968; Daly et al., 1978).

It is known (e.g., in anemone fishes of the genus *Amphipiron*) that a territory can be established due to the availability of toxins for protection (i.e., Elliott et al., 1995), but there are no reports that animals defend toxic diets. The type and quantity of alkaloids might be crucial for the survival of the strawberry poison frog and some areas may provide ants that contain valuable alkaloids. Ants are known to exhibit a mosaic distribution pattern in the leaf litter and arboreal habitats (Wilson, 1959; Leston, 1978), i.e., some ant species are absent in one, but present in another area of the forest and therefore, the occurrence of alkaloid containing ants

at a specific location may give a reason to establish the territorial core area in this frog species.

In the present study we investigated the foraging behaviour of this frog in dependence of the territory extension and distribution of ants. Ants inside and outside of the territories of the frogs were collected and classified. The presence of (1) all ants, (2) probably "less toxic" myrmicine ants, and (3) possibly "highly toxic" formicine ant genera *Brachymyrmex* and *Paratrechina* in territorial core areas in comparison to non-territorial sites was determined to find out whether the presence of ants in general or of a certain ant group containing alkaloids is a reason for territorial defence. Furthermore, a preliminary qualitative analysis of alkaloid toxins secreted by dendrobatid frogs of the same study area was performed.

Material and methods

Field observations were conducted at the beginning of the rainy season from April to July 2008 on two populations of *Oophaga pumilio* at the Biological Station Hitoy Cerere, Costa Rica (9°40'N, 83°05'W). The study areas of these two populations differed in their successional stage: (1) an old secondary forest with transition to primary forest, and (2) an old banana plantation with transition to a young secondary forest, referred to further as primary and secondary forest.

Whole day observations

Twelve frogs (six males in the primary and six males in the secondary forest) were observed for twelve hours from 6:00 a.m. to 6:00 p.m. Each frog was observed continuously and with a break of 10 minutes each hour. The distance to the frogs was at least 2 m, so that the frogs were not disturbed by the presence of the observer. Core areas of the territories were determined by observing the frogs' locations three days before the observation started between 09:00 a.m.-12:00 noon on every day. Sites where a frog called without a female in the vicinity were marked. Areas with most markings and where the same frogs were found each day were marked with nylon cords to 4 m², male core area sizes ranged from 2.7 m² (mean, primary forest) to 3.2 m² (mean, secondary forest) (Pröhl and Berke, 2001) and were considered as core areas. For each frog (1) the calling time (focal animal sampling) and (2) the time spent inside and outside of the restricted core area was measured in minutes per hour with a stop watch, and (3) the number of feeding attempts (the small prey was not visible by eye, so it could not be assessed whether the attempts were successful or not) per hour was recorded.

Observations during the main foraging time

To find out whether frogs preferred certain areas for foraging, the 4 m² core areas of twelve additional males (six in each habitat) were divided with marks and cords into 100 20 × 20 cm² grids. Each male was observed for five minutes and the number of feeding attempts in each grid was recorded. Afterwards, the next frog was observed. During the three hours of the main foraging time between 09:00 a.m. and 12:00 noon (see result section), each frog was observed for 30 minutes each day. This procedure was performed for 24 days (12 days in each habitat) and every day the order of observed males was switched.

Ant collection and classification

Ants were collected inside the 4 m² core areas of all 24 frogs. This was conducted by systematically placing 25 baits consisting of an equal amount of honey, tuna and bread into the core area of the territories. Furthermore, twelve non-territorial areas (six in each habitat) were selected for ant collection. These areas were located between the frog territories (distance <10 m) and were similar in habitat structure to the frog core areas. These areas were observed for three days before ant collection to guarantee the absence of frogs and 25 baits were placed into each 4 m² non-territorial area. In total, 25 × 36 = 900 baits were distributed.

