Ectoparasite loads of the Central American Whiptail Lizard *Ameiva festiva* (Squamata: Teiidae)

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We studied relative abundance, sex ratio, sexual maturity, body size and mite loads of the lizard *Ameiva festiva* in grassland, primary and secondary forest in the Caribbean region of Costa Rica. A total of 51 individuals were captured using drift fences with pitfall and funnel traps. With equal sampling efforts across sites, 19 individuals were caught along a gap within secondary forest, 29 individuals were caught at a grassland site and three specimens were caught in primary forest. The proportion of immature individuals differed significantly among sampling sites in the three habitats, whereas sex ratio did not. Snout-vent length did not differ between sexes or habitats. The chigger mite *Eutrombicula alfreddugesi* was the only ecto-parasite found to infest *A. festiva*. Proportionally more lizards were infested in the grassland site (86%) than in the secondary forest site (10%), a finding which is unlikely to result from differential mite densities in the two habitats. The number of mites on each individual increased with body size in grassland, but not in secondary forest where almost no individuals were infested. We found no effects of sex or stage on mite loads. The average infestation intensity was 21 mites (range 1–115) per individual.

Key words: chigger mites, Costa Rica, *Eutrombicula*, lizards, mite loads, sex ratios

INTRODUCTION

The genus *Ameiva* (Squamata: Teiidae) is restricted to the New World, and consists of 33 species of moderately large terrestrial lizards (Leenders, 2001; Savage, 2002). The genus has a broad distribution that ranges from Mexico to Ecuador and Argentina, and is also present throughout the Antilles and the Bahama Islands (Savage, 2002). In Costa Rica, the genus is represented by five species, with the Central American Whiptail *Ameiva festiva* (Lichtenstein & von Martens, 1856) being common to both the Pacific and Atlantic slopes, from lowlands up to 1000 m a.s.l. (Leenders, 2001). The species is terrestrial and diurnal, actively foraging over leaf litter exposed to direct sunlight during mid-mornings (Vitt & Zani, 1996). Sexual maturity is reached between three and six months of age (Savage, 2002). Its diet consists of small arthropods (mainly grasshoppers, beetles and spiders), in addition to small amphibians and other lizards (Savage, 2002). *Ameiva festiva* is mostly found in undisturbed forest interiors, where it basks in clearings or sun-exposed patches and ventures sporadically into open areas of vegetation (Leenders, 2001; Savage, 2002). It is also known to prefer the open edge of forests, and habitats with lower vegetation cover including disturbed areas such as roadsides (Hillman, 1969). Previous studies on teiid lizards have focused on relative abundance, size and demographic structure of populations (Smith, 1968; Hillman, 1969; Vitt & Zani, 1996). Although it has been well documented that intra-specific population structure in lizards varies significantly with isolation, vegetation cover, competition and food availability (Moermond, 1979; Schoener & Schoener, 1980; Downes & Shine, 1998; Langkilde & Shine, 2004), data on how size and age distributions in *A. festiva* change within different habitat types in the same geographical and climatic region are still scarce.

Ecto-parasitism on tropical lizards has been shown to be a frequent phenomenon (Rubio & Simonetti, 2009; Delfino et al., 2011). The chigger mite *Eutrombicula alfreddugesi* Oudemans, 1910 (Acari: Trombiculidae) is known to parasitize 39 species of reptiles, 13 of which are lizards (Zippel et al., 1996). Its reddish larva is the only parasitic stage of the life cycle, and appears in late April with peaks in late June and early July (Wharton, 1952; Sasa, 1961; Clopton & Gold, 1993). Chigger mites have been found to prefer cool, shady and moist habitats with dense vegetation cover over open and sunny areas, with high temperatures and low humidity reducing their activity levels (Clopton & Gold, 1993; Schlaepfer & Gavin, 2001).

To improve our understanding of habitat use in *A. festiva* we investigated the relative abundance of the species in grassland, primary forest and secondary forest in the Caribbean lowlands of northern Costa Rica. In order to get a more detailed picture of how the animals were spatially distributed we tested if body size, sex ratio and sexual maturity (adult vs. immature) differed among sampling sites representing one of the three habitat types. We also recorded the prevalence and infestation intensity of *E. alfreddugesi*, and examined if mite loads varied in relation to habitat, sex and sexual maturity. Since larger animals may attract more mites we also tested for body size in adults.

MATERIALS AND METHODS

All sampling sites were situated in northern Costa Rica, at La Selva Biological Station in the province of Heredia (10°26'N, 83°59'W). This study was conducted in May-June, coinciding with the local rainy season. La Selva is located between 30 and 150 m a.s.l. and receives approximately 4000 mm of annual rainfall (McDade & Hartshorn, 1994).

