

Nymphal sexual dimorphism in the sheep tick *Ixodes ricinus* (Acar: Ixodidae)

František Dusbábek

Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

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Abstract. Unfed nymphs of *Ixodes ricinus* (L.) can be divided into two morphological groups according to the length of idiosoma, scutum, hypostome and palpal segment III, and the number of dorsal alloscutal setae. Specimens of greater body dimensions and more numerous dorsal alloscutal setae moulted predominantly into females. The frequency of different nymphal length categories in field-collected ticks followed a normal distribution. The length of unfed nymphs correlates well with the length ($r = 0.7248 \pm 0.0711$, $P < 0.001$) and weight ($r = 0.6519 \pm 0.0782$, $P < 0.001$) of engorged nymphs, however, it varies in ticks of different origin. In field-collected ticks, freshly engorged female nymphs were 2.30–2.94 mm long, male nymphs 2.14–2.46 mm long. Feeding period ($P < 0.05$) and premoult period ($P < 0.001$) were significantly longer in female nymphs both in field-collected and laboratory-derived *I. ricinus*. The engorgement weight was found to be the best criterion for differentiation of male and female nymphs of ixodid ticks. In field-collected nymphs engorged on BALB/c mice, 98.6 % of females moulted from nymphs weighting more than 3.60 mg, while in laboratory-derived ticks, 98.4 % of females emerged from nymphs of 3.42 mg body mass or more.

Males of ixodid ticks differ from females and immature stages mostly in the increased sclerotization of the dorsum of the idiosoma; the scutum covers almost all the dorsum of the male but is restricted to the anterior region of the idiosoma in the female and nymph (Evans 1992). The length and form of hypostome is also different in many species, being long and acute in females, but short and blunt in males. In *Ixodes ricinus* (L.) the morphology of nymphal stages follows the morphology of females both in the form of scutum and hypostome.

Arthur and Snow (1966) reported that two weight groups occurred throughout the engorged larval and nymphal populations of *Hyalomma anatolicum anatolicum* Koch. The group of heavier immature stages produced females and that of lighter ones males. This was confirmed by many authors in different species of Ixodidae and even in Argasidae. Several authors also mentioned that male nymphs completed engorgement earlier than female nymphs and demonstrated differences in the length of premoult period between male and female nymphs (for more details see Guglielmone and Moorhouse 1985). Belozerov et al. (1993) found differences in the size and body mass between engorged male and female nymphs of *Ixodes rubicundus* Neumann. Voltzit (1986, 1987, 1988, 1989) succeeded in finding differences in morphology, such as length of scutum, gnathosoma, palps and hypostome in which engorged female nymphs of *Ixodes persulcatus* Schul-

ze, *I. uriae* White and some species of *Dermacentor* and *Hyalomma* differ from male nymphs.

Kahl et al. (1990) and Dusbábek et al. (1994, 1995) distinguished the female and male nymphs of *I. ricinus* according to their engorgement weight. While Kahl et al. (1990) considered the nymphs engorged on gerbils weighing less than 4.0 mg to be the male nymphs and those heavier than 4.4 mg to be female nymphs, in papers of Dusbábek et al. (1994, 1995) the limiting weight for distinguishing of the male and female nymphs engorged on BALB/c mice was only 3.5 mg. Because of this variability in the engorgement weight limits of female and male nymphs, we tried to find some more objective criteria for nymphal sex pre-determination in *I. ricinus*, a widely used species in many experimental studies of immunoresistance, physiology, behavioural biology and control.

MATERIALS AND METHODS

Laboratory-reared ticks

Nymphs of the third generation of pathogen-free laboratory colony of *Ixodes ricinus*, originating from localities around České Budějovice, were maintained at 20°C and 90–95 % r.h. under a 12 : 12 h light/dark cycle. Nymphal

Table 1. The main differences ($P < 0.05$ or 0.01) in measurement of unfed male and female nymphs of *Ixodes ricinus* in μm . Means \pm SD. Means followed by the same letter are not significantly different ($P > 0.05$).