Five ants from each bait station were collected by means of an insect aspirator and stored in 70% ethanol. Generally, only one ant species visited each bait station and prevented the access of other species. In cases where more ant species were found at a bait station, only the most abundant species was collected. The chance to collect most of the ant genera present in a certain area was optimised by the high number of bait stations. A total of 3075 ants were collected from 615 bait stations. This procedure enables a comparison of the species present, but it does not allow the determination of the exact abundance of ants inside and outside the frogs' core areas. All ants were classified to the genus using the taxonomic keys of Bolton (1994) and Hölldobler and Wilson (1990). Formicine ant species of the genera *Brachymyrmex* and *Paratrechina* were identified by comparing the specimens with those from the Instituto Nacional de Biodiversidad (henceforth INBio) ant collection (previously established by Dr. J.T. Longino).

Chemical analysis

Alkaloid samples of twelve territorial males and six females of *Oophaga pumilio* and six *Dendrobates auratus* specimens were collected by rubbing with a dry cotton swab over the frog's back and placing the swabs in 80% methanol. This technique was chosen to not harm or kill the frogs. The samples were analysed by gas chromatography combined with mass spectroscopy (GC/MS) as described by Mebs et al. (2008).

Data analysis

Data were analysed by using Microsoft Excel 2002 and R 2.7.1. Foraging behaviour data were tested for parametry and variance of homogeneity with the Shapiro-Wilk test and *F*-test, respectively. Since the *P*-values of all data were <0.05, Student-*t* tests were applied to compare populations with respect to the a) calling time of males, b) number of feeding attempts, c) foraging area of the core area, d) presence of ant genera/species inside and outside the core areas.

A paired Student-*t* test was performed to compare data within one population to estimate whether significant differences exist in a) time spent inside and outside the core areas and b) feeding attempts inside and outside the core areas. The sample size (*n*) given in the results section refers to the number of territories examined. In the paired *t*-test, six territories were compared including at least twelve data points (six for each site).

The connectivity of grids where frogs were observed feeding in the main foraging time was analysed with "Joins Count Statistics" (JCS) (Lee and Wong, 2001), which tests whether the spatial connection of grids differed from a random spatial distribution. The test counts the number of connected grids in a binary system; in this case a "foraging grid" is marked black and a "non-foraging grid" is marked white. The results indicate whether or not frogs favour special areas (patches) in their territories for prey hunting. If they search in patches, there should be more connections between black-black grids and fewer connections between black-white grids than in a random distribution. Data were evaluated using MS Excel with the macro "rookcase.xla" (<http://www.lpc.uottawa.ca/data/scripts/index.html>).

To determine whether there is a significant difference in the number of bait stations with *Brachymyrmex*/*Paratrechina* and other ant genera in territorial and non-territorial areas of both habitats (primary and secondary forest), a Fisher's exact test for count data was applied.

Results

Whole day observations

The main calling time of the frogs took place in the morning between 6:00 a.m. and 10:00 a.m. Thereafter, their foraging activity increased with a distinct peak between 10:00 a.m. and 11:00 a.m. in the primary forest and 10:00 a.m. and 12:00 noon in the secondary forest. They remained mainly inside their territories during this period (fig. 1).

Frogs of the primary forest called significantly less than frogs of the secondary forest (two tailed *t*-test for independent data: *P* <

