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FURTHER OBSERVATIONS ON THE LIFE CYCLE AND LIFE STRATEGY OF A TROMBICULID MITE, *HIRSUTIELLA ZACHVATKINI* (SCHLUGER, 1948) (ACARIFORMES: TROMBICULIDAE), IN THE LABORATORY

ДАЛЬНЕЙШИЕ ИССЛЕДОВАНИЯ ЖИЗНЕННОГО ЦИКЛА И ЖИЗНЕННОЙ СТРАТЕГИИ КРАСНОТЕЛКОВЫХ КЛЕЩЕЙ *HIRSUTIELLA ZACHVATKINI* (SCHLUGER, 1948) (ACARIFORMES: TROMBICULIDAE) В ЛАБОРАТОРИИ

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Ключевые слова: Trombiculidae, жизненный цикл, лабораторное выращивание, продолжительность стадий, генеративные циклы

ABSTRACT

The life cycle of the East European population of a trombiculid mite, *Hirsutiella zachvatkini* (Schluger, 1948), was carefully examined in the laboratory at a room temperature for over than four years since autumn, 1998. Eggs of springtails *Sinella curviseta* Brook were used for feeding of deutonymphs and adult mites, guinea pig — for feeding of larvae of the first laboratory generation. For the first time in Russia, adult mites of the second generation (the first laboratory generation) of this species were successfully obtained. The life cycle is characterized by more or less constant duration of quiescent instars (prelarva, proto- and tritonymphs) mostly restricted by three-four weeks and quite asynchronous development of active stages (larva, deutonymph and adult) with the obvious tendency to their maximum duration (179 days for unfed larva, 429 days for deutonymph and 956 days for adult male) so far known for trombiculids. Females show weakly outlined deposition (generation) cycles, and the deposited eggs reveal an extremely long, sporadic and asynchronous development (maximum 832 days for eggs capable to undergo further development), so that it is nearly impossible to receive a representative quantity of unfed larvae for their subsequent feeding. Nevertheless, the species studied is thought to demonstrate a univoltine or semi-univoltine life cycle in nature. Some new hypotheses concerning life strategy of boreal and eastern populations of the species are proposed.

РЕЗЮМЕ

Жизненный цикл краснотелкового клеща *Hirsutiella zachvatkini* (Schluger, 1948) из его восточной популяции был детально исследован в лаборатории при комнатной температуре на протяжении более чем четырех лет, начиная с августа 1998 г. Яйца коллембол *Sinella curviseta* Brook употребляли для кормления дейтонимф и взрослых клещей, а морскую свинку использовали для кормления личинок первой лабораторной генерации. Впервые в России были получены взрослые клещи второй генерации (первой лабораторной) этого вида. Жизненный цикл характеризуется более или менее постоянными сроками покоящихся возрастов (предличинки, прото- и тритонимфы), в основном ограниченных 3–4 неделями, и асинхронным развитием активных стадий (личинка, дейтонимфа и взрослые) с очевидной тенденцией к их максимальной длительности (179 с для голодной личинки, 429 с для дейтонимфы и 956 с для самца), известной для краснотелок. Самки обнаруживают слабо очерченные генеративные циклы, а откладываемые яйца развиваются исключительно длительно, неравномерно и асинхронно (832 дня для яйца, претерпевшего дальнейшее развитие), так, что получить достаточное количество голодных личинок для их дальнейшего кормления крайне сложно. Тем не менее, в природе этот вид, скорее всего, обладает односезонным либо полуодносезонным циклом развития. Предложен ряд гипотез относительно жизненной стратегии восточных и бореальных популяций этого вида.

INTRODUCTION

Trombiculid mites of the family Trombiculidae are well known and the only larval parasites from the cohort Parasitengona, which attack an

extremely wide spectrum of vertebrate hosts including human beings. They cause a skin eruption and itching in their hosts during feeding and are also found to serve as vectors of the causative agent (*Orientia tsutsugamushi*) of scrub typhus, or *tsutsugamushi* disease, in several parts of the Eastern Asia and in the islands of the Pacific region [see Ewing, 1944, Traub, Wisseman, 1974, etc.]. In accordance with such practical value, trombiculid larvae, or *chiggers*, have attracted a permanent interest of scientists from many tropical and subtropical countries in XX century due to their medical importance, with the pick of such an interest in the middle of the century. Deutonymphs and adult mites are soil dwellers praying upon various arthropods and especially their eggs, are rather rarely observable on the soil surface [see Wharton, 1946; Daniel, 1961, 1965, etc.] and are not known for the most species until present time. Although many successive works have been made earlier towards colonization or rearing trombiculid mites in the laboratory, particularly in the sub-tropical and tropical countries, as well as clarification of their ecology [Elton, Keay, 1936; Keay, 1937; Gunter, 1939; Jayewickeme, Niles, 1946, 1947; Melvin, 1946; Michener, 1946; Wharton, Carver, 1946; Williams, 1946; Radford, 1946a, b; Jenkins, 1947, 1948; Cockings, 1948; Mehta, 1948; Richards, 1948; Krishnan et al., 1949; Lipovsky, 1951, 1953, 1954; Sasa, Miura, 1953; Chen, Hsu, 1956, 1962; Ito et al., 1957; Minter, 1957; Wen, 1958; Hsu, Chen, 1960a, b; Neal, Barnett, 1961; Wen, Hsu, 1962; Audy, Lavoipierre, 1966; Kaufmann, Traub, 1966; Jameson, 1967, 1968, 1972; Shirasaka, Sasa, 1967; Nadchatram, 1968; Upham et al., 1971; Evefett et al., 1973; Kulkarni, Mahadev, 1973; Cunningham et al., 1975; Kulkarni, 1988; Southcott, Frances, 1991; Takahashi et al., 1993; Takahashi et al., 1995], some problematical questions are still remained unclear in respect with the life strategy of boreal species, such as *Hirsutiella zachvatkini*. Previous attempts to obtain representative laboratory generation of this species were predominantly failed in Russia [Schluger, 1949; Shoshina, 1964, 1965; Kudryashova, 1972; Vasil'eva, 1977; Shatrov, 1996, 2000]. In contrast, a Middle-European population of *H.zachvatkini* appears to demonstrate a more integral life cycle and the mites of the first laboratory generation were successively received [Simonova, 1977, 1983].

Life strategy of other Parasitengona was discussed in several works [Tevis, Newell, 1962; Singer, 1971; Böttger, 1977; Wendt et al., 1992; Eggers, 1995; Gerecke, Smith, 1995; Wohltmann, 1995, 1996; Wohltmann, Wendt, 1996; Mitchell, 1998; etc.], without paying much attention to trombiculid mites, and was finally summarized in the recent review paper by Wohltmann [2000], where, unfortunately, most of published works on trombiculids listed above as well as many others were predominantly omitted. However, the family Trombiculidae is thought to be the most specialized and even derived group among other related families of the cohort Parasitengona owing to a number of morphological and ecological evidences, not to speak about their unique larval parasitism on vertebrates. Thus they deserve a following careful consideration in comparison with other related groups.

