Variation in body size in the tick complex *Rhipicephalus appendiculatus*/*Rhipicephalus zambeziensis*

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ABSTRACT: We examined the relationship between body size and the phenology of the tick complex *Rhipicephalus appendiculatus*/*Rhipicephalus zambeziensis*. These ticks transmit *Theileria parva* in cattle. In Africa, the body size of *R. appendiculatus* increases with latitude while the body size of the morphologically similar *Rhipicephalus zambeziensis* is constant at two different latitudes. A larger body size is necessary once survival becomes a constraint. The most plausible explanation for the smaller *R. appendiculatus* in equatorial Africa is the cost to produce a larger egg. The consequences of these findings for the introduction of *R. appendiculatus* in new environments are discussed. New field observations from southern Zambia indicate that *R. appendiculatus* body size does not vary seasonally as compared to eastern Zambia. This is an additional indication of the presence of a single diapausing population of larger ticks. ***Journal of Vector Ecology*** **29 (2): 347-354. 2004.**

Keyword Index: Ticks, body size, survival, *Rhipicephalus, Theileria parva*.

INTRODUCTION

Theileriosis, caused by *Theileria parva* (Theiler), constitutes a serious threat to the traditional cattle sector in Zambia (D'Haese et al. 1999) and other parts in Africa (Norval et al. 1992). Its vectors, *Rhipicephalus appendiculatus* (Neumann) and *Rhipicephalus zambeziensis* (Walker, Norval, and Corwin), have been recognized as tick species that are most important economically throughout eastern and southern Africa (De Vos 1981).

Unlike some temporal ticks such as *Ixodes ricinus* (Linnaeus) (Gray 1991), African hard ticks have a life cycle that takes less than a year to complete. *R. appendiculatus* shows a wide distribution in extensive parts of Africa. At different latitudes different seasonal activity patterns are observed. Near the equator, ticks usually feed throughout the year and numbers vary less. Mainly south of Zambia (down to South Africa 33°S) there are marked annual cycles of abundance of each life-stage (larva, nymph and adult) punctuated by near total absence during the dry season of each year (Speybroeck et al. 2002). During this dry season (June-November), adult ticks enter into a diapause that they terminate at the beginning of the rainy season (December).

Within Zambia, the presence of a transition zone with respect to activity patterns of *R. appendiculatus* can be noticed: in southern Zambia, *R. appendiculatus* adults have a single peak of host-seeking activity (during the rainy season in February), whereas in eastern Zambia, a second generation of adult *R. appendiculatus* is possible (at the beginning of the dry season in May; Chaka et al. 1999). Interestingly, it is also in Zambia where we found a difference in body size between southern and eastern *R. appendiculatus* (Speybroeck et al. 2002). This is an indication of a link between the size and population dynamics of *R. appendiculatus.*

Berkvens (1994) was first to recognize the importance of body size when attempting to understand *R. appendiculatus* activity patterns on the population level. However, relatively little attention has been given to this until now. Chiera et al. (1985) reported lower survival rates for small *R. appendiculatus* individuals under unfavorable conditions. Madder et al. (1996) demonstrated low heritability of body size under laboratory conditions. Chaka et al. (1999) showed that in eastern Zambia, *R. appendiculatus* adults were smallest during the dry season, but specimens became larger as the rainy season progressed. Second generation (non-diapausing) adults were on average smaller than those of the first generation. We hypothesized that body size is related to the activity patterns (i.e. diapausing intensity) in *R. appendiculatus* (Speybroeck et al. 2002). This suggestion would be analogous to non-diapausing cricket populations showing a smaller body size (Mousseau and Roff 1989). However, crickets and ticks, although both arthropods, are not exactly comparable. Crickets are herbivorous and feed continuously within each nymphal stage, while ticks only take one blood meal per instar. In order to grow larger, a cricket needs to feed longer which might result in costs (e.g. risk of predation). This is not the case for hard ticks, which feed only once per stage.