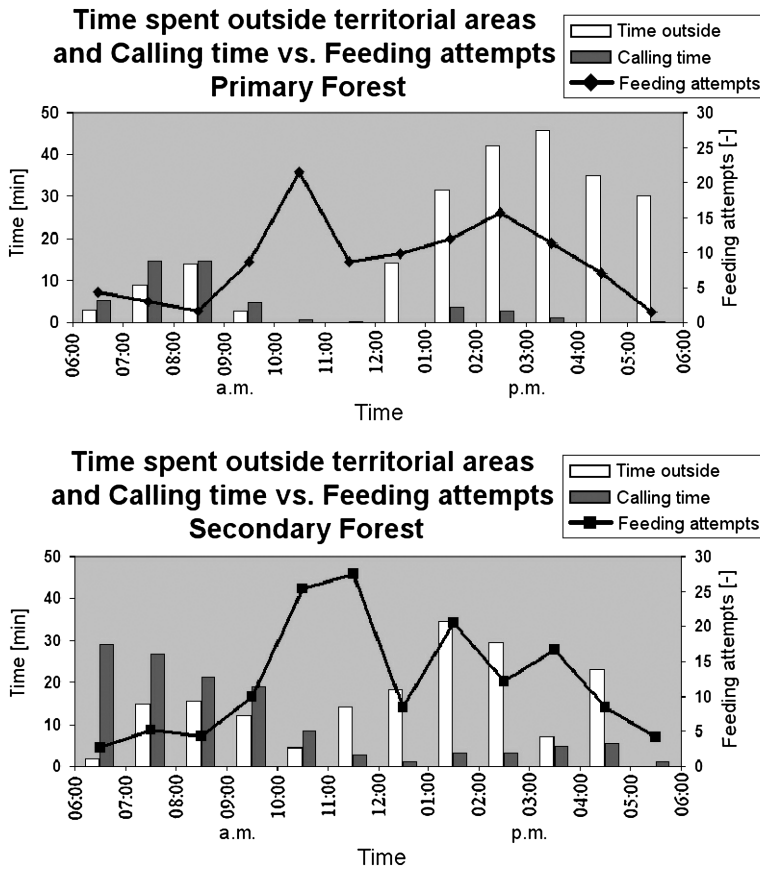


Figure 1. Time spent outside territorial areas and calling time vs. feeding attempts of frogs of the populations in the primary forest ($n = 6$) and secondary forest ($n = 6$). The white bars show the time outside the restricted areas in minutes per hour, the grey bars the calling time in minutes per hour and the line the amount of feeding attempts per hour.

0.01; $n = 12$). On average, frogs of the primary forest called for 48.33 ± 27.65 minutes (mean \pm SD, $n = 6$) and frogs of the secondary forest called 126.33 ± 52.77 minutes ($n = 6$) daily.

Frogs of the primary forest performed significantly less feeding attempts than frogs of the secondary forest (two tailed t -test for independent data: $P < 0.05$; $n = 12$). On average, frogs of the primary forest performed 105.17 ± 31.78 feeding attempts, those of the secondary forest 145.50 ± 28.36 feeding attempts per day.

Frogs of both populations remained mostly inside their core areas before noon, and left them for the purpose of clutch moistening ($n = 3$ in the secondary forest, $n = 4$ in the primary forest) or territory combat ($n = 1$ in the primary

forest) in the afternoon. The frogs left the core area quickly and directly (e.g., clutch) and foraged only on their way back to the core areas.

Frogs of both populations spent significantly more time inside their core area than outside (two tailed, paired t -test, primary forest: $P < 0.001$; $n = 6$; secondary forest: $P < 0.01$; $n = 6$). Within 720 minutes observation time, frogs of the primary forest spent on average 493 ± 45.52 minutes and those of the secondary forest 567.5 ± 97.32 minutes inside their core areas (fig. 2). The difference in the time spent in- and outside the core areas between both populations was not significant (two tailed t -test for independent data: $P = 0.12$; $n = 12$).

Frogs of both populations performed significantly more feeding attempts inside their 4 m^2

core area than outside (two tailed, paired *t*-test, primary forest: $P < 0.05$; $n = 6$; secondary forest: $P < 0.001$; $n = 6$). On av-

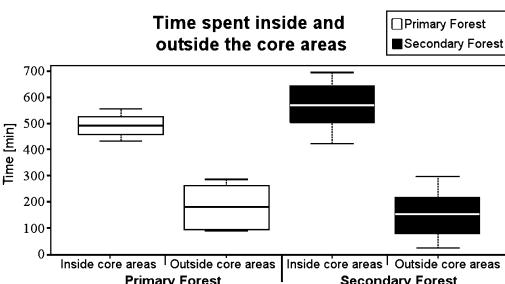


Figure 2. Average time spent inside and outside the core areas of frogs of the populations in the primary forest ($n = 6$) and secondary forest ($n = 6$) within 720 minutes observation time.

