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Noxious arthropods as potential prey of the venomous Javan slow loris (*Nycticebus javanicus*) in a West Javan volcanic agricultural system

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Arthropods play a significant role in ecosystems as prey for animals such as insectivorous primates. The venomous Javan slow loris (Nycticebus javanicus) is a nocturnal primate endemic to the island of Java, Indonesia. It remains unknown if its venom is partially sequestered from noxious arthropod prey. We studied the little-known arthropod community in a rural agriculture system in West Java, Indonesia, in order to investigate the potential prey and source for sequestration of venom. We found specimens of the partially noxious insect orders Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, Orthoptera and of the class Arachnida in slow loris foraging trees in an agricultural area in West Java, Indonesia. To examine the effects of environmental conditions on the abundance of this food source, arthropods were trapped every two weeks for five months, using sweep net transects, Malaise and pitfall traps. Trap type had a significant effect on taxa caught. Wind strength negatively affected the number of Lepidoptera captured in the Malaise trap and humidity had a confounding effect on Orthoptera caught by sweep net. Despite the short-term nature of our study, by using a combination of trapping methods, we identified a relatively high diversity of insects in a human-dominated landscape. Our results can be used as a basis to understand the proximate and ultimate factors shaping the use of venom by the slow loris as a primate.

Keywords: Arachnida; Indonesia; Insecta; Malaise trap; Nycticebus; sweep net

Introduction

Arthropods play a significant role in all forest communities, especially in the tropics (Basset et al. 2003). Arthropods are important pollinators, and affect plants through grazing, influencing primary production (Lowman 1982). Furthermore, arthropods accelerate nutrient cycling, mediate plant competition (Clay et al. 1993), and are prey for carnivores (Miller 1993). Some animals sequester toxins from noxious arthropod prey (Dumbacher et al. 2009; Saporito et al. 2009). Arthropods have shown to be an important food source for the loris genus *Nycticebus* (Streicher 2004; Wiens et al. 2006; Swapna et al. 2010; Starr and Nekaris 2013), the only venomous primates (Nekaris et al. 2013). Slow lorises activate their toxin by mixing brachial gland exudates (BGE) with saliva, resulting in a venomous bite (Alterman 1995; Hagey et al. 2007). Although the venom's evolutionary purpose remains unknown (Nekaris et al. 2013), it is likely to be a predator and ectoparasite deterrent, and effective in intraspecific competition

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(Alterman 1995; Nekaris et al. 2013). Preliminary analyses of BGE show that the chemical composition of the venom of wild lorises brought into captivity changes over time, suggesting that some venom components may be partially extracted from food sources such as arthropod prey (Nekaris et al. 2013). If this hypothesis holds true, variation in arthropod abundance could have an effect on the ability of slow lorises to sequester their venom and potentially influence slow loris fitness.

While data on venom composition of *Nycticebus* are not yet available for a comparison, here we investigated the potential arthropod prey of Javan slow lorises (*N. javanicus*) in a rural agriculture system in West Java, Indonesia. We report noxious taxa in the arthropod community and explore the effects of methodological (trap type: Southwood and Henderson 2000) and environmental abiotic factors (ambient temperature: Lessard et al. 2011; humidity: Gao et al. 2011; rainfall: Tanaka and Tanaka 1982; lunar illumination: Tigar and Osborne 1999) on the abundance of potential arthropod prey.

Methods

Study site

Research was conducted from February to June 2013 in the agricultural gardens near the village of Cipaganti, regency Garut, province West Java (7°6'6"–7°7'0"S, 107°46'0"–107°46'5"E; Figure 1). The study site was located at 1300–1750 m above sea level (asl), and was the same area where slow lorises were radio-tracked (Rode-Margono et al. 2014). Cipaganti lies on the foothill of the mountain range of the active volcano Mount Papandayan. While Mount Papandayan is recognized as a Nature Reserve (*Cagar Alam*) the agricultural areas and surrounding areas are not protected (Rode-Margono et al. 2014). The agricultural area is called *talun* by Sundanese people, and consists of a mosaic-like landscape that includes cultivated fields, abandoned fields, bamboo patches, interspersed with single trees and small tree plantations. The area was subject to a dry period from May until October and a wet period from November to April (Rode-Margono et al. 2014). We chose two adjacent trapping sites at 1425 m and 1460 m asl, c.200 m apart. Both were tea fields enclosed by bamboo (*Gigantochloa* spp.), green wattle (*Acacia decurrens*) and Cajeput trees (*Melaleuca leucadendra*).

