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DEVELOPMENTAL CYCLE OF CHIGGERS UNDER LABORATORY CONDITIONS

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Abstract. The paper describes the developmental cycle of two chigger species: *Neotrombicula autumnalis* and *N. zachvatkini*. With *N. autumnalis* the development of the generation coming from larvae collected in nature was studied, while with *N. zachvatkini* also the development of laboratory-reared generations was observed.

In order to learn the biology of chiggers, to elucidate some taxonomic obscurities and to carry out experiments with larvae as vectors of infectious agent irrespective of their seasonal occurrence, culturing of these mites under laboratory conditions is indispensable. Wharton and Fuller (1952) summarized all results obtained until the fifties of this century in their monograph. The chiggers are successfully cultured on a large-scale, with mass production of larvae, in research institutions in Japan and USA (Nadchatram 1968). The method of allowing larvae to feed on laboratory animals was used by several authors (Neal and Lipovsky 1959, Smith 1966, Baker et al. 1968). Studies on the developmental cycles of chiggers cultured in laboratory were published by André (1930) and Minter (1957) who dealt with *Neotrombicula autumnalis*, by Shoshina (1964) and Kudryashova (1972) who studied *Neotrombicula zachvatkini* and by Cunningham et al. (1975) who treated *Neoschoengastia americana*.

MATERIAL AND METHODS

The initial material for culturing *Neotrombicula zachvatkini* (Schlug.) were the engorged larvae collected from small terrestrial mammals which were trapped in South Moravia in the winter of 1971/72. The larvae of *N. autumnalis* (Shaw) came from a live host, the bank vole (*Clethrionomys glareolus*), most of them were obtained in August 1971 and 1972 by exposure to three host species mentioned below (Daniel 1969) in front gardens in the suburbs of Prague where this chigger occurs in masses.

The chiggers were kept in 5—10 ml glass chemical weighing bottles with fitted covers. The substrate maintaining a constant relative humidity was composed of a mixture of plaster and activated charcoal at the 1 : 1 ratio. Eggs of spring-tails (*Sinella curviseta*) were used as food of post larval stages. Fasting larvae were allowed to feed on the white laboratory mouse, hamster (*Mesocricetus auratus*) and on a two-week-old chicken. The culturing methods are described in detail in the paper by Simonová (1977).

The developmental cycle was studied primarily at room temperature (average 22 °C), with the species *N. zachvatkini* also at temperatures of 9 °C, 15 °C, and 28 °C. Tables were based on data obtained from chiggers reared separately.

RESULTS

NEOTROMBICULA AUTUMNALIS

The development from engorged larvae to adults was observed in three chigger groups according to different hosts, always at room temperature (Table 1).

Table 1. Development of *Neotrombicula autumnalis* from different hosts at room temperature

Stage		Host	Duration of stage in days				Total number	% of larvae	% of preceding stage
			min.	max.	most frequent	average			
Active engorged larva		hamster	3	18	5—10	8	202	100	—
		chicken	—	19	7—13	—	131	100	—
nympho-chrysalis	formation	hamster	2	11	4—7	5	186	92.1	92.1
	persistence	vole	12	27	12—14	16	41	93.2	93.2
		hamster	9	17	10—15	13	156	77.2	83.9
Active nymph		hamster	12	413	30—60	59	155	76.7	99.4
		chicken	15	85	26—35	37	76	58.0	—
imagochrysalis	formation	hamster	3	15	4—8	5	93	46.0	60.0
		chicken	2	8	3—6	5	52	39.7	68.4
	persistence	vole	15	24	20—22	20	22	50.0	57.9
		hamster	8	19	14—17	14	41	20.3	44.1
		chicken	13	21	14—17	17	47	35.9	90.4
imago		vole	—	—	—	—	7	15.9	31.8
		hamster	—	—	—	—	34	16.8	82.9
		chicken	—	—	—	—	47	35.9	100
larva → imago		vole	85	131	104—124	114	—	—	—
		hamster	80	144	90—120	109	—	—	—
		chicken	40	85	40—60	56	—	—	—

Larvae. The first engorged larvae began to drop off their hosts after 24 hours. The maximum of engorged larvae were obtained the next day (65 %), on the fourth day there were only solitary specimens (1.5 %). The time necessary for engorgement did not essentially depend on the species of host animal. The viability detected from the percentage of moulted nymphs did not show any important difference either. The majority of engorged larvae remained motile for 7—10 days.