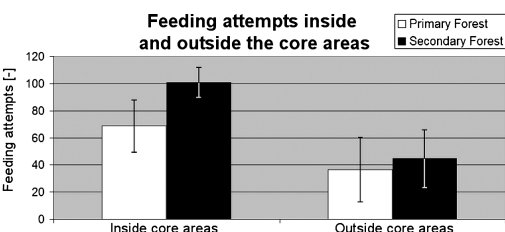


Figure 3. Average number of feeding attempts by frogs per day of both populations (primary forest ($n = 6$) and secondary forest ($n = 6$)) inside and outside the core area.

Table 1. Joins count statistics of twelve core areas (observed were each six frogs of the primary (PF) and secondary forest (SF)). Each 4 m² core area was divided in 100 20 × 20 cm² grids. *B* (black) = grid in which was hunted. *W* (white) = grid in which was not hunted. J_{BB} = number of black-black joins. J_{BW} = number of black-white joins. The difference between observed (obs.) and by a random distribution expected (exp.) values gives a *Z*-value. A confidence table (Lee and Wong, 2001) assigns a *P*-value to each *Z*-value.

	Primary forest							
	J_{BB} obs.	J_{BB} exp.	<i>Z</i> - value	Sign. (<i>P</i>)	J_{BW} obs.	J_{BW} exp.	<i>Z</i> - value	Sign. (<i>P</i>)
Frog 1 PF	34	11.81	7.49	<0.01	78	106.33	-4.20	<0.01
Frog 2 PF	56	17.48	8.20	<0.01	66	122.36	-6.57	<0.01
Frog 3 PF	37	26.12	7.30	<0.01	133	139.29	-3.74	<0.01
Frog 4 PF	51	22.45	7.37	<0.01	94	132.93	-5.07	<0.01
Frog 5 PF	43	17.48	7.30	<0.01	95	122.36	-3.74	<0.01
Frog 6 PF	57	24.25	8.20	<0.01	85	136.18	-6.57	<0.01
	Secondary forest							
	J_{BB} obs.	J_{BB} exp.	<i>Z</i> - value	Sign. (<i>P</i>)	J_{BW} obs.	J_{BW} exp.	<i>Z</i> - value	Sign. (<i>P</i>)
Frog 1 SF	59	26.12	8.00	<0.01	92	139.29	-5.99	<0.01
Frog 2 SF	51	32.13	4.24	<0.01	134	147.79	-1.69	ns
Frog 3 SF	64	26.12	9.22	<0.01	90	139.29	-6.25	<0.01
Frog 4 SF	53	22.45	7.89	<0.01	80	132.93	-6.89	<0.01
Frog 5 SF	40	13.13	8.67	<0.01	69	110.55	-6.02	<0.01
Frog 6 SF	49	28.05	4.96	<0.01	122	142.26	-2.54	<0.05

erage, frogs of the primary forest performed daily 68.67 ± 19.35 feeding attempts inside and 36.5 ± 23.92 feeding attempts outside their core areas. Frogs of the secondary forest performed on average 100.83 ± 11.21 feeding attempts inside and 44.67 ± 21.58 outside their core areas (fig. 3).

Observations during the main foraging time

Frogs of the primary forest used on average 1.11 ± 0.14 m² ($n = 6$) inside their core area, frogs of the secondary forest 0.95 ± 0.13 m² ($n = 6$) for foraging. The difference of the area between both populations was close to significance (two tailed *t*-test for independent data: $P = 0.066$; $n = 12$).

The joint count statistic (JCS), applied with the queens adjacency (also diagonal connected grids count), showed a highly significant spatial connectivity. The number of connected “foraging grids”, J_{BB} was highly significant for all territories observed ($P < 0.01$; table 1). The number of connections between the “foraging grids” and “non-foraging grids”, J_{BW} was for eleven territories less than randomly expected (table 1), which also implies that there are more

connected black-black joins than expected by a random distribution. These results indicate that frogs of both populations preferred hunting prey in specific patches of their core areas.