Characters	Laboratory-derived ticks		Field-collected ticks	
	δ nymphs n = 14	φ nymphs n = 16	δ nymphs n = 50	φ nymphs n = 50
Idiosoma length	1098.75 ^a \pm 39.52	1198.07 ^b \pm 43.73	1072.72 ^c \pm 45.89	1159.60 ^d \pm 40.13
Scutum length	599.51 ^a \pm 24.45	637.5 \pm 21.36	575.62 ^c \pm 17.11	617.08 ^d \pm 15.71
Hypostome length	204.13 ^a \pm 9.29	224.64 ^b \pm 6.41	199.62 ^a \pm 10.21	213.36 ^b \pm 8.89
Third palpal segment length	277.0 ^a \pm 13.37	294.0 ^b \pm 12.83	274.64 ^a \pm 11.83	294.58 ^b \pm 10.97
No. of dorsal alloscutal setae	47.71 ^a \pm 4.34	53.69 ^b \pm 8.33	48.88 ^a \pm 6.03	51.62 ^b \pm 5.93

ticks were allowed to feed in batches of 10 nymphs on naive BALB/c mice in plastic capsules glued on the shaved back of each mouse, according to the method of Den Hollander and Allen (1985). From 520 nymphs 426 (81.9 %) engorged. Freshly engorged nymphs were weighed on Sartorius electronic balance (precision 0.01 mg) and kept individually inside the wells of 96-well microplates closed with foam rubber plugs and inspected daily. Length of feeding and premoult periods and the sex of moulted adults were registered.

Field-collected ticks

Nymphal *I. ricinus* were collected in localities around České Budějovice by flagging on August 19 and September 1, 1994. A total number of 254 nymphs was measured between two microscopic slides and divided into 13 length categories from 0.96 to 1.32 mm. Nymphs of each length category were fed on BALB/c mice separately and maintained similarly to the ticks of the laboratory population. From 197 nymphs 121 (61.42 %) engorged. Feeding period, premoult period, engorgement length and weight of freshly engorged nymphs and the sex of adults moulted from each nymphal length category were noted.

Morphological studies

For morphological studies, unfed nymphs were mounted between microscopic slides in Swan's chloralhydrate medium (Daniel 1969) and measured in the optical microscope. Both laboratory-reared and field-collected ticks were used, the latter being collected on April 18, 1995 in localities around České Budějovice. The following 13 morphological characters were studied: idiosoma length, scutum length, width and length/width ratio, diameter of spiracular plate, number of ventral podosomal setae and pores, number of scutal and dorsal alloscutal setae, length of hypostome, palpal segment III, tarsus I and dorsal alloscutal setae.

RESULTS

The morphological studies made it possible to divide unfed nymphs into two morphological groups, which differed significantly ($P < 0.05$ – 0.01) in the length of

idiosoma, scutum, hypostome and palpal segment III, and the number of dorsal alloscutal setae (Table 1). From these, the least variable seems to be the length of idiosoma and scutum ($v = 2.55$ – 4.28 in different sexes and populations), the most variable the number of dorsal alloscutal setae ($v = 9.10$ – 15.52). In other characters, namely scutum width, scutum L/W ratio, diameter of spiracular plate, number of ventral podosomal setae and pores, length of tarsus I, number of scutal setae and length of dorsal alloscutal setae, no significant differences ($P > 0.05$) were recorded among groups of nymphs within the same origin. Nevertheless, the body dimensions in field ticks were generally smaller than in the laboratory-reared sample.

The frequency of different unfed nymphal length categories in field-collected ticks follows a normal distribution (Fig. 1). The most frequent nymphal length category 1.11 mm included 23.6 % of all measured ticks. Once engorged 52.0 % became males and another

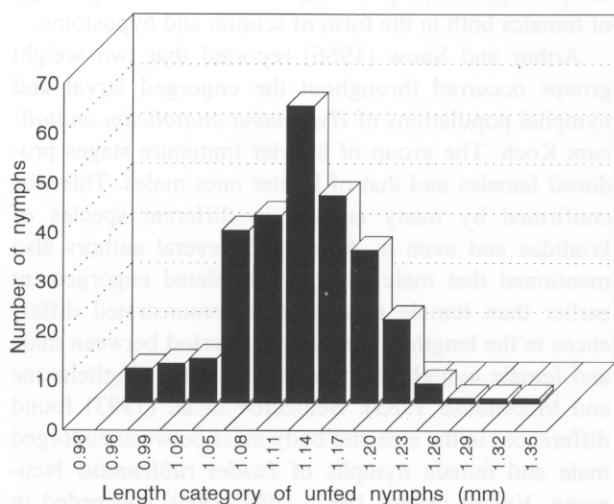


Fig. 1. Frequency of different length categories in unfed field-collected nymphal *Ixodes ricinus*.

Table 2. The main differences between male and female nymphs of *Ixodes ricinus*. Means \pm SD. Means followed by the same letter are not significantly different ($P > 0.05$).