More than six years have passed since the last experimental work on the life cycle of trombiculid mites has been published [Shatrov, 1996]. For that time, some new experimental data on the life cycle were obtained that impel me to summarize them in a separate communication. For this reason, the main purpose of this study is to provide a detail description of the life cycle of the East-European trombiculid mite, *Hirsutiella zachvatkini* (Schluger, 1948), obtained anew in the laboratory at room temperature and kept for quite a long period of time that made it possible to reveal a maximum duration of stages and to receive representatives of the first laboratory generation.

MATERIALS AND METHODS

About 30 fully engorged larvae collected from the trapped bank voles (*Clethryonomis glareolus* Schreb.) by Dr. M.K. Stanyukovich in the 2nd and 4th of October 1998 near Brykin Bor, Ryazan district were the initial material for the laboratory rearing of mites. Larvae after parallel alcohol fixation were kindly determined by Dr. A.A. Stekolnikov. Captured mites were immediately placed into the plexiglass Petri dishes 4 cm in diameter and 1 cm height with plaster of Paris and charcoal mixture in equal proportion as a substrate filling up to 0.5 cm height in each dish. Substrate was regularly moistened to saturation to provide a relatively high equilibrium humidity (around 95–100%). Grooves and holes of particular configurations were made in a moist substrate before it got hardened during preparation of the containers. The latter were also provided by tightly opposed cap to prevent decrease of moister. No any other components like cotton wool, soil, vermiculite, etc. were placed above the original substrate having nearly black color that was very helpful for observation of

Table 1

Average room temperature during maintenance of a trombiculid mite, *Hirsutiella zachvatkini,* in laboratory

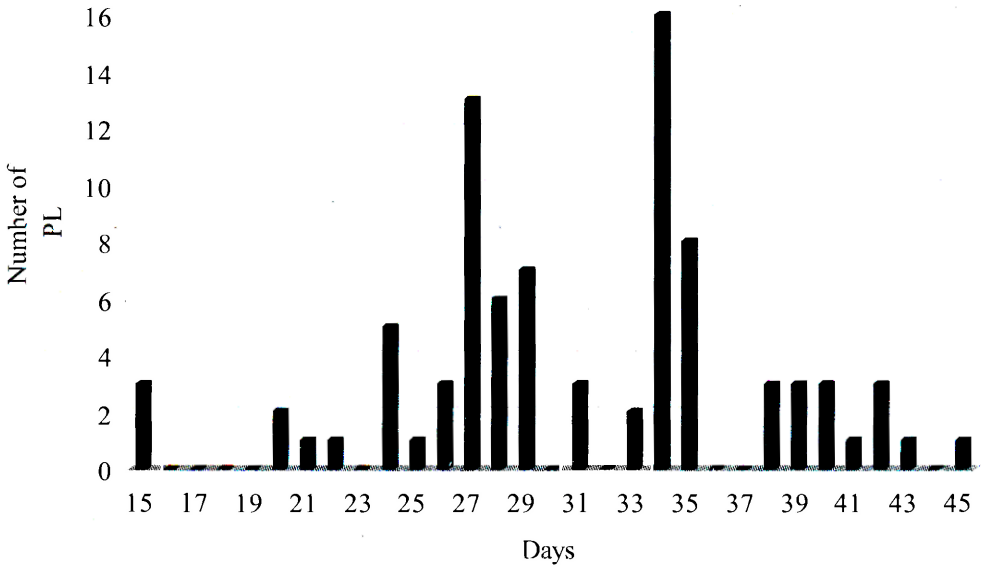
Таблица 1

Средняя комнатная температура за время содержания краснотелковых клешей *Hirsutiella zachvatkini* в лаборатории.

mites. Such containers are found to be the only suitable laboratory glassware for culturing of trombiculid mites for their whole life cycle. The Petri dishes with mites were usually kept in darkness and examined under dissecting microscope every day, twice or once a week during the course of the experiment. A room temperature was predominantly kept within the interval 15–25°C (Table 1). Eggs of springtails *Sinella curviseta* Brook, which culture was maintained in the laboratory since 1980, served as a food for active deutonymphs and adult mites. Collembolan eggs were added into Petri dishes with active postlarval instars approximately twice a week in a number of 30–50 eggs per dish with 2–5 mites, empty eggshells and various debris as well as newly emerged insects were regularly removed from the containers. Young guinea pig provided with a special capsule on its back was used for feeding of the unfed larvae. After emergence, the adult mites were placed individually into newly prepared Petri dishes, and the substrate was examined on the presence of spermatophores, which usually appeared within the next 3–5 days. If there were no spermatophores found in a Petri dish after ten days have passed, the mite was believed to be a female that was always confirmed by a subsequent egg deposition. After a sex determination has been done, females and males were typically placed in pairs into another Petri dish to provoke an egg deposition. When mites of one sex were dominated in number, they have been remained vacant for some time as long as mites of the opposite sex have become free. A more detail description together with historical review of the laboratory methods used for rearing of trombiculid mites was published earlier [Kudryashova, 1972; Shatrov, 1993a].

Another important question, which is inevitably arisen during examination of the life cycle of trombiculids is a determination of sequential stages of the mite ontogenetic development. The problem is that, as it is already well-known, the visible documented periods of the trombiculid life cycle do not correspond to the true developmental stages, so, for instance, the organism from immobilization of the fed larva up to emergence of the active deutonymph, that is usually called nympho-chrysalis, actually includes the immobilized larva, the quiescent protonymph *per se*, and the developing pharate deutonymph being within the old protonymphal cover and, sometimes, even within the old larval cast. The sequence of developmental events is seen equally the same for both deutovum (prelarval) and imago-chrysalis (tritonymphal) pe-

Table 2
Duration of quiescent prelarval stage in *Hirsutiella zachvatkini*
Таблица 2
Продолжительность покоящейся предличиночной стадии *Hirsutiella zachvatkini*



riods, and may be exactly documented only in terms of molting cycles and deposition of new cuticle of an every given stage during its internal examination [for detail account, see Shatrov, 1999a, b, 2000, 2001]. However, when observing mites in culture, it is doubtless more convenient to determine not the true ontogenetic stages, but the periods marked by distinctly visible morphological and behavioral processes, such as immobilization of the previous instar and emergence of the subsequent one. Thus, the egg period is defined from the egg deposition up to the cleavage of the eggshell when the development of prelarva has started; the prelarval period is defined from the egg cleavage up to the ecdysis of the active larva; the protonymphal period is defined from the immobilization of the fed larva up to the ecdysis of the active deutonymph; the deutonymphal period is limited by the immobilization of this instar after its active life; the tritonymphal period is defined from the immobilization of the active deutonymph up to the emergence of the active adult; and, finally, the period of adult stage is limited by its death. The life of the active larva is also marked from its emergence up to the death. This gradation appears to be the only applicable to characterize the life cycle in the laboratory, seems to use by all authors working with trombiculid's life cycle and corresponds conventionally to the process of true ontogenetic development, especially in the case of active instars.

RESULTS

Biology of the trombiculid mite *Hirsutiella zachvatkini* in the laboratory, in particular its molting, feeding and mating behavior, was described in detail previously [see Shatrov, 1996, 2000]. In this communication I will focus my consideration on the proposed realization of the life cycle and life strategy, maximum duration of stages, characterization of the deposition (generation) cycles as well as some physiological aberrations (schizeckenosy), and, finally, receiving of the first laboratory generation.