Sampling took place in three habitats: 1) dense old growth primary forest with no sunny patches on the ground, 2) a 13–17 year old secondary forest with a dense canopy that allows for sun-exposed clearings, 3) a grassland area consisting of pastures and abandoned plantations with tall grasses and sporadic trees. A transect was established randomly in each of the three habitat types. The primary forest transect was set up 1 km to the south-east of the research station's main grounds (the "Camino Circular Lejano" trail between the 50 and 170 m marks). The secondary forest transect was located 3.1 km away from the station along the "Sendero Tres Ríos" trail between the 3,090 and 3,100 and the 3,120 and 3,210 m marks. The grassland transect was situated approximately 800 m from the station along the "Sendero Arriera-Zompopa" trail, between the 300 and 420 marks.

Individuals of *A. festiva* were taken alive, examined and released in situ between 13 May and 27 June 2008. The trapping methodology followed Blomberg & Shine (1996), Crosswhite et al. (1999) and Savage (2002), and consisted of drift fences with pitfall and funnel traps, 120 m in length and containing 12 traps placed 10 m apart. For the secondary forest transect the fence was erected along a trail due to drainage problems and soil structure (12 traps distributed over 100 m). Distances between sampling sites exceed *A. festiva*'s putative home range of 100–400 m² (see Verwaijen & Van Damme (2007) for other lizard species). Pitfalls consisted of 9 litre buckets, 40 cm wide at the top, buried flush with the ground. Funnel traps were constructed with thin aluminium mesh or mosquito screen, three feet in length and had approx 30 cm wide openings at both ends. Two funnel traps on each side of the fence, were installed approximately every 30 m. Drift fences were built using 10 m x 0.5 m plastic foil. Traps were checked twice a day at 0730 hours and at 1500. Pitfalls and funnels were covered and closed every afternoon at 1600.

A total of 11 lizards were captured using funnel traps and the remaining 40 were caught with pitfalls. There were no significant differences in capture efficiency with regards to sample site (χ^2=1.27, df=2, p=0.53), sex (χ^2=0.89, df=1, p=0.35), sexual maturity phases (χ^2=0.49, df=1, p=0.48) or size (Mann-Whitney test Z=0.24, p=0.80). Therefore, all individuals were combined into one set of data.

Snout-vent length of lizards was measured to the nearest mm, using a 150 mm plastic calliper. Sex and stage (mature vs. immature) were determined following Leenders (2001) and Savage (2002). Immature lizards have a distinct colouration which differs from adult colour patterns but nevertheless readily allows sex identification (Savage, 2002), and comprises age classes ranging from about three weeks to three months. We marked each lizard individually with a black permanent marker pen on the animal's ventral side, and recaptured animals were not included in our analysis.

When mites were present on a lizard, their total number was counted by visual inspection. Random samples were removed from different parts of the body using forceps with a fine tip. In lizards with a high number of mites, up to approximately 80% of these were collected; all chiggers were removed from lizards infested by a maximum of 10 ectoparasites. Mites were preserved in a 5 cc vial containing 70% alcohol that was assigned to each particular lizard. They were subsequently mounted on microscope slides for identification (Brennan & Jones, 1959).

Statistical analyses were performed with the JMP software version 7.0 (Sall et al., 2005) and the R software version 2.9.0 (Crawley, 2005). Due to small sample size in primary forest (*n*=3), statistical analyses only include data from secondary forest and grassland unless otherwise stated. We used Generalized Linear Models (GLM) in R to test 1) how body size (measured as snout-vent-length) is affected by habitat, sex and maturity status, and 2) how infestation intensity of the lizards is affected by habitat, sex, maturity status and body size. The GLMs used a quasipoisson distribution because of overdispersion in the data. We carried out the model selection procedure in a stepwise and forward manner. In each step the fit of a model including the variable with lowest residual deviance was compared with the model selected in the previous step (the null model in the first step) using ANOVA (Crawley, 2005). The procedure was repeated until the model fit did not improve by adding further variables. In the final step we tested if including the interaction between single variables in the selected model significantly improved the model fit. Due to a lack of replicates for the two habitat types, habitat was considered a random factor.

Table 1. Number of captured *A. festiva* according to habitat type, sexual maturity and sex. Note that the sex is given for both immature and mature individuals (see Materials and Methods).

