DIFFERENCES IN NYMPHS OF *AMBLYOMMA TRIGUTTATUM TRIGUTTATUM* KOCH MOULTING TO MALES OR FEMALES

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TICKS
NYMPHS
NUTRITION
WEIGHT
PREMOULT
PERIOD
SEX

SUMMARY : The mean weight of engorged female nymphs was greater than for male nymphs ; after feeding on rabbits the mean weights were 51.55 and 33.18 mg respectively, and on rats 50.96 and 34.28 mg. The peak of detachment was seven days after infestation for nymphs of either sex fed on rabbits and eight days for those fed on rats. The final weight of nymphs of either sex was not related to the feeding period. The premoult time at 30°C and 2-3 mm Hg saturation deficit in darkness was longer in female nymphs (19.7 days) than in males (17.7 days). Heavier engorged larvae produced heavier engorged nymphs which, in turn, gave rise to more females than males. Prediction of the sex of nymphs using engorgement weights was unreliable due to weight overlap. The different sizes of male and female nymphs must create problems in developing a technique for accurately evaluating field populations on hosts of bisexual three-host ticks. The different premoult periods and weights of male and female nymphs must be taken into account in experimental designs involving engorged nymphs.

TIQUES
NYMPHES
REPAS
POIDS
PRÉ-MUE
SEXE

RÉSUMÉ : Le poids moyen des nymphes femelles gorgées s'est montré plus élevé que celui des nymphes mâles ; après un repas sur des lapins les poids moyens ont été respectivement de 51,55 mg et 33,18 mg, et sur des rats de 50,96 mg et 34,28 mg. Le pic de détachement s'est produit sept jours après l'infestation chez les nymphes de l'un et l'autre sexe nourries sur des lapins, huit jours après chez celles nourries sur des rats. Le poids final des nymphes de l'un et l'autre sexe n'a pas été en relation avec la durée du repas. Le temps de pré-mue, à 30° et 2-3 mm de Hg en déficit de saturation à l'obscurité, a été plus long chez les nymphes femelles (19,7 jours), que chez les nymphes mâles (17,7 jours). Les larves les plus fortement gorgées ont fourni les nymphes les plus fortement gorgées qui, en retour, ont engendré plus de femelles que de mâles. La prévision du sexe des nymphes au moyen de leur poids après engorgement s'est révélée inefficace en raison du chevauchement de ces poids. Les tailles différentes des nymphes mâles et des nymphes femelles peuvent créer des problèmes pour le développement d'une technique satisfaisante d'évaluation des populations naturelles des hôtes de tiques bisexuées à trois hôtes. Les périodes de pré-mue différentes et les différences de poids des nymphes mâles et nymphes femelles doivent être prises en considération dans les projets expérimentaux mettant en œuvre des nymphes gorgées.

CARRAPATAS
NINFAS

RESUMEN : El peso medio de repleción fue mayor en ninfas que mudaron a hembras que las que lo hicieron a machos : 51,55 y 33,18 mg, respectivamente, cuando

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COMIDA
PESO
PRE-MUDA
SEXO

alimentadas sobre conejos ; 50,96 y 34,28 mg en las alimentadas sobre ratas. Sin embargo la predicción del sexo de las ninfas por su peso es de escaso valor aplicado debido a superposición en los rangos. La moda del período parasitario fue de 7 días en ninfas de ambos sexos alimentadas sobre conejos y de 8 días sobre ratas. No se detectó relación entre el período parasitario y el peso final de las ninfas. El período medio de pre-muda, a 30°C, 2-3 mm Hg de déficit de saturación en la oscuridad fue más largo en ninfas que mudaron a hembras (19,7 días) que las que lo hicieron a machos (17,7 días). Las larvas repletas de mayor peso produjeron ninfas repletas más pesadas que a su vez originaron mayor cantidad de hembras. Las diferentes dimensiones de las ninfas de distinto sexo puede crear problemas para el desarrollo de técnicas precisas para evaluar poblaciones de garrapatas bisexuales de tres hospedadores sobre animales. Además, las diferencias en peso y en período de pre-muda de ninfas de distinto sexo debe ser tenida en cuenta en diseños experimentales que involucren a este estadío evolutivo.

INTRODUCTION

ARTHUR and SNOW (1966) reported that two weight groups were present throughout the larval and nymphal populations of *Hyalomma anatolicum anatolicum*. The group of heavier immature stages produced females and the lighter, males. Later investigations involving various tick species showed that nymphs moulting to females were heavier than those moulting to males, for example *Argas arboreus* (HAJJAR, 1972) ; *Amblyomma tuberculatum* (COONEY and HAYS, 1972) ; *H. marginatum rufipes* (KNIGHT, et *al.*, 1978) ; *Rhipicephalus glabroscutatum* (RECHAV and KNIGHT, 1981). Although many species remain to be studied, the occurrence of heavier female nymphs seems to be a common feature in the suborder Ixodoidea.