As it is seen from Tables 2–4, the duration of quiescent periods, irrespectively of a season, the longevity of the previous active stages (or egg diapauses) or an ambient temperature (see Table 1), is relatively constant and predominantly restricted by two to four weeks for proto- and tritonymphal periods and by four to six weeks for prelarval ones. These periods for the East-European population of *H.zachvatkini* are significantly longer than those found for other trombiculids reared in the laboratory including the Middle-European population of this species [Sasa, Miura, 1953; Neal, Barnett, 1961; Shirasaka, Sasa, 1967, Nadchatram, 1968; Everett et al., 1973; Cunningham et al., 1975; Simonova, 1977, 1983; etc.]. Some protonymphs appear to be unable to complete their development supposedly due to an unsatisfactory larval feeding and have died within

Table 3
Duration of quiescent protonymphal stage in *Hirsutiella zachvatkini*
Таблица 3
Продолжительность покоящейся протонимфальной стадии *Hirsutiella zachvatkini*

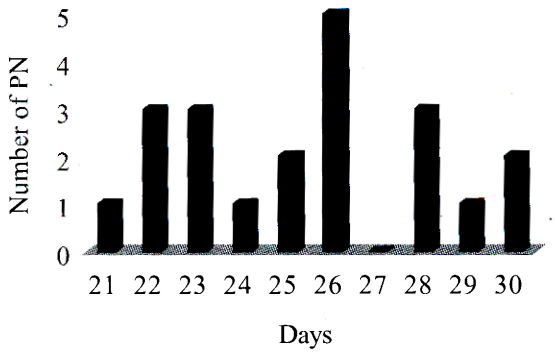
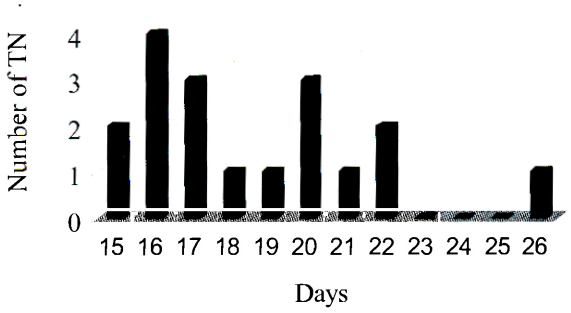


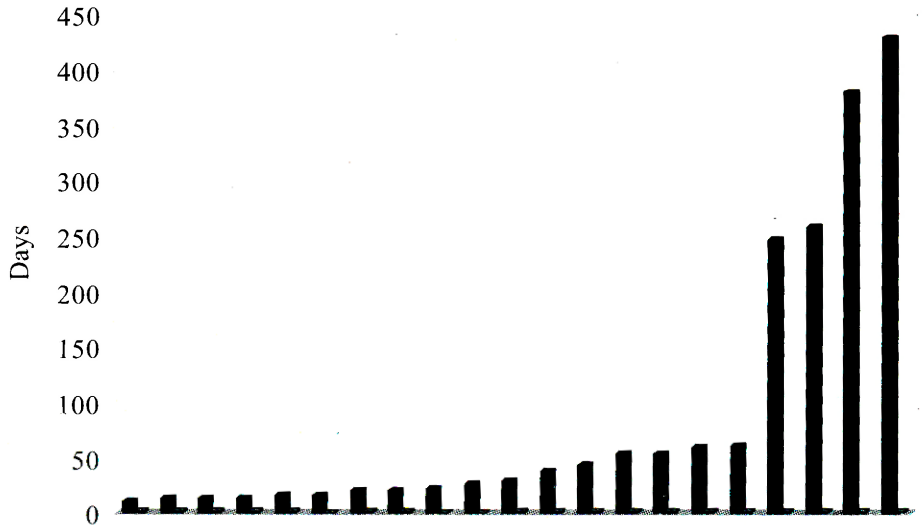
Table 4
Duration of quiescent tritonymphal stage in *Hirsutiella zachvatkini*
Таблица 4
Продолжительность покоящейся тритонимфальной стадии *Hirsutiella zachvatkini*



10–20 days after the immobilization of a fed larva (total 6 PNS in this experiment). Duration of the deutonymphal period has never been shorter than 10 days (only 1 DN) and normally may last up to 61 days (Table 5) showing a great variability in relatively constant climatic conditions thus reflecting a particular mode of the life strategy. Moreover, the lifetime of 4 deutonymphs capable to undergo a further development was exceedingly prolonged without obvious external reasons with a maximum duration of 429 days. Such variations in the deutonymphal development have been also recorded for this species previously — 14–258 days in the first laboratory generation for the Middle-European population [Sinonova, 1983] and even much greater — 9–597 days for the boreal population [Shatrov, 1996]. However, the duration of the subsequent tritonymphal period of such specimen did not differ significantly from normal meanings. For instance, the deutonymph, which had lived for 258 days, has undergone tritonymphal development within 15 days; in other cases corresponding periods were 380 and 15 (winter 1999), and 429 and 26 days (winter 2000).

After capturing on October 2nd and 4th, 1998 as engorged larvae, some mites could demonstrate a relatively rapid subsequent development, so that the first adult mites already appeared on November 26th that year — there were two males, another one male appeared on November 30th, the first female appeared on December 15th. The following appearance of adult mites, however, has prolonged for over than one year, so that the last adult mite appeared only on February 13th, 2000 from the deutonymph that has lived for 429 days. To that time, the first adult mites began to die, particularly, the female, which appeared on December 15th, 1998, died on February 20th 2000 after it had lived for 424 days. The first eggs in all three Petri dishes (that mark, correspondingly, the beginning of the deposition cycles), where were one female in each dish with one, two and three males per female (per dish), appeared on January 25th 1999, and the first prelarvae in these dishes ap-

Table 5
Individual life duration of active deutonymphs of *Hirsutiella zachvatkini*, which undergone further development
Таблица 5
Индивидуальная продолжительность жизни активных дейтонимф *Hirsutiella zachvatkini*, претерпевших дальнейшее развитие