Habitat	Immature	Mature	Males	Females
Grassland	20	9	14	15
Secondary forest	9	10	9	10
Primary forest	1	2	1	2
Total	30	21	24	27

RESULTS

Relative abundance and sex ratio

We captured 51 lizards across the sampling sites in the three habitats (Table 1). Numbers of captured individuals varied significantly among the three sites (χ^2=13.04, df=2, $p<0.01$). A Bonferroni-corrected partitioning procedure revealed significant differences between the primary forest site and the two other sites (grassland: χ^2=6.72, df=1, $p<0.01$; secondary forest: df=1, χ^2=3.86, $p<0.05$) but not between the site in grassland and secondary forest (χ^2=1.05, df=1, n.s.). The overall proportion of male and female *A. festiva* across all sites was 47% and 53%, respectively. The sex ratio did not deviate from overall unity (Binomial test: p=0.78), or between grassland and secondary forest sites (χ^2=0.004, df=1, p=0.95).

Sexual maturity

In total, 58.8% of all captured individuals were immatures (Table 1). The majority of immatures were recorded in the grassland site (68.9% from a total of 29 individuals), whereas in the secondary forest the proportion of immature individuals was similar to that of adults (47.4% and 52.6% respectively, *n*=19; χ^2=3.07, df=1, p=0.021). In the primary forest, we recorded one female, one male and an immature female (Table 1).

Body size

There were no sex–specific differences in snout-vent length when comparing immature (Mann-Whitney-Test: Z=-4.89, *n*=29, p=0.62) and adult individuals (Z=-1.74, *n*=21, p=0.08). We found a significantly positive effect of sexual maturity on snout-vent length ($F_{1,46}$=72.88, $p<0.001$). However, there were no effects of sex or site and hence these variables were excluded from the final model.

Factors affecting mite loads

The chigger mite *E. alfreddugesi* was the only ecto-parasite found. The most frequently infested body parts were the depressions where the extremities meet the body, and the base of the tail. Overall, 57% (29/51) of the captured lizard individuals were infested by mites. There was no difference in the proportion of infested individuals with respect to sex (χ^2=0.58, df=1, p=0.44, Table 2) and sexual maturity (χ^2=0.37, df=1, p=0.53, Table 2). Prevalence
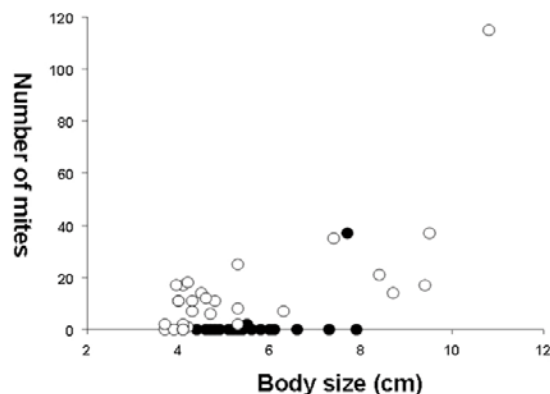

Fig. 1. Effect of the interaction between size and sample sites on mite loads of *A. festiva* for the 29 individuals captured in grassland (open symbols) and the 19 individuals caught in secondary forest (closed symbols).

varied significantly between grassland and secondary forest (χ^2= 26.71, df=1, $p<0.001$, Table 2). In total 68% of the 620 recorded mites were found on lizards from grassland. In primary forest, two of the three captured lizards were infested with *E. alfreddugesi*, accounting for 26% of the total parasite load observed in this study. Prevalence was lowest in secondary forest, where two individuals of *A. festiva* were parasitized and accounted for 6% of the total mite numbers found. Infestation intensity was affected by body size ($F_{1,46}$=64.42, $p<0.001$), sample site ($F_{1,45}$=23.73, $p<0.001$) and by the interaction between body size and sample sites ($F_{1,44}$=10.20, p=0.003). Thus, lizards from grasslands showed relatively high parasite loads which increased with body size; individuals from secondary forests, on the other hand, always had few mites regardless of body size (Fig. 1). Sex and maturity did not have any effect on this relationship (data not shown).

DISCUSSION

Our sample size is concordant with other field studies on this species (Hillman, 1969; Vitt & Zani 1996). The relative abundance of *A. festiva* differed among sites, with the majority of the lizards found in grasslands and secondary forest and very few individuals occurring in primary forest. Moreover, immature lizards appeared to

Table 2. Numbers and percentages of mites and infested male, female, immature and mature *A. festiva* sampled across habitats at La Selva Biological Station, Costa Rica. Note that the sex is given for both immature and mature individuals (see Materials and Methods).