Nymphochrysalis. The period of the resting stage between the active engorged larva and nymph was divided into two different phases. The phase of the nymphochrysalis formation was calculated from the moment of immobilization of the engorged larva (the first pair of legs stretched upright, idiosoma swollen, rounded) to the appearance of first symptoms of changed body shape (evacuation of the anterior part of idiosoma, tapering of the idiosoma end, legs sticking out of venter). The interval between the morphological formation of nymphochrysalis and the hatching of nymph was called the phase of persistence. The formation phase was always shorter than that of persistence and together they mostly did not exceed three weeks.

Nymphs. The duration of the period of active nymph was very variable. Although in the major part of material it was no longer than two months, there were also sporadic cases with nymphal activity six times longer. With chiggers from a bird host the variance in the persistence of nymphal activity was lesser and most cases

were shifted towards lower limit. The values of the duration of nymphal stage activity being quite different, the relationship to the amount of food taken was studied. The nymph consumed on the average 1—3 eggs of *S. curviseta* per day. It took 3—5 minutes to suck up one egg. In the group of chiggers fed on hamster the nymphs consumed on the average less per day in comparison with the group initially fed on chicken and due to the total average consumption during nymphal stage being very close (80 and 96 eggs) their period of activity was also longer. Due to the considerable fluctuation of particular values of total consumption throughout the period of nymphal activity (45—224 eggs) and of specimens from all three hosts, however, the duration of the nymphal stage did not seem to depend on the amount of the food taken. The mortality of nymphs was high enough (up to 40 %) and was not caused by any dominant reason.

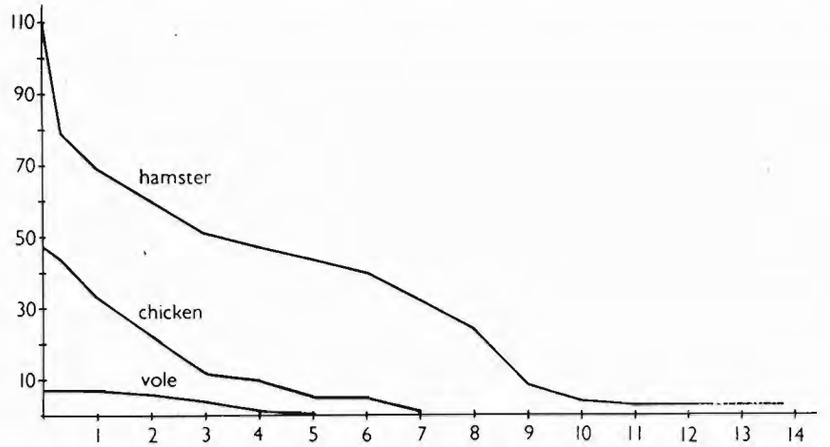

Fig. 1. Survival of *Neotrombicula autumnalis* adults at room temperature. Abscissa — months, ordinate — number of adults.

Imagochrysalis. The duration of the resting stage could be divided again into the formation phase, which was calculated from the nymph inactivation to the beginning of morphological changes and the persistence phase, which, being calculated from the formation of imagochrysalis to the hatching of adult, was always longer. In the second restring stage 81 the too phases usually did not exceed the period of three weeks.

Adults. In the most numerous group of chigger larvae fed on hamsters 36 % of adults survived after six months, 2.7 % after twelve months. The average food consumption per day was always higher than with nymphs (3—5 eggs). On the first day after moulting the adults almost always reached the maximum of daily values of food taken (as many as 18 eggs). The adults were kept in both the darkened and undarkened weighing bottles with the culture medium surface both smooth and rough; in one group natural hibernation was simulated. The deposition of spermatophores could not be ascertained. Only two sterile eggs were found which had been deposited by chiggers aged 30—60 days. In all groups observed both sexes occurred, the sex ratio being 1.7 in favour of females. No female was found with eggs (Fig. 1).

The whole cycle was observed in 88 specimens from three host species. The duration of development from engorged larvae to adults collected from voles and hamsters was not essentially different in minimum, maximum nor any most frequent values.

The total percentage of adults obtained was also similar (16 and 17 %). The developmental cycle of larvae fed on chicken was one half shorter in minimum and most frequent values, and the percentage of adults obtained was double (36 %). For laboratory culturing it is therefore more profitable to allow larvae to feed on chicken. Sterile eggs were obtained from these adults only.