There is another marked difference between southern and eastern Zambia: *R. zambeziensis* is present in the first but lacking in the latter. *R. zambeziensis* and *R. appendiculatus* adults cannot easily be distinguished morphologically but are considered as two separate species (Walker et al. 1981).

Silver and Renshaw (1999) reviewed studies on the relationship between body size and latitude in insects. They found that mosquito body size, as estimated by wing length, increases with latitude when all species of the Nearctic mosquito fauna are pooled. For ticks, to our knowledge, no study discusses the relationship between body size and latitude.

The aim of this study is to investigate variation in space and time of the body size of the complex *R. appendiculatus* and *R. zambeziensis*. To enhance our understanding of this variation, we studied the relation between female weight and clutch size.

MATERIALS AND METHODS

Study of the effect of latitude (laboratory ticks)

Laboratory stocks of *R. appendiculatus* originated from Kenya (Muguga), eastern Zambia (Wafa) and southern Zambia (Nkonkola and Keemba), Zimbabwe (West-Mashonaland), and South Africa (the Rietvlei strain, provided by the Onderstepoort Veterinary Institute). The latter institute also provided a stock of *R. zambeziensis* (from Swartwater in the Limpopo Province). All ticks were reared under similar conditions (21-23ˆC, 85-87% RH). They were used to investigate the body size of ticks collected at different latitudes without interference of local factors. The body size was measured by determining the maximum distance between the tip of the scapular processes and the distal end of the conscutum on about 30 randomly selected male ticks per stock (locality) using a micrometer mounted on a stereoscopic dissection microscope, allowing a resolution of 1.25 μm. The statistical analysis of the body sizes used the Scheffé multiple-comparison test in Stata (StataCorp. 2003).

Study of seasonality in body size in southern Zambia (field ticks)

To verify the seasonality in body size that was also suggested by Chaka et al. (1999) for eastern Zambia, in 1997 and 1998, *R. appendiculatus* males were collected monthly from traditionally owned cattle at three different locations (see Figure 1) in southern Zambia. This was done over 14 (Livingstone), 13 (Nteme), and 10 (Siabwengo) mo. Livingstone, Nteme, and Siabwengo are areas with respectively pure *R. appendiculatus* populations, mixtures of *R. appendiculatus* and *R. zambeziensis* populations, and a majority of *R. zambeziensis* populations (Speybroeck et al. 2002). Ticks were collected, stored, and measured as described in Chaka et al. (1999). The body size was measured as described above on about 30 to 100 male ticks per mo, randomly selected from collections on about 20 animals. If less than 30 ticks were collected in a month, all available ticks were measured.

The effect of host resistance on tick size in the field could not be measured and makes comparisons of field and laboratory ticks impossible. Therefore, the results obtained from colony-raised ticks are not compared with those from field ticks.

Reproduction Study

To better understand the advantages and disadvantages of smaller tick size, the number of eggs in relation to female weight (weights established after molting) was investigated. From 270 randomly selected *R. appendiculatus* adults (141 females) from eastern Zambia, the females were ranked with respect to weight. According to the selection criteria described in Madder et al. (1996), the 13 smallest [average weight (aw) = 5.0 mg, standard deviation (sd) = 0.2] and 12 largest females (aw = 7.8, sd = 0.4) were selected and separately applied to two rabbits with, respectively, the 10 smallest (aw = 3.2, sd = 0.8) and 14 largest males (aw = 10.5, sd = 0.63). Each egg batch was weighed. Also, the number of eggs of 3 batches from the group of small females and four batches from the group of large ticks was determined under a stereoscopic microscope, using a dissecting needle to separate the eggs. For these batches an average egg weight was determined. This average egg weight and the weights of egg batches were used to calculate the numbers of eggs in the remaining egg batches.