Habitat	Total no. of infested lizards (%)	Immature (%)	Mature (%)	Males (%)	Females (%)	Total no. of mites (%)	No. of mites per infested individual
Grassland	25 (86.2)	16 (64)	9 (36)	13 (52)	12 (48)	422 (68)	average 16.8±22.5
Secondary forest	2 (10.5)	0	2 (100)	1 (50)	1 (50)	37 (6)	2 and 37
Primary forest	2 (66.6)	0	2 (100)	1 (50)	1 (50)	161 (26)	64 and 96

select pastures, whereas mature lizards stayed closer to the edge of secondary forest. The disparity in occurrence of immature individuals among habitat types, however, indicates that the picture is more complex than previously described. One possible reason why immature individuals occur more often in open areas may be habitat effects on thermoregulatory requirements. Teiids generally depend on sunlight and adequate ground temperatures to acquire the necessary energy for foraging (Hillman, 1969; Vitt & Carvalho, 1992; Vitt & Colli, 1994; Kerr et al., 2005). Because juvenile lizards are smaller and lighter, they cannot absorb nor retain as much energy from the sun as adults. Juveniles compensate for this by establishing themselves in sun-exposed habitats such as grassland (Hillman, 1969; based on *A. leptophrys*). Differences in behaviour of adult and juvenile *A. festiva* could also affect their habitat preferences. Adults have been observed to be cannibalistic in times of low invertebrate prey abundance and show aggressive displacement behaviour towards juveniles in thermally favourable and resource-rich locations (Kerr et al., 2005).

In total 60% of the 51 captured lizards were immature. However, the largest mature individuals may have been able to escape from the pitfall traps. Nonetheless only a single escape was observed, and capture rates did not differ from funnel traps, a size independent method. Our findings match well with results reported by Smith (1968) who found 64% juveniles in his sample of 225 *A. festiva* in Pandora, Costa Rica. A possible explanation for a higher proportion of juveniles in the sample is that the time of our study coincided with the hatching of eggs from an earlier mating season (Simmons et al., 2005). Despite the different conditions that lead to habitat partitioning between immature and adult lizards, individuals in grassland and secondary forest attain a similar size. Our range of body size coincide with those of Vitt & Zani (1996) in their study of a population in Nicaragua and with those described by Smith (1968) for the Atlantic region of Costa Rica.

Sex differences in length and mass have been reported for *A. festiva* and other teiids (males are larger; Hirth, 1963; Smith, 1968; Censky, 1996; Vitt & Zani, 1996; Kerr et al., 2005) and has been attributed to sexual selection (Anderson & Vitt, 1990; Vitt & Carvalho, 1992; Vitt & Colli, 1994; Vitt & Zani, 1996; Censky, 1997; Kerr et al., 2005). Nevertheless, there is a considerable overlap in size (Leenders, 2001; Savage, 2002), occasionally leading to non-significant differences in average length between the sexes (this study; Vitt & Zani, 1996). Head measurements (which were not studied here), rather than snout-vent length, have been suggested to be better indicators of intrasexual selection (Anderson & Vitt, 1990; Kratochvíl & Frynta, 2002; but see Kratochvíl & Frynta, 2007). The prevalence of mites was much higher in grassland than in secondary forest. This was surprising, since chiggers generally prefer cool, moist and shaded habitats (Schlaepfer & Gavin, 2001). Infestation rates might have been low in the forest because few mites were present, although this seems unlikely given the high infestation rates of the two individuals captured in this habitat. In concordance with other studies on lizards (Poiani, 1992;

Bull & Burzacott, 1993; Schlaepfer & Gavin, 2001; Cunha-Barros et al., 2003; Carvalho et al., 2006, Rubio & Simonetti, 2009), infestation intensity at our grassland study site was significantly related to body size. Our largest male (mass: 46 g, snout-vent length: 10.8 cm) had about 115 mite larvae. However, whether size *per se* affects mite loads is debated (Cunha-Barros et al., 2003; de la Peña et al., 2004). Despite the habitat preferences of *E. alfreddugesi*, a possible alternative is that heavier individuals are more sedentary and move with their body closer to the ground, acquiring mites more readily than lighter individuals (Cunha-Barros et al., 2003). Moreover, larger individuals may have bigger territories which may increase the probability of mite exposure (Hirth, 1963; de la Peña et al., 2004; Simmons et al., 2005).

We did not find differences in infestation intensity between the sexes. Because the interaction of sex and sexual development was included in our analysis, this finding was true for immatures and adults. Future studies should focus on replicating results over a larger area with different lizard abundances among and within habitats. This would shed light on the species' habitat preferences and reveal which factors drive mite loads in this host-parasite system.

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