Data sampling

We trapped arthropods at each area once every two weeks during three consecutive nights. As the effect of arthropod traps varies per order and forest stratum (Basset et al. 2003), we used different trap types to cover different arthropod orders and multiple forest strata. We placed the Malaise trap in the centre of each area and used it to capture flying arthropods. Intercepted arthropods moved upwards inside the trap and subsequently fell into a collection pot filled with a 70% alcohol solution (Campos et al. 2000). At each site, we established a line transect of 50 m in length between the rows of tea bushes to the left and right of the Malaise trap. At each step we took a sweep on each side of the researcher. After five steps (10 sweeps) we emptied the net and recorded the contents. We generated three random GPS locations in each area and placed in total six pitfall traps to target ground dwelling arthropods (Topping and Sunderland 1992). We left the Malaise and pitfall traps for 12 hours



Figure 1. Map of the study location Cipaganti near Garut, West Java.

(18:00–06:00) before collecting the contents. We walked each sweep net transect once every trapping night. All collected arthropods were included in the research. Orders were identified following McGavin (2001). We collected the environmental factors minimum ambient temperature, rain (both calculated for the previous 24 h), ambient humidity, cloud cover (estimated to the nearest 10%) and wind using the weather station Nexus TFA 30. 1075, recording one data point per hour. We retrieved the exact lunar illumination in percentage from the software NEW MOON 1.0 for lunar illumination (Thomas 1998). When the moon was below the horizon an illumination of 0 was recorded.

Data analysis

As there was no significant differences in total arthropod abundances captured with different trap types between the two areas (Mann–Whitney U tests; Malaise trap: U = 40.5, p = 0.345; n = 21, sweep net: U = 19, p = 0.114; n = 27; pitfall trap: U = 9, p = 1; n = 9) data were merged. We focused on the most abundant arthropod taxa that are also reported to be eaten by lorises (adult Lepidoptera, Hymenoptera, Orthoptera and Arachnida) for statistical tests. As environmental factors we used minimum temperature per night, average humidity in the previous 24 h (0600–0600), rainfall in the previous 24 h, wind in the previous 24 h, average lunar illumination

during this night (percentage of the night with moon over the horizon multiplied by the moon phase). Because abundance data were not normally distributed and sample size per trap type and order too small, we used non-parametric statistics. We performed Kruskal–Wallis tests for comparing samples of different trap types (number of insect specimens caught), and Mann–Whitney U post hoc tests with Bonferroni corrections. We computed Spearman rank correlations to test for correlations between environmental variables and trap type–order combinations (Lepidoptera adults–Malaise trap, Lepidoptera adults–sweep net, Orthoptera–sweep net, Arachnida–sweep net). All tests were performed with SPSS 20.0 and significance level set to 0.05.

Results

We conducted 21 trap nights for Malaise traps, 17 for sweep nets and nine for pitfall traps. The total abundance of different arthropod taxa caught is shown in Table 1, with potentially noxious taxa specified.

Focusing on the most abundant taxa, trap type has a significant influence on the number of animals captured (Figure 2 and Table 2). For each combination of the taxa and their most efficient trap type, we used Spearman Rank correlations to test their correlation with the different environmental variables.

When checking the correlations between the different arthropod groups-trap type combinations and environmental factors, the abundance of adult Lepidoptera captured by Malaise trapping correlated significantly negatively with wind strength ($\mathbf{R} = -0.824$, p = 0.006, n = 9). The abundance of Orthoptera captured with sweep nets correlated significantly negatively with average humidity ($\mathbf{R} = -0.790$, p = 0.011, n = 9). All other combinations showed no significant correlations with any of the abiotic factors collected.