The females used in this trial were maintained in an incubator at 24°C (daytime) and 18°C (nighttime), a 14:10 h light: dark regimen and 85% RH. Engorged females were transferred to a second incubator (26°C, 85% RH and total darkness) until eclosion of the eggs.

RESULTS

Study of the effect of latitude (laboratory ticks)

The results of body sizes of laboratory ticks are shown in Table 1 and visually represented in Figure 1. They indicate that *R. appendiculatus* ticks from eastern Zambia (Wafa) (average size (as) = 3.48, standard deviation (sd) = 0.30) were significantly larger than the Kenyan ticks and significantly smaller than the more southern *R. appendiculatus* ticks from southern Zambia (Nkonkola) (as = 3.96, sd = 0.25), Zimbabwe (West-Mashonaland) (as = 3.86, sd = 0.31), and South Africa (Rietvlei strain) (as = 3.74, sd = 0.22). The latter three groups of ticks did not show a significant difference in body size despite the increasing latitude. The body size of Kenyan *R. appendiculatus* ticks (as = 3.16, sd = 0.32) was not significantly different from the body sizes of *R. zambeziensis* ticks from southern Zambia (Keemba) (as = 3.17, sd = 0.30) and South Africa (Limpopo Province) (as = 3.04, sd = 0.41).

Seasonality in body size in southern Zambia (field ticks)

The statistical analysis indicates that the body size of *R. appendiculatus* in Livingstone and that of *R. appendiculatus* and *R. zambeziensis* in Nteme and Siabwengo did not vary seasonally (P>0.05). In 1998 compared to 1997, body sizes were larger in Livingstone only (Figure 2).

Reproductive effects

The results of this study show that the average number of eggs for the group of ticks with low weight was 4,456 (range: 3,176-5,787) and for the group of heavy ticks 7,223 (3,650-9,832). From the small sample size, it was observed that individual eggs of heavy and light ticks have a similar weight: an average weight of 0.0418 mg and 0.0416 mg respectively. This indicates smaller numbers of eggs for heavier females relative to their body weight.

DISCUSSION

The results in this study indicate that the body size of *R. appendiculatus* increases with increasing latitude. This trend stabilizes from southern Zambia onwards. The latitude ties strictly to conditions that determine selection. We argue that the selection pressure is both "juvenile" (eggs and larvae) as well as adult survival. From southern Zambia (15°S) onwards, *R. appendiculatus* is forced in a phenology that combines a compulsory diapause (Madder et al. 2002) with a larger body size. Both attributes support the survival of *R. appendiculatus*. The diapause results in production of eggs and larvae synchronized with favorable conditions (rainy season), while a larger body size allows adults surviving harsh conditions during diapause as demonstrated by Chiera et al. (1985). From southern Zambia (15°S) onwards but not in eastern Zambia, a diapause is also required by the extreme cold temperatures during the cold season that follows the rainy season. Eggs and larvae develop slowly when temperatures are low and would be exposed much longer to dry unfavorable conditions (Speybroeck et al. 2002).

Near the equator, ticks usually feed throughout the year and numbers vary less in time than at higher latitudes. The size of these ticks is smaller than the size of ticks in southern Africa. A straightforward explanation for smaller body sizes at low latitudes does not exist. While the evidence for the selection of larger body size is overwhelming, counterbalancing selection favoring small body size is often masked, and evidence for any of

Table 1. Body sizes of *Rhipicephalus appendiculatus*/*Rhipicephalus zambeziensis* in different localities.

Locality (origin of laboratory ticks)	Average body size (S.D)	Grouping*
Kenya, *Rhipicephalus appendiculatus*	3.16 (0.32)	a
Eastern Zambia, *Rhipicephalus appendiculatus*	3.48 (0.30)	b
Southern Zambia, *Rhipicephalus zambeziensis*	3.17 (0.30)	a
Southern Zambia, *Rhipicephalus appendiculatus*	3.96 (0.25)	c
Zimbabwe, *Rhipicephalus appendiculatus*	3.86 (0.31)	c
South Africa, *Rhipicephalus appendiculatus*	3.74 (0.22)	c
South Africa, *Rhipicephalus zambeziensis*	3.04 (0.41)	a

*Grouping according to the Scheffé multiple-comparison test.

the mechanisms that drive organisms to become smaller is lacking (Blanckenhorn 2000).