NEOTROMBICULA ZACHVATKINI

GENERATION COMING FROM LARVAE COLLECTED IN THE FIELD

Larvae. Engorged larvae which had dropped off their hosts and were kept in culture jars, ceased to move within 6—10 days (Table 2). Larvae collected from dead hosts at the moment when they were still attached to the animal, were not suitable for culturing because almost always they were attacked by fungi. Collecting of engorged larvae in different winter months did not affect the length of developmental cycle in any way.

Table 2. Development of *Neotrombicula zachvatkini* collected from hosts in nature at room temperature

Developmental stage		Duration of stage in days				Total number	% of larvae	% of preceding stage
		min.	max.	most frequent	average			
active engorged larva		6	34	6—10	11	289	100	—
nympho-chrysalis	formation	3	17	3—8	7	227	78.5	—
	persistence	7	21	11—15	13	200	69.2	88.1
active nymph		11	220	27—40	32	181	62.6	90.5
imago-chrysalis	formation	2	11	3—5	5	145	50.2	80.1
	persistence	7	23	11—14	13	134	46.4	92.4
imago		—	—	—	—	131	45.3	97.8
larva → imago		52	240	60—90	75			

Nymphochrysalis. The duration of the resting stage did not exceed three weeks. The formation phase was shorter and varied within about one week, the persistence phase used to be double.

Nymphs. The duration of the nymphal stage was quite variable. The largest number of nymphs remained active for 30 days, but there were also cases, when a nymph metamorphosed into imagochrysalis after seven months and an adult moulted from it in due time. This markedly prolongated nymphal period was manifest primarily in those chiggers which had come from the October collecting of engorged larvae. A nymph consumed three spring-tail eggs daily, during the entire active period

50 eggs on the average. Also distinct variability in size of nymphs was observed which was caused by the fact that larvae engorged to repletion. Small nymphs were always less resistant and only in sporadic cases they completed their development to adulthood.

Imagochrysalis. The formation phase of imagochrysalis was again twice to three times shorter than the persistence phase. To complete the development of adult 18 days were necessary.

Adults. A statistically significant difference in the length of development of females and males was ascertained. The development of males was one quarter shorter on the average (66 days) than that of females (84 days). The adults consumed on the average 5 eggs of *S. curviseta* daily, this consumption being more than double on

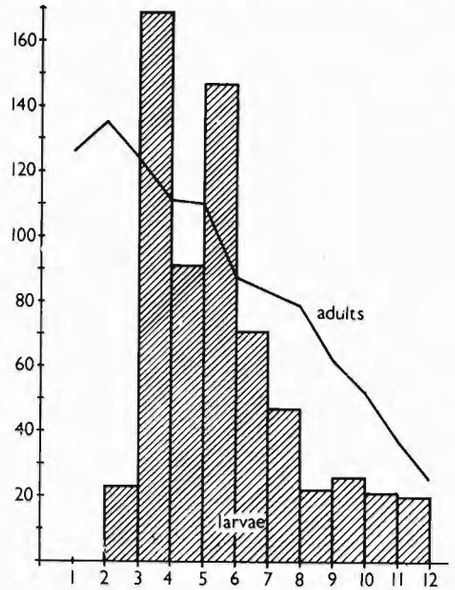

Fig. 2. Production of *Neotrombicula zachvatkini* larvae and survival of adults in their joint culturing. Abscissa — age of adults in months, ordinate — number of chiggers.

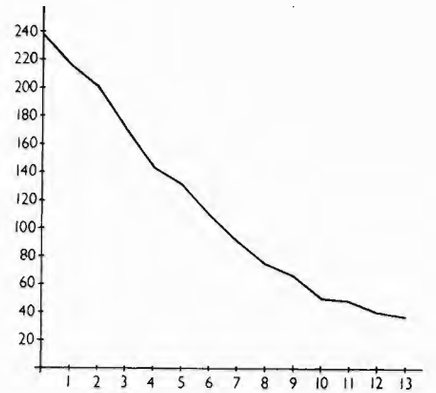

Fig. 3. Survival of *Neotrombicula zachvatkini* adults at room temperature. Larvae obtained from free-living rodents. Abscissa — months, ordinate — number of adults.

the first day after moulting. The complete consumption of one egg took about 13 minutes. To determine the sex, the adults were kept separately at first. Males deposited spermatophores mostly 2—5 days after moulting, sporadically as early as the first day. They began to deposit after the 15th day only rarely and therefore the optimal time for the differentiation of sexes was about 20 days. Older males began to deposit spermatophores again after they were transferred to a clean substrate in a new culture jar, mostly on the second day.