GLADNEY, et *al.* (1977), KNIGHT et *al.* (1978), OSBURN (1981), and RECHAV and KNIGHT (1981), dealing with *A. inornatum, H. marginatus rufipes, Dermacentor albipictus* and *R. glabroscutatum* respectively, noted that the male nymphs completed engorgement earlier than female nymphs, while AUBERT (1981) stated that male and female nymphs of *Ixodes rugicollis* dropped from the host at the same rhythm. The premoult periods of male and female nymphs form patterns which can be divided into three groups. In the first, the males moult earlier than the females. The majority of ticks so far studied fall into this category, including *Ornithodoros rostratus* (GUGLIELMONE and HADANI, 1980) ; *A. inornatum* (GLADNEY et *al.* 1977) ; *Aponomma hydrosauri* (BULL, et *al.*, 1977) ; *Boophilus microplus* (BOERO and D'ANGELO, 1947) ; *R. e. evertsi* (RECHAV, et *al.*, 1977). In the second group no differences have been found in the premoult period of males or females, for example *A. tholloni* (NORVAL et *al.*, 1980) ; *I. rugicollis* (AUBERT, 1981) ; *R. glabroscutatum* (RECHAV and KNIGHT, 1981). In the final group the female nymphs moult earlier than the male, as in *H. dromedarii* (FELDMAN-MUHSAM and MUHSAM, 1966) ; *R. appendiculatus* (BRANAGAN, 1973) and *R. zambeziensis* (WALKER, et *al.*, 1981). COLBORNE and NORVAL (1982) noted that the premoult pattern of male and female nymphs of *I. matopi* varied according to the time of year when the nymphs were feeding. The present investigation was carried out to help elucidate the biology of *A. triguttatum triguttatum* ; this is a three-host-tick peculiar to Australia, and its natural hosts are the larger macropods, although it also attacks domesticated animals and man (ROBERTS, 1970).

Throughout this article the abbreviation *A.* applies to *Amblyomma*, *Aponomma* is written in full. Similarly *Hyalomma* is abbreviated to *H.*, while *Haemaphysalis* is in full.

MATERIALS AND METHODS

■ TICKS : Larvae and nymphs were collected in Durikai State Forest (SE Queensland) with carbon dioxide as the attractant, between July and

October 1981. Ticks were used within 24h of capture.

HOSTS AND FEEDING : Adult male rats (Wistar strain) and adult male rabbits (White Cloud X Dutch) were used as hosts and each animal was used for only one feeding trial. Hosts were restrained to prevent the ticks being shaken off. Unless otherwise stated, cages containing rats were covered with muslin and the ears of rabbits with cotton bags for 24h after infestation to prevent the escape of ticks. Experiments were conducted at 25 ± 2°C and 75 ± 10 % relative humidity, with a photoperiod of 12h dark and 12h light.

The majority of *A. t. triguttatum* detach during daylight (GUGLIELMONE, unpublished data). Engorged ticks were collected at 2-3h intervals during the light period.

■ AFTER FEEDING : Engorged ticks were held in glass tubes (5 × 1 cm) with the ends covered with muslin held by perforated plastic lids. They were held in glass jars of 1.4 litre capacity. Containers of sulphuric acid solution, prepared according to SOLOMON (1952), were placed in the jars both above and below the tick tubes to provide the necessary humidity, and to avoid humidity gradients within the jars.

Apart from the first trial, where the temperatures used are mentioned later, all ticks were held in the dark at 30° ± 1°C and 2-3 mm Hg saturation deficit. Ticks were weighed on an analytical balance with can accuracy of 0.1 mg.

In the first trial, one rabbit was parasitized with 75 unfed nymphs, two rabbits and four rats with 50 nymphs each, and two rats with 25. Feeding periods and engorgment weights were recorded and nymphs were then individually held at temperatures ranging from 25 to 35°C until moulting, when their sexes were recorded.

In a second trial, two rabbits were each parasitized with 100 nymphs ; later the premoult period and the sex of the resulting adults were determined from 140 of the 175 engorged nymphs recovered.

Finally, one rabbit was infested with 113 larvae. Forty-two undamaged engorged larvae from the 48 recovered were weighed and divided into two groups according to their weights.

The first group comprised 23 larvae weighing from 0.5 to 1.45 mg, while the second comprised 19 larvae weighing from 1.5 to 2.45 mg.

Twenty-one nymphs from the first group moulted and 16 from the second. These were subsequently allowed to feed on another rabbit when approximately 15 days old. Each group of nymphs was fed on a different ear and was covered with an ear bag that remained in place until the detachment of the last tick, so as to prevent mixing of the groups. The engorgement weight of nymphs and sex, after moulting, were recorded.