Although a larger sample size is needed to reach a final conclusion, we have indications from our reproduction study that individual eggs of large and small ticks have a similar weight and that the weight of non-engorged females is positively correlated with the number of eggs laid. These results suggest that large ticks lay more eggs than small ticks, but the eggs of both groups are similar in size which is also reflected in the resulting adults (data not shown). This can explain the low heritability of size (regression towards the mean), which was found in one population of eastern Zambian ticks by Madder et al. (1996). Like Madder and his colleagues, we used only one population of eastern Zambian ticks in our reproduction study. Thus it is possible that the size of an egg is approximately constant within a population (=stock) but not between different populations. More detailed investigations are necessary to tackle this question. It will be essential to conduct a trial investigating numbers and average egg weights from populations from different origins, e.g. comparing the large southern and the smaller northern African populations could be very important.

However, at least for one population, we can assume that every tick has a certain amount of energy, designated as X, which it can use to produce a number of eggs (n). We can further assume that large ticks have a higher amount of available energy (X_{large}). These large individuals lay n_{large} eggs with size X_{large}/n_{large}. The small individuals lay eggs with size X_{small}/n_{small}. Within a population, we demonstrated eggs are of similar size for both groups meaning that: X_{large}/n_{large} H•X_{small}/n_{small}. For the studied population, we found on average: n_{large}/n_{small} = 7223/4456 H•1.6. Combining our assumption about the respective amount of usable energy with the average individual weight in both groups, we find: female-weight$_{large}$/female-weight$_{small}$ = 7.8/5.0 H•1.6. This agreement in the empirically determined ratios is striking, particularly because an association between the individual body weight and the assumed amount of reproductive energy is very parsimonious.

It could now be hypothesized that the average size of a population remains similar from one generation to another if no selection is acting. Although field and our laboratory data cannot be compared, Kenyan ticks might remain small because conditions in Kenya are so favorable that no selection takes place. Indeed, it would require more energy to produce larger eggs that result in generally larger offspring. In equatorial Africa, this does not provide compensation as no selection against smaller ticks takes place.

Other less probable selection forces against being large might exist. If small ticks would develop faster (shorter generation interval) this could be an advantage (more generations possible in a year) counterbalancing, for example, the number of eggs laid by large ticks. However, as yet no evidence exists for a faster development of small ticks. The influence of host resistance on *R. appendiculatus* body size has been studied extensively under experimental conditions (Nuttall 1913, Fivaz and Norval 1989, Walker et al. 1990), reaching the general conclusion that increasing host resistance significantly decreases the body size of all instars. Here, it is important to distinguish phenotypic and genotypic effects. Whether or not high levels of host resistance in field conditions could affect the genotype determining the size of *R. appendiculatus* is not known, but it seems doubtful that it does.

It appears that at present, *R. appendiculatus* populations are adapted to their local environment and show a phenology of one generation a year (in southern Zambia), two generations a year (in eastern Zambia), or a continuous presence throughout the year (in equatorial Africa). If the small non-diapausing equatorial ticks would be transported to an unfavorable environment where a diapause is necessary, the small ticks would probably not survive in the long term. If large ticks from southern Zambia for example, were transported to equatorial Africa with favorable condition throughout the year, there is no reason for them not to survive, but they would probably be absorbed in the large pool of small non-diapausing ticks.