The oviposition began on the 20th to 40th day since the adults were placed together in the culture jar. Females oviposited separately, most frequently one, maximally three eggs daily. The oviposition lasted with intervals 1—14 days. Probably not every female was able to oviposit. The number of eggs deposited varied a great deal. Individual females deposited 1—91 eggs each, the last did so at the age of six months. After accepting the spermatophore the female could deposit both the fertile

and sterile eggs (even in the batches of the same day) minimally for as long as 3—4 months. In our experiments the females deposited a greater number of sterile eggs.

In the mass culturing of adults in 50 ml weighing bottles larvae began to appear when the adults were almost three months old and the greatest production took place in the following three months (Fig. 2). The survival of adults was essentially regular, after six months 50 %, after 12 months 17 % of them remained. (Fig. 3).

FIRST LABORATORY GENERATION

Larvae. Deutovum in the egg envelope formed mostly in 19—27 days and from almost each a larva developed (the loss was only 8 %). In 4—10 days the created deutovum began to stain yellow, in 13—20 days orange. Larva hatched in 7—14 days from the stained deutovum. The whole development of larva in the egg took 40—60 days, but the maximum values could amount to as many as 109 days (Table 3).

Table 3. *Neotrombicula zachvatkini* development of the first laboratory generation

Developmental stage		Duration of development in days				Total number	% of larvae	% of preceding stage
		min.	max.	most frequent	average			
active engorged larva		3	21	3—5	9	57	100	—
nympho-chrysalis	formation	3	15	4—6	7	45	80.0	80.0
	persistence	8	14	10—14	12	43	75.4	94.2
active nymph		14	258	22—31	63	27	47.4	63.2
imago-chrysalis	formation	2	5	3	3	14	24.6	52.3
	persistence	6	16	11—14	12	12	21.1	85.8
imago		—	—	—	—	12	21.1	100
larva → imago		47	217	63—83	80			

The hatched larvae were allowed to feed on hosts at monthly intervals and fasting specimens survived this period with minimum losses. In our experiments a total of 518 larvae of the first laboratory generation were fed on hosts and out of them less than 35 % engorged successfully. The engorged larvae dropped off on the 2nd to 4th day, the maximum of them engorged on the 3rd day (62 %). An almost ten-fold weight increase was ascertained after feeding. The length of activity of the laboratory generation larvae in comparison with larvae engorged in free nature gradually decreased. This was probably caused by the retardation of life processes due to low temperatures in larvae collected from dead hosts in the field during the first days of their activity.

While studying the viability of the first laboratory generation larvae in their further development, the two-month-old hamster seemed to be the most suitable host. The

viability of larvae decreased with the increasing age of the ovipositing females, the percentage of larvae capable of engorgement being low, but the percentage of moulted nymphs being high.

Nymphochrysalis. The duration of the phase of formation or persistence did not vary in any way compared to chiggers collected in the field. There was a marked difference, however, in the percentage of moulted nymphs. There was a high mortality of nymphochrysalis during feeding in the autumnal months (October, November), when they desiccated in 7—12 days, while during the spring feeding (April, May) almost 100 % of nymphs moulted from them.

Nymphs. The duration of the active nymphal stage was extended in extreme values, but in the most frequent values it was reduced, roughly by one quarter. In sporadic cases the nymphal activity was prolonged to as many as 10—14 months and only afterwards the development to adulthood took place (not included in Table). With some nymphs, which completed their development to adulthood, also the food consumption was studied. The average consumption of spring-tail eggs (3.5) did not differ much from the value obtained in chiggers observed in the field.

Imagochrysalis. A marked difference between the laboratory generation and that produced from larvae collected in the field was observed only in the decreased maximum values.

Adults. Fresh moulted males deposited their spermatophores immediately, the eggs could be obtained from the adults with retarded development, with the phase of nymphal activity prolonged by 12 months. A total of 18 adults and from them 143 larvae of the second laboratory generation were obtained.