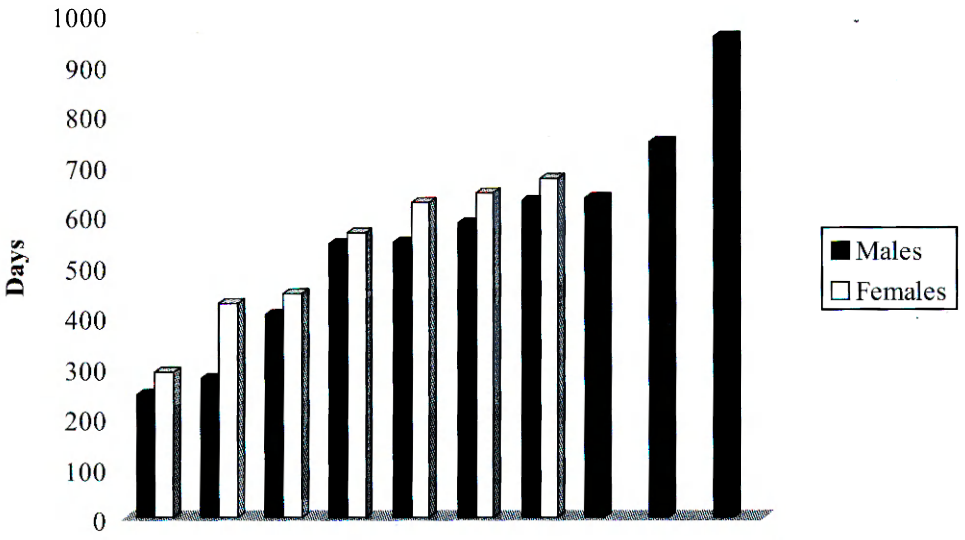


peared on April 7th and 26th and on May 11th 1999 (one, two and one prelarva, respectively). It is interesting to note that for the most cases the early-appeared deutonymphs gave subsequently rise to males, whereas females were predominantly developed from the late-appeared deutonymphs. The last adult mite, female, died on November 22nd 2001 (lived 674 days), and with its death the first generation (deposition) cycle of this female has completed, which lasted (together with generation cycle of one another female seating in the same Petri dish) for 437 days (about generation cycles — see later). Thus, from the foundation of the culture in October 1998 till the death of the last adult mite in November, 2001 over than three years have been passed. The pick of the prelarvae formation observed in all Petri dishes (up to 12 prelarvae in each observation) was achieved in April-May and August-September, 2002 in one Petri dish, where the last female used to live earlier. Correspondingly, the unfed larvae in this dish have become gradually appeared some time later. As it is clearly seen, to that time there were already remained no adults in the culture. A minimum nominal age of such developing eggs being dormant during this period of time was rather large — from November 22nd 2001, the time of the formal completion of the deposition cycle and simultaneous death of the female, — from a half of the year to one year. However, these ages recorded for eggs in the culture are far from reaching maximum (see later).

A sporadic death of some adult mites in their early ages (one mite of unknown sex has died in 4 days after emergence) or within the first year of their life could be a result of a general weakness of an organism after unsatisfactory nymphal feeding or, contrary, due to a general dysfunction of an organism provoked by an exceeding feeding, exceeding fluid contents in the tissues, or impossibility to remove the excretes. However, the same reasons usually cause mortality in mites in their old ages after one year of life (diseases of aging). In contrast, some mites may live in culture over than 600 and more days and die remaining "healthy" (Table 6). In general, however, after the one-year life in culture, mites, permanently feeding and being active all the time, turn, however, into their old ages, when the risk of diseases is high. In an occasional decrease of the ambient temperature up to 12–15°C, developmental periods of mites are correspondingly increased (retarded), and deutonymphs and adult mites may even die, especially under a low humidity and unsatisfactory feeding conditions. It was not a case for long periods during work of the author with mites.

Normally, both deutonymphs and adult mites eat (suck out) on average 1–2 collembolan eggs per day. Some deutonymphs, which do not undergo

Table 6
Individual life duration of active adult mites of *Hirsutiella zachvatkini*
Таблица 6
Индивидуальная продолжительность жизни активных взрослых клещей *Hirsutiella zachvatkini*



further development for more than one month and, sometimes, adult mites, irrespectively of season, eat badly and consume only 1–2 eggs per week or even with less intensity. Conversely, some adult mites may demonstrate an exceeding abnormal appetite and suck dry 3 and more eggs per day. Only in these cases, mites may seemingly attack their own eggs attempting to pierce them and separating them apart from the place they have initially lied. This phenomenon may be termed as a *"**facultative cannibalism**"* (see Discussion). The easiest and nearest consequences of such abnormal feeding behavior may be stretching of the integument and flattening of a proterosomal (dorsosejugal) groove that may even lead to appearance of tiny drops of metabolic water on the integument among setae. These external alterations may be accompanied by an abnormal expression of the excretory organ having become visible through the integument in various parts of the body. There is nothing to say that such a situation may only lead to a significant deterioration of living conditions of mites that is usually observed after a year of their lifetime and sometimes earlier. In particular, all above-mentioned alterations of mite physiology, when the body has become swollen from the exceeding concentration of metabolic water with drops of fluids on the integument ("dropsy"), have led to a quite early death of one female on the 289th day after its emergence that has happened on May 11th, 2000. This mite was the only female, which has not been depositing eggs during its life. In another case, female has died due to the same reasons (diseases) after a relatively long life (566 days) on the 5th of July 2001. The exceeding feeding and a high tension of the integument from the pressing of the metabolic fluids may lead to a rupture of the integument and to a phenomenon called the *"**provocation of the gut defecation**"* [Shatrov, 1993b, 2000]. In particular, the male that died on July 3rd 2000 on the 583rd day of its life fed exceedingly, its body has become swollen and a split has formed on the lateral side of the protero- and histerosoma right before its death.

In three cases, exceeding feeding has led to a true "schizeckenosy" [Mitchell, Nadchatram, 1969], or the *"**gut defecation**"* [Shatrov, 1993b, 2000], when the integument split and a part of the midgut with accompanied fluids and tissues evacuate outside the body. In some instances, parts of the integument may be also involved in this process and tear off the body. In the young ages, however, the gut defecation may take place easily, without nearest consequences, as it has happened on November 19th 1999 with the 340 day old female, which has only slightly grown thin. In the old ages, however, such a process may be accomplished more dramatically and generally leads to a death of mites, when the physiological deteriorations seem to be incompatible with the life. For instance, 518-day-old male has undergone the caudal gut defecation with accompanied alterations of the integu-

ment and uncovering the midgut caudally. This male has died 5 days after the gut defecation on May 30th 2000 and was producing spermatophores up to its death. The third case of the gut defecation in the ventro-caudal part of the body has led to an immediate death of the female that was naturally week during all its life, in the age of 624 days that has happened on November 22nd 2000.

Males deposit spermatophores during their whole life generally regardless of the season, individual age or number of mites in a Petri dish that is actually determined not by external reasons, but by physiological conditions of mite organism *per se*. At the same time, a spermatophore deposition takes place with a great irregularity from male to male. For instance, three males seating together with one female in the same Petri dish after a one-year life, in a winter period, deposited up to 21 spermatophores per 2–3 days for a relatively long time. In some other cases, males seating alone or together with one female were depositing only 1–2 spertamophores per one-two weeks immediately from the beginning of the spermatophore deposition. It is important to note that the individual life of spermatophore may be rather long. One spermatophore situated in a Petri dish after the mites have already died having been examined for the period of 878 days and all that time revealed no changes in its appearance.

Females generally demonstrate weekly outlined deposition cycles in their egg laying. To stimulate the oviposition, males and females are usually placed in pairs or one female is placed with two or three males into one Petri dish, otherwise the deposition did not take place. Within one-three weeks since the time the adults were placed together the first eggs appeared in the containers. Females regardless of either sitting with one or several males naturally produce a quite variable number of eggs, among which, however, a varied and sometimes great number of sterile eggs were usually identified. Some evidences apparently indicate that the character of the deposited eggs depends not on a number of males or spermatophores, but, again, on the physiological conditions of a given female. Usually, females deposit eggs separately with a rather varied intensity. Rather rarely, some of the freshly laid eggs may be found near one another in small batches. There were two such cases when 13 and 6 eggs have been found disposed side by side during the first deposition cycle by two females seating with three and one males, respectively. However, after a deposition has started, a general number of eggs could not be increased for a long time due to a large number of sterile eggs.

The second deposition cycle usually takes place sporadically and irregularly, and may be expressed by the appearance of only 1–2 eggs in one to three months after completion of the first cycle, as in the case observed in August, 1999 in one Petri dish, where the second cycle was reduced to appearance of two eggs on July 27th and on August 9th, three months later when the first cycle has finished.