Ant collection and classification

Table 2 summarises the ant genera and species/morphospecies collected. We differentiated between myrmicine ant genera, which are suspected to contain histrionicotoxins, and formicine ant species of the genera *Brachymyrmex* and *Paratrechina*, which may contain pumiliotoxins. All other subfamilies (respectively the formicine ant genus *Camponotus*) were only occasionally found.

There was no significant difference in the total presence of ants between primary and secondary forest (two tailed *t*-test for independent

data: $P = 0.21$; $n = 36$). Of the twenty five baits distributed per 4 m², ants were found on average in 15.72 ± 6.22 bait stations in the primary forest and in 18.44 ± 6.62 bait stations in the secondary forest.

The difference in the total presence of ants between territorial and non-territorial areas in each habitat was not significant (two tailed *t*-test for independent data, primary forest: $P = 0.470$; secondary forest: $P = 0.086$; each habitat: $n = 12$ territorial and $n = 6$ non-territorial areas; table 2), neither was the difference in the presence of myrmicine ants between territorial and non-territorial areas (two tailed *t*-test for independent data, primary forest: $P = 0.773$; secondary forest: $P = 0.150$; each habitat: $n = 12$ territorial and $n = 6$ non-territorial areas; table 2), whereas the difference in the presence of formicine ants of the genera *Brachymyrmex*

Table 2. Summary of bait stations with specific ant genera/species from territorial (each habitat: $n = 12$) and non-territorial areas (each habitat: $n = 6$) in the primary and secondary forest. The average of each type of ant in territorial areas was compared with the average in non-territorial areas of both habitats by means of a *t*-test for independent data.

Ant genera/species	Primary forest		Secondary forest	
	Territorial (<i>n</i> = 12)	Non-territorial (<i>n</i> = 6)	Territorial (<i>n</i> = 12)	Non-territorial (<i>n</i> = 6)
<i>Brachymyrmex/Paratrechina</i>				
<i>Brachymyrmex longicornis</i>	6	0	0	0
<i>Brachymyrmex JTL-004</i>	4	1	0	0
<i>Brachymyrmex JTL-007</i>	6	1	1	0
<i>Brachymyrmex spp.</i>	0	0	2	0
<i>Paratrechina steinheili</i>	5	1	7	0
<i>Paratrechina caeciliae</i>	1	0	0	0
<i>Paratrechina JTL-006</i>	0	0	4	0
Mean \pm SD per area (<i>n</i>)	1.83 \pm 1.27	0.50 \pm 0.55	1.17 \pm 0.94	0.00 \pm 0.00
Significance (<i>P</i>)	<0.05		<0.01	
Myrmicinae				
<i>Solenopsis spp.</i>	73	47	186	76
<i>Pheidole spp.</i>	79	33	38	7
<i>Crematogaster spp.</i>	19	0	2	0
<i>Cyphomyrmex spp.</i>	3	1	3	0
<i>Pyramica spp.</i>	1	1	0	0
<i>Sericomyrmex spp.</i>	1	1	0	0
Mean \pm SD per area (<i>n</i>)	14.67 \pm 5.47	13.67 \pm 7.61	19.08 \pm 4.54	13.83 \pm 9.50
Significance (<i>P</i>)	0.773		0.150	
Other genera				
<i>Camponotus spp.</i>	0	0	0	2
<i>Pseudomyrmex spp.</i>	0	0	1	1
<i>Azteca spp.</i>	0	0	0	2
Total mean \pm SD per area (<i>n</i>)	16.50 \pm 5.49	14.17 \pm 7.81	20.33 \pm 4.48	14.67 \pm 8.89
Significance (<i>P</i>)	0.470		0.086	

and *Paratrechina* between territorial and non-territorial areas was significant (two tailed *t*-test for independent data, primary forest: $P < 0.05$; secondary forest: $P < 0.01$; each habitat $n = 12$ territorial and $n = 6$ non-territorial areas; table 2). Most of the formicine ants that were collected in territorial areas belong to the species *Brachymyrmex longicornis* and *Paratrechina steinheili*, in which pumiliotoxins were detected (Saporito et al., 2004).