SECOND LABORATORY GENERATION

Hungry larvae survived the intervals between feedings as long as 60 days without any losses. The length of the developmental cycle did not differ from that of the first laboratory generation, except for the extended period of active nymph. Most nymphs metamorphosed to imagochrysalis in more than 100 days. A total of 7 adults could be obtained. The deposition of spermatophores took place normally, no eggs were laid during the three months of our studies.

DEVELOPMENTAL CYCLES AT DIFFERENT TEMPERATURES

A group of *N. zachvatkini* was reared at different temperatures. The temperature of 9 °C stopped the development at the stage of nymph. They survived without any change as long as 14 months. They took nourishment only during controls at a higher temperature, when they started to move actively immediately. The development of nymphochrysalis was 5—7 times longer than that at room temperature.

The temperature of 15 °C proved to be suitable for slowing down the development and for a more long-lasting maintenance of active and inactive stages. An extended development was manifest particularly in the resting stages, and its length was three-fold length of the development at room temperature. Eleven adults were obtained. The deposition of spermatophores as well as eggs was considerably delayed, but was not smaller in volume. The eggs laid at 15 °C did not develop after 104 days, only after they were transferred to room temperature deutova began to be formed, the first one after 25 days. On the other hand, eggs laid at 22 °C and kept at 15 °C for 1—7 days developed in normal time. The duration of the entire development of the first laboratory generation at this temperature amounted to 12 months on the average, in comparison with the development of less than four months at room temperature.

The temperature of 28 °C was not suitable for chigger culturing. The entire development lasted 69 days, corresponding to that at 22 °C, but the mortality in all stages was much higher. Only in eggs no greater losses were observed and the development of larvae was somewhat accelerated.

DISCUSSION

We could not obtain the whole developmental cycle of the chigger *N. autumnalis* in our laboratory, we only succeeded in obtaining its portion from engorged larvae to adulthood. The reports in literature about culturing this species are quite scarce, the data on obtaining the first laboratory generation have not yet been published. Previous studies describe only nymphs obtained from engorged larvae without any statement of the duration of development. A more detailed paper was published by Minter (1957) who recorded the developmental period of the nymphs from the engorged larvae to be 20—30 days. Our data confirmed these results. He also paid a close attention to the amount of food consumed by post-larval stages. Our observations were similar to his results. André (1937) reported the duration of larval feeding on host to be 70 hours. In our experiments 65 % of engorged larvae on the average dropped off their hosts after 48 hours, and only 22 % after 72 hours. On the basis of extensive ecological studies Daniel (1961) reported reconstruction of the developmental cycle of *N. autumnalis*. The results obtained in laboratory culturing fully confirmed the conclusions reconstructed from the field observations.

Information on the developmental cycle observed in laboratory culturing of *N. zachvatkini* was only reported by Shoshina (1964) and Kudryashova (1972). While the latter author recorded the duration of particular developmental stages corresponding to our results, Shoshina's experiments showed the development to be much shorter. She obtained adults from nymphs at temperatures of 20—25 °C in 3—7 days, while in our observations the development of adults from nymphs at a room temperature lasted 40—60 days. Similar results were only in the duration of the resting stage of nymphochrysalis. Both authors included in the resting stage only the section from the morphologically created nymphochrysalis, called by us "the persistence phase". In Kudryashova's cultures (1972) the adults succumbed after three months without ovipositing. Shoshina (1964) observed oviposition and hatching of larvae in chiggers collected in nature. She obtained larvae from adults as early as in 30 days at the temperature of 15—17 °C, the development from eggs in 20—25 days. Our data on oviposition and larval development were approximately double, but they concerned adults hatched in laboratory and with larvae of the first laboratory generation. The results also differed in the duration of larval feeding: Shoshina reported 4—6 days, in our experiments larvae of the first laboratory generation dropped off in 2—4 days. No reports on obtaining more laboratory generations were published.

ЦИКЛ РАЗВИТИЯ КРАСНОТЕЛОК В ЛАБОРАТОРНЫХ УСЛОВИЯХ

В. Симонова

Резюме. В работе описан цикл развития двух видов краснотелок: *Neotrombicula autumnalis* и *N. zachvatkini*. У вида *N. autumnalis* изучали развитие генерации, происходящей от личинок собранных в природе, а у вида *N. zachvatkini* также исследовали развитие генераций, выращенных в лаборатории

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Note: According to recent taxonomic concepts within the family Trombiculidae the species *Neotrombicula zachvatkini* is placed in the genus *Hirsutiella* (Schluger et Vysotzkaja, 1970).