Due to impossibility to provide an adequate formalization of the generation (deposition) cycles, the following verbal characterization of the first deposition cycles may be performed.

1. Petri dish No. 1a (Fig. 1). Three males from 26.11.98 (2) and 06.01.99 (1) and one female from 06.01.99 were placed together 18.01.99.

Beginning — 25.01.99 (there was 1 spermatophore in the container) — 1 egg. Maximum egg number of the newly laid eggs — 10 and 13 — was observed 22.02.99 and 24.03.99 respectively. First prelarvae — 07.04.99 — on the 73rd day after beginning of the cycle. Completion — 26.04.99. Duration — 92 days. Total egg number — 63, sterile eggs — 17. To the time of the cycle completion the first pick of the prelarvae formation has also finished — 30.04.99. Another prelarva has formed only 17.05.99. The first unfed larva was found 26.05.99 — on the 121st day after beginning of the cycle. The second deposition cycle was not clearly identified.

2. Petri dish No. 1b (Fig. 2). One male from 03.01.99 and one female from 15.12.98 were placed together 06.01.99.

Beginning — 25.01.99 (2 spermatophores in the container) — 4 eggs. The first sterile eggs were identified 03.02.99. Maximum eggs number — 8 — was observed 14.04.99 and 20.05.99. Two first prelarvae appeared 26.04.99 — on the 92nd day after the cycle has started. The first unfed larva appeared 07.06.99 — on the 133rd day after the cycle has begun; the second larva was found only 27.07.99, and the next two larvae have emerged 09.08.99. In July and August there were intervals in the egg deposition, and 16.08.99 five prelarvae newly developed from the early deposited eggs were found. During September and October egg deposition was regular — up to 4 eggs per week, but in late October intensity of deposition has failed even in increasing of the ambient temperature. Prelarvae in numbers of 1 to 3 have formed rather irregularly with large intervals up to 121 days.

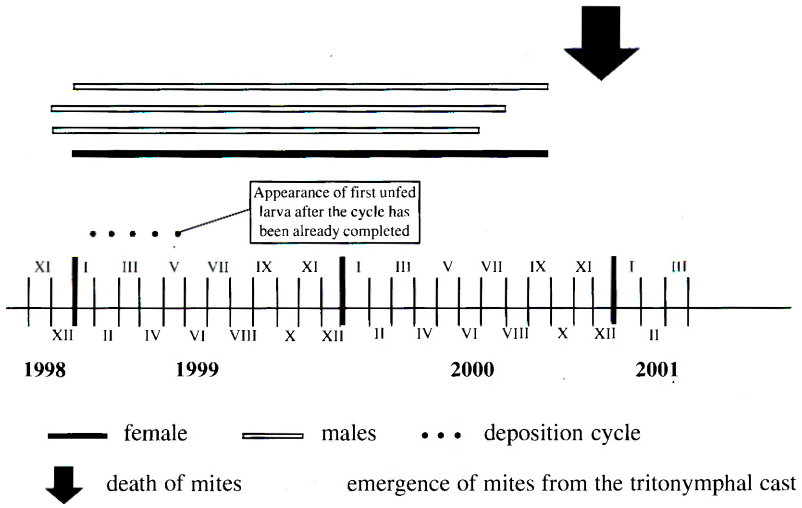


Fig. 1. Approximate correlation between individual lifetime of adult mites *Hirsutiella zachvatkini* and deposition cycle of a female in Petri dish No. 1a. Second generation cycle has not been observed in this dish.
Рис. 1. Приблизительное соотношение индивидуального срока жизни взрослых клещей *Hirsutiella zachvatkini* и генеративного цикла самки в чашке Петри № 1а. Второй генеративный цикл в этой чашке Петри не был отмечен.

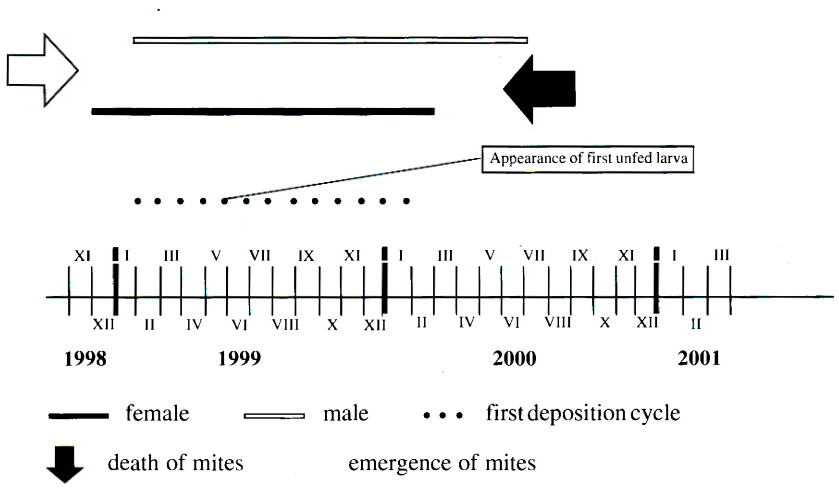


Fig. 2. Approximate correlation between individual lifetime of adult mites *Hirsutiella zachvatkini* and deposition cycle of a female in Petri dish No. 1b. Only one generation cycle is very prolonged.
Рис. 2. Приблизительное соотношение индивидуального срока жизни взрослых клещей *Hirsutiella zachvatkini* и генеративного цикла самки в чашке Петри № 1b. Единственный генеративный цикл оказался очень продолжительным.

Completion — 25.01.00. Duration — 365 days (1 year). Total egg number — 153. Sterile eggs — 86 (to the time of the cycle has finished). The second cycle was not observed with certainty.

3. Petri dish No. 2c (Fig. 3). Two males from 11.12.98 and one female from 03.01.99 were placed together 15.01.99.

Beginning — 25.01.99 (6 spermatophores in the container) — 2 eggs. The first sterile eggs were determined 03.02.99 — on the 10th day after appearance of the first eggs. During late February-March oviposition was very intensive — up to 9–11 freshly laid eggs per 3–5 days, although there were also very many sterile eggs as from newly so from early deposited eggs. It is important to note, that despite there were two males in the dish, intensity of spermatophore deposition was very low for all winter and spring, when only one spermatophore has appearing weekly. The first prelarva was formed 11.05.99 — on the 106th day after beginning of the cycle. Correspondingly, the first larva has appeared from this prelarva 07.06.99 — on the 133rd day after the cycle has started, precisely as in the Petri dish No. 1b. The second new prelarva has formed only 20.07.99 with the interval of 70 day after the appearance of the first one and