Discussion

Although arthropods in their various roles in tropical forest communities are of great interest to science, studies have generally focused on natural forest areas (e.g. Basset et al. 2003). The arthropod communities on Java have been particularly neglected (Maschwitz et al. 2000; Tati-Subahar et al. 2007), possibly because more than 90% of Java's natural vegetation has been converted into human-dominated landscapes, agricultural areas and to some degree forest plantations (Smiet 1992; Lavigne and Gunnell 2006). Research on Java on the effect of arthropods on other wildlife is equally absent, as little research has been done on wildlife in general, except for some island endemics such as the Javan rhino (Fernando et al. 2006), Javan gibbon (Supriatna 2006), Javan langur (Nijman 2002) and Javan hawk-eagle. The widespread *talun* agriculture system on Java that consists of a patchwork of various habitats and is often located adjacent to more natural vegetation, such as national parks, may hold interesting arthropod communities. Likewise, many species of wildlife show a certain degree of flexibility and cope well with the semi-natural habitats left on Java, e.g. Javan slow lorises (Rode-Margono et al. 2014), common palm civets (Corlett 1998), or leopard cats (Rajaratnam et al. 2007). Our results show for the first time that the *talun* agricultural system on Java harbours a diverse arthropod community. During 21 capture nights, using three different trap types, we captured 1185

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Arthropods	Specimens caught	Potentially noxious* taxa	Source
Insect order	Ν		
Blattodea(cockroaches)	9	Blattidae	Wallbank and Waterhouse (1970)
Coleoptera (beetles)	70	Tenebrionidae	Seena and Thomas (2013)
Hemiptera (true bugs)	71	Heteroptera: Reduviidae	Ishikawa et al. (2007)
Hymenoptera (bees, ants, wasps)	118	Formicidae, Formicinae: Polyrhachis	Torres et al. (2013)
Lepidoptera adult/caterpillar (butterflies and moths)	289/104	Arctiidae	Sutrisno (2010)
Mantodea (mantids)	21		
Odonata (dragonflies)	ŝ		
Orthoptera (grasshoppers, crickets, katydids)	266	Tettigoniidae	Rentz (2001)
Dermaptera (earwigs)	14	Arixeniidae	Eisner et al. (2000)
Other arthropod classes			
Arachnida (arachnids)	220	Araneae	Murphy and Murphy (2013)
Chilopoda (centipedes)	1	Geophilomorpha: Mesoschendyla	Edgecomb and Giribet (2007)
Diplopoda (millipedes)	7	Spirostreptidae	Eisner et al. (1978)
*Potentially novious' taxa are known to produce toxic	secretions		

Table 1. Abundance of all arthromods caught during five months in the arriculture tabun fields of Cinaganti. West Java.

taxa are known to produce toxic secretions. Potentially noxious



Figure 2. Mean abundance of the most frequently captured arthropod taxa per trap type. Sample size: Malaise trap n = 21, sweep net n = 17, pitfall trap n = 9. Error bars: ± 1 SE.

Table 2. Mean abundance and standard deviation of the most frequently captured arthropods per trap type.

	Malaise trap	Sweep net	Pitfall trap	Kruskal–Wallis	р	n
Lepidoptera adult	12.1 ± 11.7^{a}	4.8 ± 3^{a}	0.1 ± 0.3^{b}	22.747	< 0.001	47
Hymenoptera	0.1 ± 0.5^{a}	0.4 ± 0.8^{a}	11 ± 10.1^{b}	29.013	< 0.001	47
Arachnida	0.1 ± 0.7^{a}	11.3 ± 7^{b}	0.9 ± 1.2^{a}	36.916	< 0.001	47
Orthoptera	0.2 ± 0.5^{a}	15.4 ± 9.1^{b}	$0.6 \pm 0.5^{\mathrm{a}}$	36.060	< 0.001	47

Different superscript letters indicate significant differences between capture rates of the different trap types according Kruskal–Wallis tests, while the same letters indicate no significant differences. Asterisks show results of Mann–Whitney U post hoc tests with Bonferroni corrections. *n*: total sample size.

arthropod specimens from nine different insect orders as well as spiders, millipedes and centipedes.