Significantly more ants of the genera *Brachymyrmex* and *Paratrechina* were found in bait stations in territorial areas than in non-territorial areas of both habitats in comparison to all other genera (two-tailed Fisher's exact test for count data, primary forest: $P < 0.05$; secondary forest: $P < 0.05$; each habitat: $n = 12$ territorial and $n = 6$ non-territorial areas; table 3).

Ants of the genera *Brachymyrmex* and/or *Paratrechina* were found in ten of twelve territories and in three of six non-territorial areas of the primary forest. In territorial areas *Brachymyrmex longicornis*, *B. JTL-007* Longino ms, *B. JTL-004* Longino ms, *Paratrechina steinheili* and *P. caeciliae* were recorded, whereas in non-territorial areas *Brachymyrmex JTL-007* Longino ms, *B. JTL-004* Longino ms and *Paratrechina steinheili* were found. In the secondary forest, these genera were identified in eight of twelve core areas, but none of these genera occurred in non-territorial areas. In the territorial areas, *Brachymyrmex JTL-007* Longino ms, *Paratrechina steinheili*, *P. JTL-006* Longino ms and

two non-identifiable species of *Brachymyrmex* (*B. spp.*) were collected (summarized in tables 2 and 3).

Chemical analysis

Alkaloids were detected in skin swabs from seven frogs, in four specimens of *Oophaga pumilio* and in three specimens of *Dendrobates auratus*. Both dendrobatid species exhibited a similar alkaloid profile. One *O. pumilio* and two *D. auratus* contained allopumiliotoxin 323B in their skin swabs. The majority of alkaloids identified belong to the histrionicotoxin group (table 4).

Discussion

The population of *Oophaga pumilio* in the primary forest called less and conducted less feeding attempts than frogs in the secondary forest. Frogs of both populations spent more time inside their core areas and hunted predominantly there for prey. They also stayed mainly inside their core areas during the main foraging time and after the main calling time in the morning. The frogs preferred a specific patch for searching for prey inside the restricted area. Inside core areas there were significantly more ants of the possibly pumiliotoxins containing genera *Brachymyrmex* and *Paratrechina* present than outside.

Table 3. Summary of bait stations with ants of each twelve territorial and six non-territorial areas in the primary and secondary forest. Ant genera were divided into possibly pumiliotoxin containing ants (*Brachymyrmex* and *Paratrechina*) and probably not pumiliotoxin containing ants (all other genera). A Fisher's exact test was performed to determine whether there is a significant difference in the presence of ants of the genera *Brachymyrmex/Paratrechina* in territorial and non-territorial areas in comparison to all other ant genera of each habitat.

	Primary forest			Secondary forest		
	<i>Brachymyrmex/Paratrechina</i>	Other genera	<i>n</i>	<i>Brachymyrmex/Paratrechina</i>	Other genera	<i>n</i>
Territorial areas	22	176	12	14	230	12
Non-territorial areas	3	82	06	0	88	06
P	<0.05			<0.05		

Table 4. Alkaloids detected in dendrobatid frogs from Hitoy Cerere, Costa Rica. Allopumiliotoxin **323B**, Isodihydrohistrionicotoin **285A**, Isotetrahydrohistrionicotoin **287A**, Allotetrahydrohistrionicotoin **287D**, Octahydrohistrionicotoin **291A**, Histrionicotoin **283A**, Allodihydrohistrionicotoin **285C**.