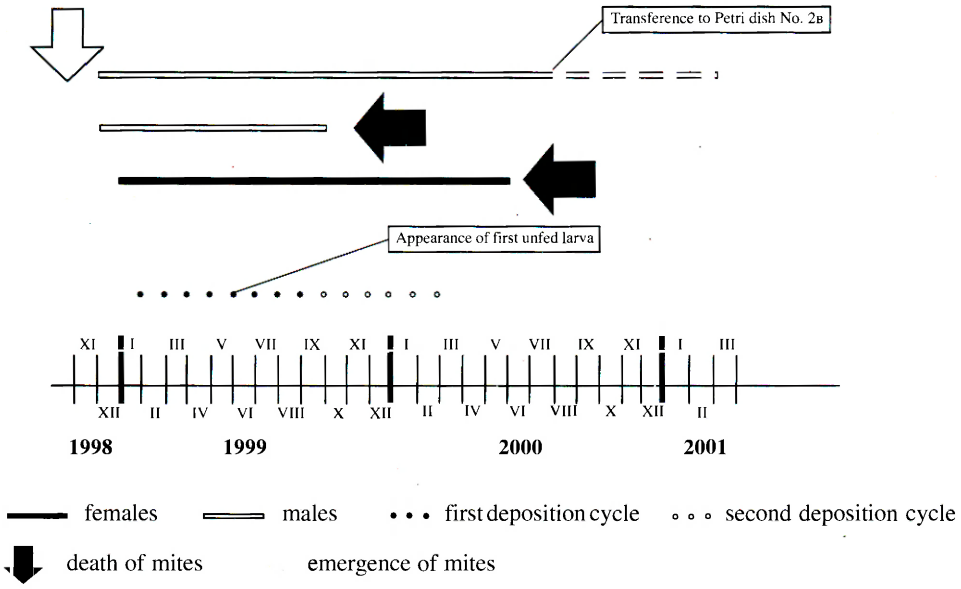


Fig. 3. Approximate correlation between individual lifetime of adult mites *Hirsutiella zachvatkini* and deposition cycles of a female in Petri dish No. 2c.
Рис. 3. Приблизительное соотношение индивидуального срока жизни взрослых клещей *Hirsutiella zachvatkini* и генеративных циклов самки в чашке Петри № 2с.

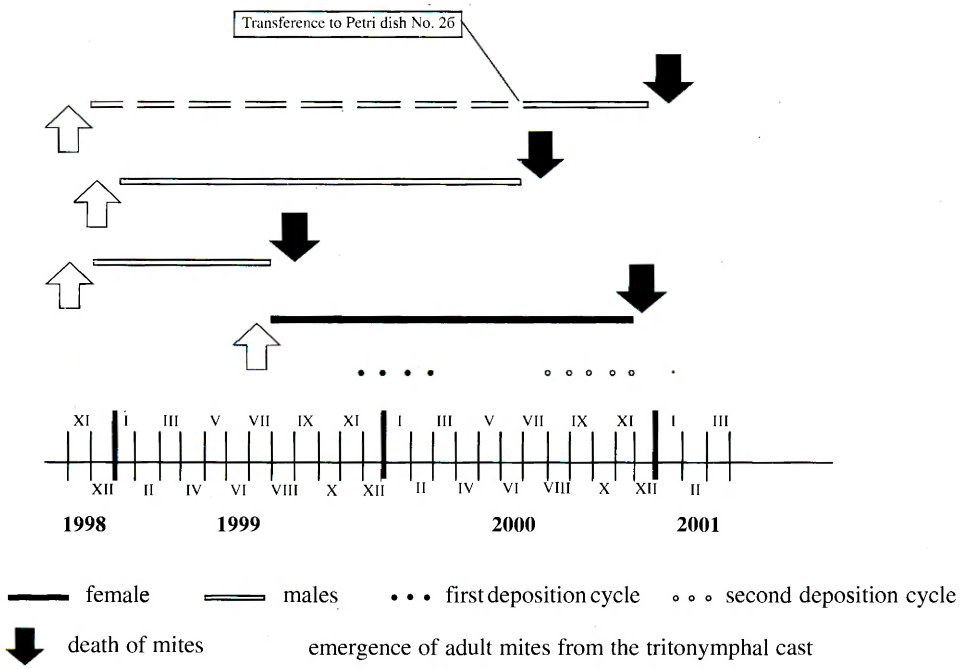


Fig. 4. Approximate correlation between individual lifetime of adult mites *Hirsutiella zachvatkini* and deposition cycles of a female in Petri dish No. 2d. There were no unfed larvae found in this dish.
Рис. 4. Приблизительное соотношение индивидуального срока жизни взрослых клещей *Hirsutiella zachvatkini* и генеративных циклов самки в чашке Петри № 2d. Голодные личинки не были зарегистрированы.

176 day after beginning of the cycle. For that time, mites revealed a good appetite, but the male deposited spermatophores rather badly. Another, the third, prelarva and the second unfed larva have appeared in the dish at the same day — 09.08.99 — already after the cycle has finished. Completion — 03.08.99. Duration — 160 days. Total egg number — 166. Sterile eggs — 140 (to the time of the cycle has finished). The second deposition cycle (the only integral second cycle) has started in this dish 21.09.99 — 49 days after the first cycle has completed (see later).

4. Petri dish No. 2d (Fig. 4). One male from 21.12.98 and one female from 08.08.99 were placed

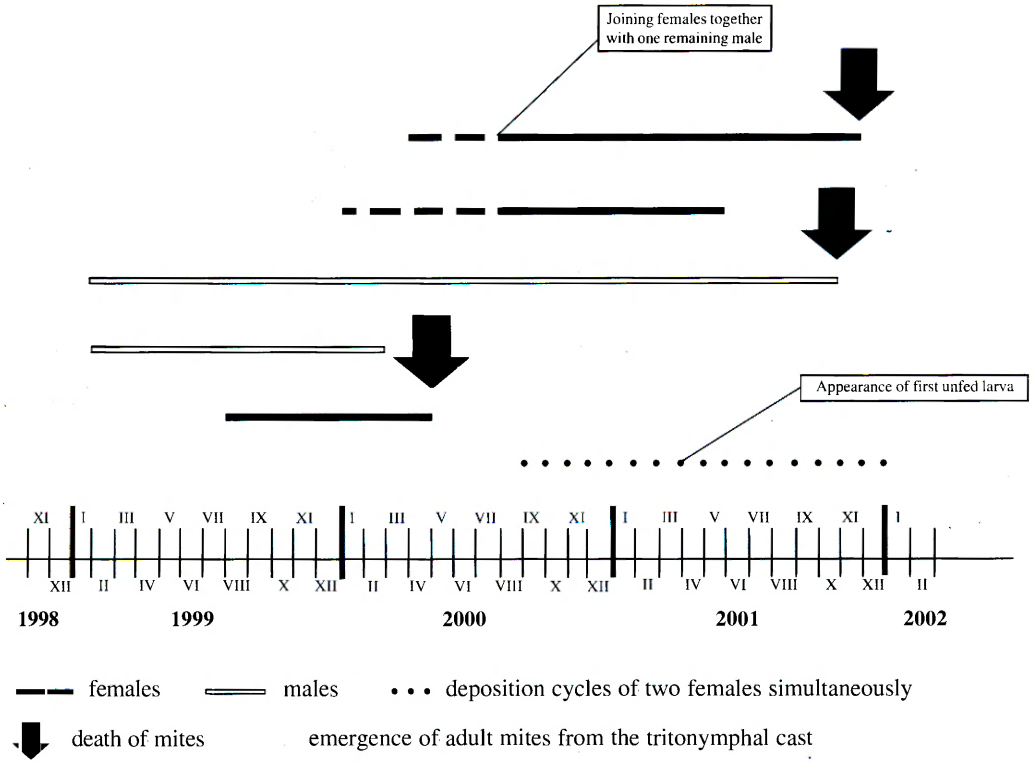


Fig. 5. Approximate correlation between individual lifetime of adult mites *Hirsutiella zachvatkini* and deposition cycle of two females in Petri dish No. 1e. Before these two females have been placed to one remaining male they were vacant and did not deposit eggs. One female being in this dish earlier did not undergo deposition cycle.