The theories developed in this research apply especially to ticks, but relate to more general theories: at the evolutionary stable equilibrium point, the proportional increase in fitness resulting from the production of slightly larger offspring just equals the proportional decrease in fitness resulting from the fewer offspring that result from the increased expenditure on each offspring (Lloyd 1987). The concept of trade-offs between progeny size and number is an integral part of life history theory (Fox and Czesak 2000). Resources directed to reproduction can be divided into either many small progeny or a few larger progeny. In general, trade-offs have been detected in most studies of relatively semelparous arthropods that use larval-acquired resources for egg production and exhibit no parental care (Fox and Czesak 2000). In studies of more complex systems (especially vertebrates), in which females are iteroparous, use adult-acquired resources for reproduction, use adult-acquired resources for reproduction (e.g. shrimp, mosquitoes), or exhibit parental care (e.g. birds), a trade-off has been difficult to demonstrate (Glazier 1998) leading some authors to suggest that such trade-off is not universal (Bernardo

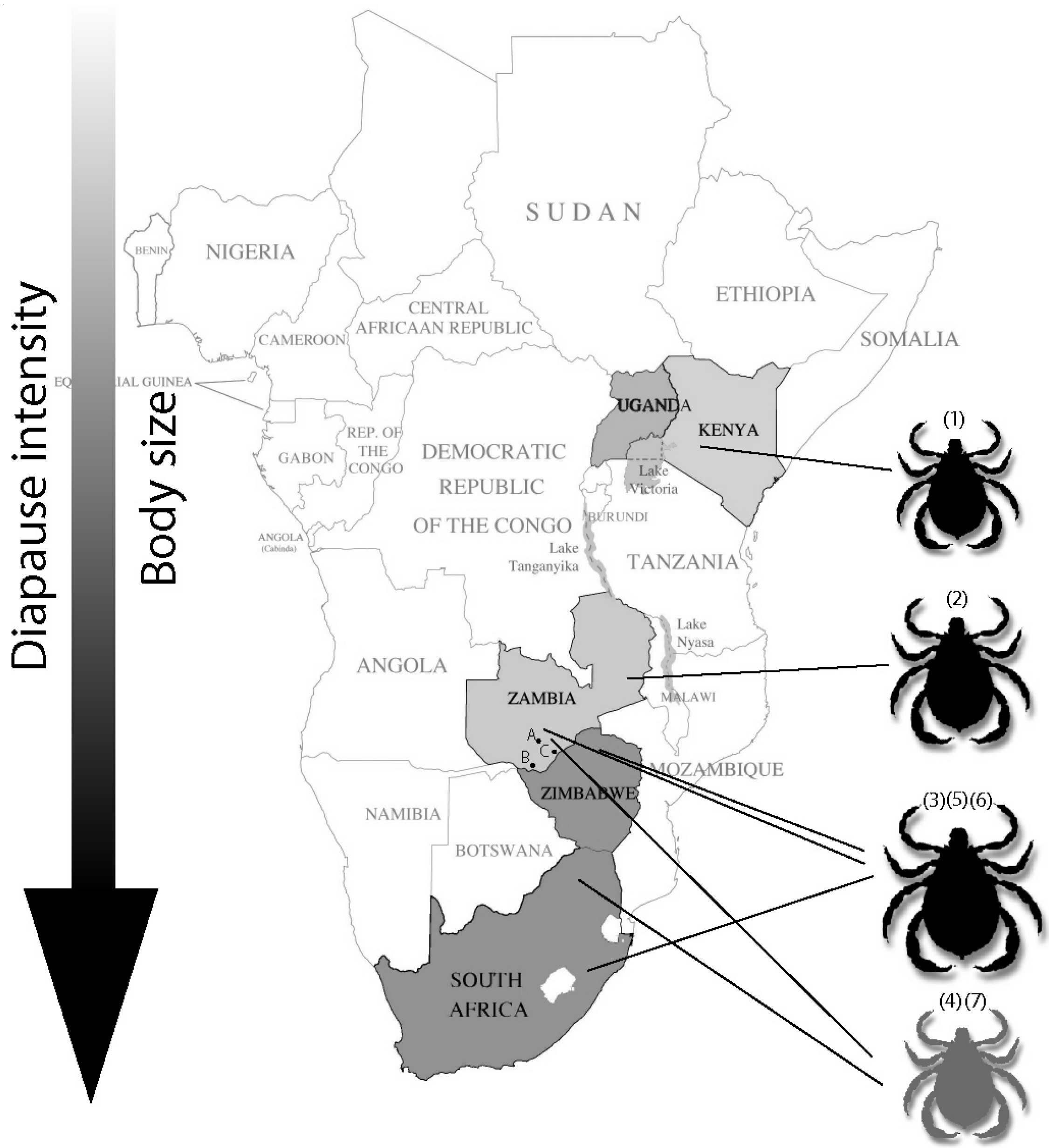

Figure 1. Body size (mm) of laboratory populations of *Rhipicephalus appendiculatus* (black) and *Rhipicephalus zambeziensis* (grey) originating from different countries in Africa: (1) Kenya (Muguga), (2) eastern Zambia (Wafa), (3) southern Zambia (Nkonkola), (4) southern Zambia (Keemba), (5) Zimbabwe (West-Mashonaland), (6) South Africa (Limpopo province), and (7) South Africa (Rietvlei strain). Also indicated: localities in which monthly collections were done: A: Nteme, B: Livingstone, C: Siabwengo.