Sample	323B	285A	287A	287D	291A	283A	285C
<i>O. pumilio</i> (Female, primary forest)		●			●		●
<i>O. pumilio</i> (Male, primary forest)		●	●				
<i>O. pumilio</i> (Male, primary forest)		●				●	●
<i>O. pumilio</i> (Male, primary forest)	●	●	●			●	●
<i>D. auratus</i> (Secondary forest)	●	●	●		●	●	
<i>D. auratus</i> (Secondary forest)	●	●	●	●	●		
<i>D. auratus</i> (Primary forest)		●		●		●	●

Comparison between primary and secondary forest

Earlier studies conducted with the same populations showed that the sex ratio of the population in the secondary forest is more female biased (Pröhl, 2002) and that frogs are larger and heavier than frogs of the population in the primary forest (Pröhl and Berke, 2001). Our data indicate that males called more often in the secondary than in the primary forest. The reason for the higher calling activity might be that males encounter more females which in turn motivate them to call more. Moreover, we also observed that more feeding attempts were performed in this habitat. A higher abundance of prey in the secondary forest may account for differences in size and weight and for maintaining higher calling activities.

Territoriality

Males of both habitats spent most of their main calling time (between 6:00 a.m. and 10 a.m., see fig. 1) inside their core areas and left them only in order to follow females or territorial combat. Males foraged less during the main calling time in the morning, when females approached males for mating. The decreased foraging activity during the time of high reproductive activities in the early morning suggests that the

territory is mainly established for the purpose of mating. This is in accordance with the findings by Pröhl and Berke (2001), which indicated that males compete for areas with a high female density. However, males performed most feeding attempts inside the core areas and also remained there during the main foraging time in the late morning (fig. 1). It seems that males, besides compelling rivals, remain also inside their territories to obtain valuable diet when there are no potential mates approaching. In this context, it is interesting to note that females also defend small areas of their home ranges, probably because of the presence of food sources (Meuche, Linsenmair and Pröhl, in press).

The results of the Joins Count Statistic show, that only a connected, small part of the territories in both habitats was used for hunting prey. Calling sites were almost always located in the vicinity to these locations. In general, more ants were found to be present in territorial than in non-territorial areas. Although no significant difference in the total presence of ants was recorded, this result confirms the findings by Donnelly (1991) that there are more ants inside a frog's territory than outside. Formicine ants of the possibly pumiliotoxin containing genera *Brachymyrmex* and *Paratrechina* are significantly more present at territorial than at non-territorial sites. These observations suggest that

calling as well as feeding sites are defended, which provide mates as well as high value nutrition to male frogs.

Toxic nutrition as limited resource

Previous chemical analyses revealed that frogs in populations of South Costa Rica, close to the present study area, generally contain major amounts of histrionicotoxins (HTX) (Saporito et al., 2007b). Our chemical analyses revealed that most of the alkaloids detected belong to this structural class. Myrmicine ants are suspected to be the dietary source for histrionicotoxins (Saporito et al., 2004; Saporito et al., 2007b) and most of the collected ant genera belong to this subfamily (table 2). While there were more myrmicine ants present in territorial than in non-territorial areas (especially in the secondary forest), no significant difference in the presence of any myrmicine ant genera was found. Previous studies indicate that quinolizidines, decahydroquinolines, pyrrolidines, piperidines, 3,5-pyrrolizidines and 3,5-indolizidines are derived from myrmicine ants of the genus *Solenopsis* (Jones et al., 1999), which was also the most common ant genus in the areas examined. It can be assumed that at least some species of this genus supply the frogs with alkaloids and thus may induce the selection of a specific territory area. Pumiliotoxins, which had been identified in both, formicine ants and oribatid mites (Saporito et al., 2004; Takada et al., 2005) were also detected in frogs from Hitoy Cerere, however, as minor components only (Saporito, unpublished data). This finding is also confirmed by the present chemical analysis, which indicates that pumiliotoxins are present both in *O. pumilio* and *D. auratus* from Hitoy Cerere. However, they seem to be minor components in comparison to histrionicotoxins. The allopumilio 323B detected is an oxidised pumiliotoxin 307A (and thus most likely a metabolite), present in formicine ants of the genera *Brachymyrmex* and *Paratrechina* (Saporito et al., 2004). Alkaloids of the pumiliotoxin group are highly toxic in comparison to

histrionicotoxins and most other toxins (Daly and Myers, 1968; Daly et al., 1978). One possible source of pumiliotoxins, formicine ants of the genera *Brachymyrmex* and *Paratrechina*, seem to be rare in Hitoy Cerere while being more abundant in the frogs' territories than outside. Pumiliotoxins may represent a very limited, but very important alkaloid rendering frogs more unpalatable than others, less toxic alkaloids. Therefore, frogs may preferably establish their territories within areas of high female density where (next to histrionicotoxins and other alkaloids) these highly toxic pumiliotoxins are also available.