Рис. 5. Приблизительное соотношение индивидуального срока жизни взрослых клещей *Hirsutiella zachvatkini* и генеративного цикла двух самок в чашке Петри № 1е. Перед тем, как эти две самки были посажены к одному оставшемуся самцу, они были вакантными и яиц не откладывали. У самки, находившейся в этой чашке Петри раньше, генеративный цикл не был отмечен.

together 24.08.99. Before that time, deutonymph that gave rise to female has lived for 258 days, although the period of tritonymph was short (15 days). Production of spermatophores by male was low.

Beginning — 16.12.99 (2 spermatophores in the container) — 2 first sterile eggs — on the 114th day after placing the mites together. Completion — 19.01.00. Duration — 35 days. Total egg number — 10. Sterile eggs — 9. Such low egg production may be supposedly caused by a rather long period of the deutonymphal life.

On the 21st of March there were found one more sterile egg in pursuit of the first cycle — 60 days after the cycles has stopped. On the 30th of May the male has died for the reason of gut defecation, which has happened 24 of May. On the 10th of July 2000 another male from a Petri dish No. 2c (has emerged 11.12.98) (see above) was placed into this dish to a native female being not very hard all the time. Conversely, the male was active and deposited spermatophores intensively — up to 10 spermatophores per 5–7 days. The second cycle in this dish has started 31.07.00 (see later).

5. Petri dish No. 1e (Fig. 5). One male from 18.01.99 and two females from 16.12.99 and 15.02.00 were placed together 18.07.00. Up to that time the females were vacant and did not deposit eggs. These two females were placed into a Petri dish to a remaining native male. With this male there were lived earlier another one male from 15.01.99, which has died 29.02.00 owing to dysfunction of the excretory system (total lifetime 402 days) and also female from 27.07.99 died 11.05.00 (lifetime 289 days) in consequence of a general dysfunction, and deposited no eggs (see above). The native male, before that time, deposited a various, but not low number of spermatophores — up to 7–8 per 5–7 days, but after placing it together with females its activity has decreased.

Beginning — 11.09.00 — 2 eggs and immediately 2 sterile eggs — on the 55th day after placing the mites together and in the ages of 208 and 269 days of females (here a deposition cycle was ascertained for two females simultaneously). The first prelarva has immediately appeared (without a recorded egg development) 16.11.00 — 66 days after the cycle has started. Another new prelarva (from

the freshly laid eggs) has formed only 14.02.01 — 156 days after beginning of the cycle. From late January 2001, during each examination of the dish there were revealed many eggs — up to 14, 8 and 10 new eggs 06.03.01, 20.03.01 and 10.04.01, respectively etc. that apparently indicate the participation of two females in the egg deposition. The first larva has appeared 20.03.01 — 190 days after the mites were placed together. The intensive oviposition has been accompanied by a large number of new spermatophores, for instance, up to 16 newly deposited spermatophores 14.06.01. After a death of one of the females that has happened 05.07.01 (total lifetime 566 days), the remaining female continued the oviposition with smaller intensity. Spermatophores also continued to appear in quantity of 9–10 ones per examination. Another new prelarvae have formed 30.07.01 and 06.08.01. The male has died 05.09.01 without evident alterations (lifetime was the maximum one — 956 days) and continued to deposit spermatophores in a large number (8 spermatophores 06.08.01) up to its depth. After that, the remaining female continued to deposit eggs slowly, but simultaneously there were observed new spermatophores (in all up to 4) that suggested a hermaphroditism of the female. Another new unfed larvae appeared on September 14th and 19th 2001. In October the ambient temperature has decreased up to 12–15°C and the mite stopped eating, but continued to deposit eggs gradually. This last mite has died 22.11.01 (total lifetime 674 days), and the first deposition cycle has finished simultaneously with its death. Completion — 22.11.01. Duration — 437 days (for two females). For this time, two females have laid 242 eggs. Sterile eggs — more than 150. This female was the last adult mite in the culture.

The second deposition cycle may be characterized as it follows.

1. Petri dish No. 2c. One remaining male from 11.12.98 and one female from 03.01.99.

Beginning — 21.09.99 — 49 days after the first cycle has finished (03.08.99) and 261 days after emergence of the female — 2 fresh eggs. To that time, there were also 19 eggs remaining from the first cycle in the dish. Another male being in this container earlier (see above) has died shortly before the second cycle — 14.09.99 and lived only 275 days. This male was large and seems to eat exceedingly, though gut defecation was not recorded. Altogether, these two males were laying few spermatophores, and to the moment of the second cycle has started no spermatophores were found in the dish. After the first two eggs, the female was not laying eggs for a month up to 21.10.99 but after that the cycle has continued. On December 10th and 16th there were found 4 and 5 freshly laid eggs, respectively, in the presence of 7 and 4 newly appeared spermatophores in the container. During winter, the male has increased its activity when the room temperature was relatively high — 19–23°C. The decrease of temperature up to 12–15°C that has happened 25 of January did not affect the egg and spermatophore deposition essentially. The second cycle has finished by one sterile egg when there were 9 spermatophores in the dish. Completion — 07.03.00. To that time there were 19 eggs in the container -- precisely the same quantity as in the beginning of the cycle. Duration — 168 days. Total egg number — 42. Sterile eggs — 35. After the completion of the cycle, the male continued to lay a relatively large number of spermatophores, whereas the female has died 03.04.00 — 25 days after the finishing of the cycle (total lifetime equaled 444 days).

2. Petri dish No. 2d. One male from 11.12.98 being earlier in the Petri dish No. 2c (see above) and one native weak female from 08.08.99.

Beginning — 31.07.00 — 1 egg 21 days after the mites were placed together, 192 days after the first cycle has finished and 358 days after the emergence of the female. Completion — 09.11.00. Duration — 132 days. Total egg number — 16 eggs. Sterile eggs — 13, although the male was active and laid up to 4 spermatophores per 5–7 days. 22.11.00 the female has died (lifetime 624 days) due to not very large ventro-caudal gut defecation. During two deposition cycles, this female has laid in total 4 fertile and 22 sterile eggs. The remaining male has died 27.12.00 (lifetime 745 days).

It is interesting to note that in two cases during the experiment there was the suspicion of presence of the ***"facultative hermaphroditism"*** both in male and female when mites after the loss of the companion of the opposite sex have provisionally changed their sexuality to deposit eggs instead of spermatophores and spermatophores instead of eggs for a short time. Such a phenomenon is seen to correspond to the internal anatomy of mites [Shatrov, 2000], is concealed deep in the mite physiology and ecology and needs to be further thoroughly examined.