Capture success was not equally distributed amongst trap types and arthropod taxa (Campos et al. 2000; Southwood and Henderson 2000), a phenomenon that is often regarded as trivial but rarely documented. Both the Malaise trap and pitfall trap use a passive method of sampling, whereas the sweep net represents an active sampling method (Southwood and Henderson 2000). Malaise traps are flight intercept traps, pitfall traps focus on ground-dwelling animals, and sweep nets on animals resting on the vegetation (Townes 1962; Southwood and Henderson 2000). In our study, adult Lepidoptera (butterflies and moths) were mainly caught in the Malaise trap and to a lower degree in the sweep net, Orthoptera and Arachnida in the sweep net, and Hymenoptera (mainly ants) in the pitfall trap. We have not included other methods of measuring insect abundance or activity that might be effective, such as bait traps for ants (Yanoviak and Kaspari 2000) or acoustic recordings for Orthoptera (Riede 1997). The results suggest that studies must consider the effectiveness of trap types in respect to the arthropod group in question, and be carefully designed. If food sources of insectivorous species are investigated, studies should include various types of traps (e.g. Lang et al. 2006).

We found only a small number of significant correlations between trap type-taxa combinations and environmental factors. Fewer Lepidoptera were captured in the Malaise trap in strong wind, while humidity had a suppressing effect on the abundance of adult Orthoptera caught with the sweep net (see also Gilbert 1985). Wind has shown to have a negative effect on the activity of flying insects in several studies (e.g. Wolda 1977). No relation between arthropod abundance and rainfall in the previous 24 h was found in our study. This agrees with previous research indicating a three-week lag between rainfall and a numerical response in arthropod communities (Tanaka and Tanaka 1982), and seasonality, rather than short-term rainfall, being the major driver of arthropod abundance in the tropics (Bigger 1976; Wolda 1978). Studies on the effect of temperature on insect activity found a lower temperature threshold below which flight will be inhibited (Taylor 1963) and an increase in wingbeat frequency and activity in Lepidoptera and Diptera with increasing temperature (Sotavolta 1947). Most studies on the effect of lunar illumination found that flying insects are more active on dark nights (reviewed in Nowinszky 2004; Lang et al. 2006). However both studies warn against reaching premature conclusions, as the effectivity of light trapping (although Malaise traps are regarded as passive traps, most traps include a white-coloured tent that may enhance attraction to insects) may be improved in dark nights due to a stronger contrast. As with trap type, effects of environmental factors were not consistent between different arthropod taxa. We encourage further longer-term studies in the area to examine further relations with environmental factors.

Many animals are known to sequester toxins from a noxious arthropod diet and accumulate them in their tissue. Examples include pitohui birds (*Pitohui* sp.) that sequester batrachotoxins from melyrid beetles (Cleroidea) (Dumbacher et al. 2009), dendrobatid poison-dart frogs that incorporate toxins from a variety of alkaloid-containing arthropods such as mites, myrmicine ants, coccinellid beetles or siphonotid millipedes (reviewed in Saporito et al. 2009), and the Asian snake *Rhabdophis tigrinus* that uses toxins from toads (Hutchinson et al. 2012). Arthropod prey is a vital part of the diet of slow lorises, the only venomous primates (Alterman 1995; Hagey et al. 2007; Nekaris et al. 2013). Dietary choice that includes noxious arthropods may play a role in the chemical composition of the venom (Nekaris et al. 2013). Animals are known to feed on gum and other tree exudates, nectar, arthropods, and to a lower degree on fruit, eggs and vertebrates (reviewed in Nekaris 2014; Rode-Margono et al. 2014). Lorises prey upon Lepidoptera (larvae and imagines), Hemiptera, Coleoptera, Hymenoptera and Arachnida (Hladik 1979; Wiens

et al. 2006; Streicher 2009), all of which include a variety of noxious taxa (Table 1). Wiens et al. (2006) report 40% of the faecal samples of lorises with remains of ants and 12.8% with remains of caterpillars. These findings are confirmed by faecal samples taken at our sites (unpublished data). Although many caterpillars are noxious, we could not include them in our analysis due to insufficient sample size. Future studies will be needed to solve the question of whether noxious arthropods are dietary sources for the toxic compounds found in the venom of slow lorises. Our fieldwork indicates that multiple catching methods are needed to sample the range of potential noxious prey for Javan lorises in field conditions. The field data may additionally serve as a first step in understanding proximate and ultimate factors that shape the use of venom in these primates.

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Disclosure statement

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