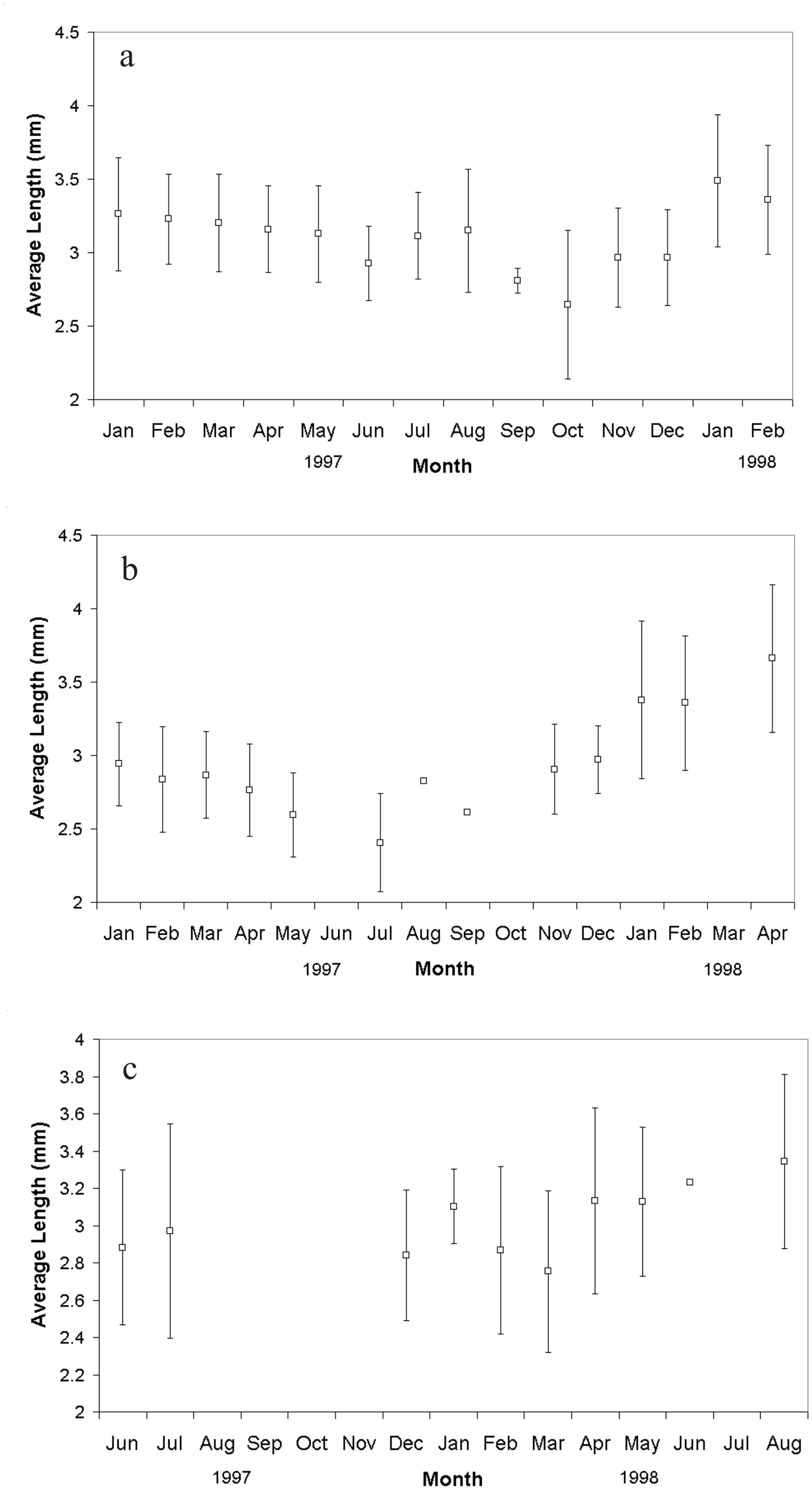

Figure 2: Average monthly male *Rhipicephalus appendiculatus* / *Rhipicephalus zambeziensis* conscutal lengths (and standard deviations when more than one observation) at Livingstone (a), Nteme (b), and Siabwengo (c).

1996).

In eastern Zambia, Chaka et al. (1999) reported smaller ticks at the beginning of the adult tick season (October to November, before the rains) compared to ticks feeding later during the rains (December to April). This might be explained by a faster diapause completion, due to an increased physiological age, in small ticks at the beginning of the rains. The sharp drop of tick body size observed at the start of the dry season in eastern Zambia (Chaka et al. 1999) can be explained by the dwindling numbers of larger ticks during the rainy season and an increasing number of smaller second generation emerging ticks that result from the ticks that terminated diapause earlier. The possible link between diapause and larger body size may explain this observation.

In southern Zambia, large ticks terminate diapause, showing a sudden upsurge in numbers at the start of the rainy season and are not replaced by small ticks from a second generation to the same extent as in eastern Zambia. This may explain the less obvious decrease in body size when the dry season draws nearer. The low numbers of small ticks at the end of the rains are probably non-diapausing adults that do not result in a viable population due to reasons explained in Speybroeck et al. (2002). From the start of the dry hot season onwards (September), there is an increasing trend in body size in time probably due to the higher survival of the questing larger ticks. Here it has to be noticed that only very low numbers of adult *R. appendiculatus* are found during this period. In 1998, ticks in Livingstone and Nteme were larger than those found in 1997. This might be explained by worse weather conditions in the dry season preceding 1998 as compared to the one preceding 1997. During a harsh dry season, larger numbers of smaller diapausing ticks might die.

Although a body size component will have to be included when designing new field trials in the future, one has to be careful when drawing conclusions comparing body sizes of field collections of ticks from different localities as these can be influenced by local factors.

It remains essential to elucidate the important relationship between phenology and body size in order to understand the species' ecology in the framework of integrated *T. parva* control programs. An understanding of the possibility of a second generation of adults is important from an epidemiological point of view (Billiouw et al. 1999). Firstly, it assures a more continuous presence of transmitting instars throughout the year, with adult tick peaks in January and May and nymph peaks in May and September. Secondly, the second wave of adult ticks is non-diapausing, which transmits *T. parva* infection soon after molting, when the parasite is still highly infective. Thirdly, they result in an overlap of transmitting instars, leading to highly efficient transmission of *T. parva* since fast transmission from cattle to cattle is possible and, perhaps more importantly, because infection may be caught from clinically ill cattle.

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