Parameter	Laboratory-derived ticks		Field-collected ticks	
	♂ nymphs	♀ nymphs	♂ nymphs	♀ nymphs
Number of nymphs	239	187	48	73
Unfed body length (mm)	1.09 ^a \pm 0.04	1.20 ^b \pm 0.05	1.07 ^a \pm 0.06	1.15 ^b \pm 0.06
Unfed body weight (mg)	—	—	0.13 ^a \pm 0.02	0.19 ^b \pm 0.04
Feeding period (days)	4.02 ^a \pm 0.59	4.33 ^b \pm 0.64	4.13 ^a \pm 0.33	4.33 ^b \pm 0.55
Premoult period (days)	119.94 ^a \pm 18.78	121.07 ^b \pm 21.62	137.84 ^c \pm 32.19	141.85 ^d \pm 34.57
Engorged body length (mm)	—	—	2.31 ^a \pm 0.10	2.71 ^b \pm 0.14
Engorged body weight (mg)	2.61 ^a \pm 0.32	4.22 ^b \pm 0.50	2.97 ^c \pm 0.44	4.94 ^d \pm 0.76

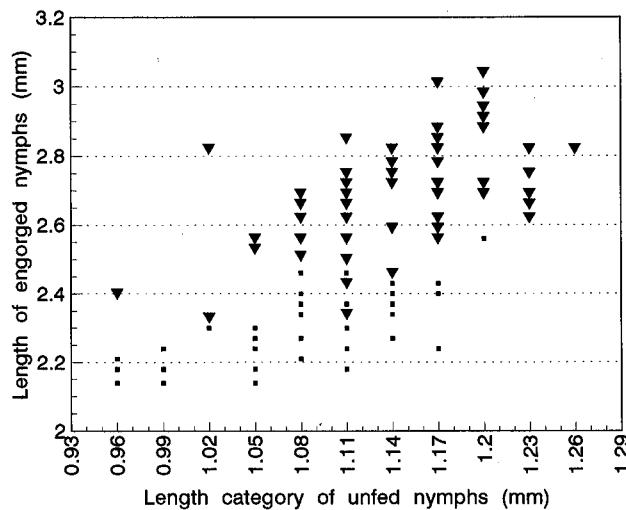


Fig. 2. Relationships between body length of field-collected nymphal *Ixodes ricinus* in the unfed and engorged state and the sex of moulted adults (■ – males, ▼ – females).

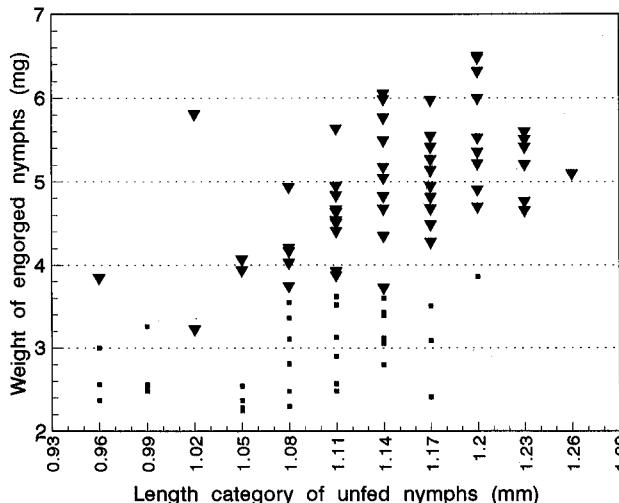


Fig. 3. Relationships between body length of unfed nymphs, the weight of engorged nymphs and the sex of moulted adults in field-collected samples of *Ixodes ricinus* (■ – males, ▼ – females).

48.0 % females. Males moulted from nymphs of 0.96–1.20 mm unfed length categories, females emerged from those nymphs with 1.02–1.26 mm unfed length (with one exception measuring 0.96 mm) (Figs. 2 and 3). A good correlation occurred between unfed nymphal length categories and the weight or length of freshly engorged nymphs. The correlation coefficients (r) and slopes (b) were as follows: $r = 0.7248 \pm 0.0711$, $b = 2.6645 \pm 0.2612$, $P < 0.001$ (length of unfed and engorged nymphs) and $r = 0.6519 \pm 0.0782$, $b = 12.1364 \pm 1.4561$, $P < 0.001$ (length of unfed nymphs and the weight of engorged nymphs).