RESULTS AND DISCUSSION

The feeding period of male nymphs fed on rabbits ranged from 6 to 10 days, while the range for female nymphs was 6 to 11 days. The peak of detachment of each sex was day 7. The range of feeding female and male nymphs on rats was 7 to 11 days and the peak of detachment was on day 8 with nymphs of either sex.

Female nymphs (Table 1) were significantly heavier ($P<0.01$ — standard normal test) than males, regardless of the kind of host used, and no differences were found in the weights of male and female nymphs fed on rabbits or rats.

In spite of the highly significant difference (Table 1) between the mean engorgement weights

TABLE 1 : Engorgement weight (mg) of male and female nymphs * fed on rats and rabbits.

HOST	RABBIT	RAT	
Mean weigth + S. D. of female nymphs	51.11 ± 10.864	50.96 ± 8.686	n. s.
Range	33.1 — 72.2	31.0 — 68.9	
n	70	55	
Mean weight + S. D. of male nymphs	33.18 + 5.708	34.28 + 4.663	n. s.
Range	21.2 — 44.2	24.2 — 46.6	
n	63	58	
	P<0.01		

* Sex determined after moulting.
n. s. — non-significant.

of male and female nymphs, the prediction of sex, using the engorgement weight, is difficult due to the overlap of the weights, as seen with *A. variegatum* (CENTURIER and KLIMA, 1979) and *H. a. anatolicum* (ARTHUR and SNOW, 1966). They differ from species such as *A. tuberculatum* (COONEY and HAYS, 1972), *A. inornatum* (GLADNEY et *al.*, 1977), and *A. americanum* (KOCH, 1981) where the engorgement weights can be used with accuracy to predict the sex of the nymphs.

As in *H. a. anatolicum*, studied by ARTHUR and SNOW (1966), the difference in weight of engorged nymphs of *A. t. triguttatum* was related to the weight of the engorged larvae and that was reflected in the proportion of males and females obtained from lighter or heavier larval groups (Table 4). A technique for indicating ticks that will engorge and detach during the following 24h, using body length, was developed by WHARTON and UTECH (1970) to sample the one-host-tick, *B. microplus*, while WAGLAND et *al.* (1979) adapted this in the study of the parthogenetic race of *Haemaphysalis longicornis*. No such method has yet been developed for the different stages of economically important, bisexual, three-host-ticks. The present investigation shows that the development of such a technique for larvae and nymphs of these ticks would be complicated by the difference in size of the two sexes, reflected in the disimilar weights of pre-imaginal stages moulting to females or males, making the accurate field evaluation of pre-imaginal populations on their hosts more difficult.

The capacity of nymphs of each sex to gain weight was not related to the duration of the feeding period (Table 2) but the factor(s) which regulate it, although probably genetic, are unknown.

TABLE 2 : Correlation between feeding period and weight of female and male nymphs * fed on rabbits and rats.

HOST	RABBIT		RAT	
Sex of the nymphs	female	male	female	male
	70	63	55	58
Correlation cœfficient	0.123	0.226	0.021	— 0.092
	n. s.	n. s.	n. s.	n. s.

* Determined after moulting.
n. s. — non-significant.

TABLE 3 : Pre-moult period (days) of male and female nymphs * fed on rabbits and kept at 30 ± 1°C, 2-3 mm Hg saturation deficit in darkness.

	Male nymphs	Female nymphs	
Mean pre-moult period ± S. D.	17.7 ± 1.23	19.7 ± 1.59	P<0.01
Range	15 — 21	16 — 23	
n	51	85	

* Sex determined after moulting.

TABLE 4 : Engorgement weights (mg) of nymphs and sex of adults resulting from two groups of larvae arranged according to their engorgement weights.

	Larval group I	Larval group II	
Range of larval engorgement weight	0.5 — 1.45	1.5 — 2.45	
n	23	19	
Mean weight ± S. D. of engorged nymphs	28.88 ± 6.556	44.51 ± 10.488	P<0.01
Range	20.5 — 44.3	28.4 — 61.3	
n	16	15	
Sex — Males	9 (64 %) *	5 (36 %)	
— Females	6 (37.5 %)	10 (62.5 %)	

* The percentage (in parentheses) of the total number of that sex recovered.

The short premoult period of male nymphs (Table 3) places *A. t. triguttatum* in the most common group of ticks where males moulted earlier than females. With this species the different premoult periods of male and female nymphs and the impossibility of predicting, with accuracy, the sex of nymphs from the weight, indicates the importance of distributing nymphs of different weights evenly between experimental groups in scientific studies. RECHAV and KNIGHT (1981) stated that the mechanisms which regulate the early emergence of males can be either a shorter feeding period for male nymphs or a shorter premoult period ; the present investigation provides evidence that the latter mechanisms act with *A. t. triguttatum*.

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