Surprisingly little is known about the true source of the various alkaloids. Many oribatid mites feed on certain soil fungi such as *Alternaria* and *Ulocladium* species (Schneider, 2005), which may be the original producers of many alkaloids (Dettner, 2007; suggested by Mebs and Daly). For instance, one *Alternaria* species has been shown to produce the indolizidine swainsonine (Gardner et al., 2003). Indolizidine alkaloids have been detected in mites as well as frogs (Saporito et al., 2007b). Further investigation may reveal a sequestration of toxic alkaloids from fungi over mites to ants, whereas both ants and mites are foraged by the frogs. The co-occurrence of formicine ants of the genera *Brachymyrmex*/*Paratrechina* and frogs might thus be linked to the presence of an original pumiliotoxin producer. Pumiliotoxins had been detected in some, but not in all samples of *Brachymyrmex* and *Paratrechina* from different sites (Saporito et al., 2004), indicating that certain alkaloids are present or absent in specific areas. In this context it is interesting to note that both dendrobatid species of Hitoy Cerere (*O. pumilio* and *D. auratus*) exhibit a similar alkaloid profile. Therefore, the relationship between differences in territorial behaviour of dendrobatid frogs as a response on the locally variable availability of certain alkaloids in certain ant populations needs further attention.

Conclusion

The presence of valuable alkaloid sources like formicine ants of the genera *Brachymyrmex* and *Paratrechina* in areas with a high female density may induce male strawberry poison frogs to establish the core area of their territory, which can provide them with two limited resources: mates and toxic diet. We hypothesize that the distribution of alkaloid containing fungi species might play a role in determining the distribution of poisonous oribatid mites, which are themselves consumed by ants. Toxins may be sequestered to the frogs by both ants and mites and accumulated in each species for predator deterrence.

To support the importance of toxic diet for territoriality it is necessary to examine the stomach content of the frogs and identify more arthropods inside and outside of core areas. Additionally, alkaloid analyses of organisms potentially containing alkaloids (fungi, mites, formicine and myrmicine ants, poison frogs) in a particular ecosystem should be performed, and combined with ecological and behavioural studies to elucidate the complex interplay between food resources, toxicity and territoriality of strawberry poison frogs.

Acknowledgements. We thank the *Ministerio del Ambiente y Energía, Area de Conservación La Amistad Caribe* (MINAE, ACLAC-C) for permission to perform this study (*Resolución 002-INV-ACLAC-2008, Resolución 012-INV-ACLAC-2008*); the *Ministerio del Ambiente, Energía y Telecomunicaciones, Comisión Nacional para la gestión de la Biodiversidad* (MINAET, Conagebio) for permission to take alkaloid containing samples (*Resolución R-008-2008-OT-CONAGEBIO*); Ing. Javier Guevara Sequeira for export permission (*08CR000020/SJ*) and Dipl.-Ing. Stefan Rath from the *Bundesamt für Naturschutz* for import permission of alkaloid containing samples (*E-3740/08*). Furthermore, we thank the *Instituto Nacional de Biodiversidad de Costa Rica* (INBio), especially Manuel Solís for providing invaluable help and instruments; Werner Pogoda, Ivonne Meuche, Mario Cerdas Gomez, Janina Eulenburg, Earl W. May and Thomas Erlinghagen for their support, suggestions and helpful comments. We especially appreciate the valuable suggestions of two anonymous referees and the editor that helped to improve the manuscript.

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Received: April 20, 2009. Accepted: October 24, 2009.

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