Sexual dimorphism seems to be best expressed by the length and weight of freshly engorged nymphs. In field-collected tick males moulted from engorged nymphs 2.14–2.46 mm long and with 2.25–3.62 mg body mass, females emerged from nymphs 2.30–2.94 mm long and weighing 3.21–6.98 mg (Fig. 4). About 97.9 % of males and only 2.1 % of females moulted from nymphs weighing 3.60 mg or less, while 98.6 % of females and only 1.4 % of males moulted from nymphs weighing more than 3.60 mg. In the laboratory-reared ticks, about 97.5 % of males moulted from nymphs of 2.03–3.43 mg engorgement weight, while 98.4 % of females moulted from nymphs with 3.42–5.82 mg engorgement weight (Fig. 5). The engorgement weight was also different between laboratory and field ticks, with the former being heavier (Table 2). The feeding and premoult period were longer in nymphs which moulted into females. The premoult period also differed between laboratory-reared and field-collected ticks. Although nymphs of both samples underwent winter diapause during the experiment, diapause in the laboratory-derived nymphs was terminated earlier than in the field-collected companions (Table 2).

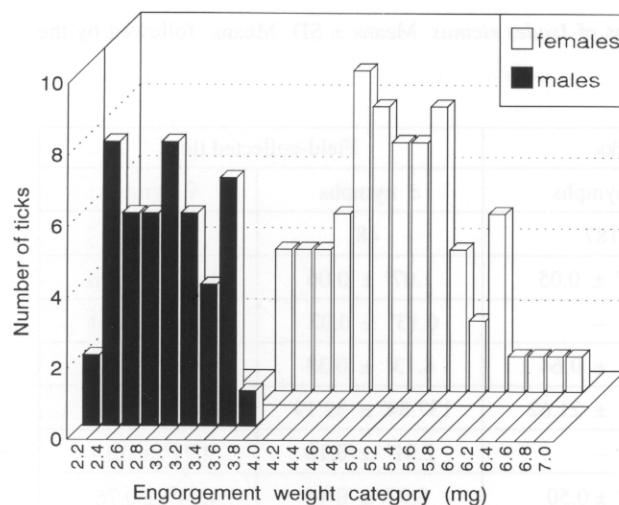


Fig. 4. Relationships between nymphal engorgement weight and the sex of moulted adults in the field-collected samples of *Ixodes ricinus*.

DISCUSSION

There are good morphological features for differentiation of unfed male and female nymphs of the sheep tick, *I. ricinus*, i.e. the length of idiosoma, scutum, hypostome and palpal segment III, and the number of dorsal alloscutal setae. Although there is a great variability in some of these characters, the combination of all five characters makes it possible to distinguish the sex predetermination of nymphs with a high probability. The limiting values of measurements for sex determination, however, frequently coincide and can be different in ticks from different populations.

The best criterion for differentiating male and female nymphs of *I. ricinus* seems to be the nymphal engorgement weight (Kahl et al. 1990, Dusbábek et al. 1994, 1995). Nevertheless, the results indicate that the body

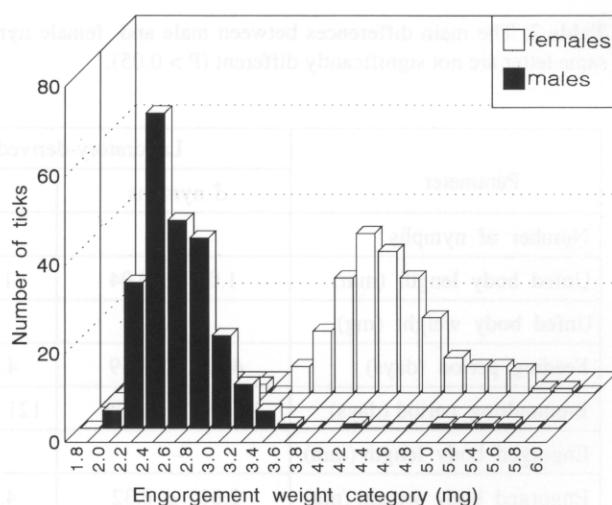


Fig. 5. Relationships between nymphal engorgement weight and the sex of moulted adults in the laboratory colony of *Ixodes ricinus*.

weight limits for sex determination may be also different in different tick populations and in ticks engorged on different host animals. According to Kahl et al. (1990) the maximal weight of freshly engorged male nymphs was 4.0 mg and the minimal weight for female nymphs 4.4 mg. In our experiment with nymphs engorged on BALB/c mice, the body weight limit for male and female nymphs differentiation was 3.6 mg in the natural population and 3.4 mg in the laboratory one. The body length of engorged nymphs and the feeding and premoult periods, although significantly different in female and male nymphs, are of less importance for nymphal sex determination because of their great variability.

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