In three first deposition cycles in Petri dishes No. 1a, No. 1b and No. 2c, after the simultaneous beginning of the cycles 25.01.99, the oviposition

was intensive in March and April, and the first prelarvae were also noticed in April of the next year after the collecting of fed larvae in autumn of the previous year has been made (on October 2nd and 4th). The next peak of the prelarvae formation was registered in August 1999 partly from the freshly laid eggs, when the first cycle in one dish having been continued and the second cycle in another dish has already finished. Thus, the period of the egg development in these cases could be either long, from the beginning of the cycle, or, on the contrary, short in the case of the mite development from freshly laid eggs. However, the subsequent interval in the prelarvae formation was extremely large — in the Petri dish No. 1b during the first cycle such interval lasted from August 24th till December 23rd 1999, and in the Petri dish No. 1a already after the completion of the facultative second cycle interval lasted also from August 24th till December 29th 1999. In general, prelarvae having been formed rather irregularly and measured out in a maximum number of 5–7 individuals for 1–2 week with a subsequent interval of month or even more both during a given cycle and mostly long after its completion from the eggs having been laid a long time ago. Such intervals irrespectively of various seasons might embrace from 38 to 113 and more days, but period of the prelarva *per se*, as a separate ontogenetic stage, did not differ significantly (Table 2) after the formation from both recent and old eggs. Sometimes, due to unknown reasons, prelarvae, which were already began to develop, were unexpectedly losing viability and finally died. The registered minimum time of the eggs development from the formal completion of a given cycle until prelarvae have formed, regardless of a season and an ambient temperature, were typically extremely long and measured from 100 to 550 days with a maximum definite duration of 832 days, the period, which has never been identified in the literature before. It should be especially noted that such duration was only found for eggs, which were capable to subsequent development.

After the first cycle has simultaneously started 25.01.99 the first unfed larvae in three Petri dishes have appeared 26.05.99 and 07.06.99 on the 121st and 133rd day after the appearance of the first eggs. Thus, in general, the minimum cycle from the fed larvae of previous to unfed larvae of the first laboratory generation in culture was estimated as approximately eight months, or, being exactly, 236 days. Individual longevity of unfed larvae was also extremely high and typically continued more than 3–4 months with the maximum value of 179 days. After that period the larvae died without feeding. The lifetime of male comprising 956 days that was demonstrated in this experiment appears to be the maximum life duration reported for the adult mites of this species [Simonova, 1977, 1983; Shatrov, 1996, 2000] and only little yield to data received earlier for adults of the Far-Eastern species *Leptotrombidium pallidum* (970 days) [Takahashi et al., 1993] and *Leptotrombidium orientale* (1030 days) [Shatrov, 1996]. Adult mites appeared in winter from the autumn larvae and being physiologically normal could successfully live through the next year, so the life cycles of individual mites were rather asynchronous in culture, especially if the larvae of the next generation are appeared gradually and irregularly.

Thus, for instance, the peak of the prelarvae formation, up to 12 newly formed prelarvae per a given examination, in the Petri dish No. 1e was registered in March-April and in August-September, 2002 from the very old eggs — the cycle has formally completed 22.11.01 simultaneously with the death of the last female. However, the unfed larvae have begun to appear during summer and autumn that year and attained maximum quantity, in total 30 larvae, to November, firstly for all the time of the experiment. As a result, in total 24 unfed larvae at the age of a week or more were placed on the back of the young guinea pig under a special capsule for feeding on November 9th 2002. Output of the successfully fed larvae was 5 larvae (around 21%), which fed for about 4 days. These fed larvae of the first laboratory generation have become immobilized within one day (2 prelarvae) or three days (3 prelarvae). Subsequent development of mites was typical, although, unfortunately, one protonymph has lost its viability due to attack of fungi and has died. Duration of the quiescent protonymphal periods was 17 days (3 mites) and 19 days (1 mite). One weak deutonymph has died on the 10th day after emergence. Two active deutonymphs lived for 18 and 24 days, whereas one remaining deutonymph was living from 03.12.02 till 31.10.03 (lifetime 333 days) with a very low feeding activity and finally successuvely transforms to a quiescent tritonymph. Periods of two quiescent tritonymphs were 18 and 21 days. Unfortunately, adult mites emerged on January 5th and 15th 2003, were females.

DISCUSSION

As it is seen from this study, the trombiculid mites *H.zachvatkini* are capable to develop succes-

sively even from the very old eggs being dormant (diapaused) for more than 300–400 days, and larvae may feed on the host after relatively long individual life. In contrast to the Middle-European population of this species [Simonova, 1977, 1983], the duration of all stages found in the author's research was significantly longer, and, moreover, there was no any possibility to receive a representative quantity of unfed larvae of the first laboratory generation mostly due to a very long diapauses on the egg stage and to a quite irregular prelarvae development. There are another sufficient differences between the representatives of the eastern and western populations of *H.zachvatkini*. For instance, Simonova [1977, 1983] pointed to cannibalism of mites and the deposition of eggs frequently in batches that were not shown in the current research. As well as that the males did not deposit spermatophores only within the first several days after emergence. However, in the attempts of rearing of *H.zachvatkini* from the Moscow district [Vasil`eva, 1977], the periods of the mite development were found to be also shorter than those in the present work, but deposited eggs in the experiments of Vasil`eva [1977] did not undergo further development and soon died. Whereas it is very difficult to find active postlarval instars on the soil in nature in various northern countries [Daniel, 1961], adults of some tropical species may be collected immediately from the soil in nature [Michener, 1946a, b; Radford, 1946a; Wharton, 1946; Cockings, 1948; Jenkins, 1948; Nadchatram, 1968]. Moreover, Radford [1946b] reports that he has found adults of *Neoschoengastia indica* within the crown of coconut palm *Cocos nucifera* L. between the bases of its leaves. In contrast, Elton and Keay [1936] were not able to find adults of *Neotrombicula autumnalis* in nature in England.

In respect to the question on what stage mites undergo the hibernation in winter, the various speculations were proposed [Williams, 1946; Mehta, 1948; Hsu, Chen, 1960a, b; Shoshina, 1965; Jameson, 1967, 1968; Vasil`eva, 1977; etc.] and only few natural experimental works have been made [Takahashi et al., 1993; Takahashi et al., 1995]. In the latter experiments, it was clearly demonstrated that mites retard their development on any stage in the cold winter period when the temperature decreases below 10°C, and all larvae fed in late autumn do not undergo further development until spring [Takahashi et al., 1993]. However, in another work there was shown that larvae, which fed in October, have rapidly developed into active deutonymphs, which have become frozen in winter, and adults have already appeared only in next May [Takahashi et al., 1995]. Mites cultivated since spring were transformed into adults already in August, and the next generation gave rise to adults, which again become frozen in winter.

It is doubtless, however, that mites may become frozen and retard their development in any season when temperature falls below 3–5°C and also hibernate and successively survive unfavorable period practically in all stages of their life cycle with the exception, supposedly, unfed larvae [Vasil`eva, 1977]. In constant temperature in the laboratory, there are no such natural surrounding climatic conditions and seasonal changes, and mites continue their development without any conspicuous diapauses on any developmental stage except for eggs. However, to initiate their awakening it seems there is no need to expose them by a cold impact [Shatrov, 1996] even if this procedure leads to continuing development of some eggs. More likely that just a short increase of temperature may provoke their further development. Boreal mites, being in nature in the soil where temperature rarely increases above 15–17°C even in summer, appear to demonstrate rather slow activity and obviously reveal a univoltine or even semi-univoltine life cycle, when adult mites being emerged in summer produce only one generation of larvae or, in the case of late appearance, do not produce larvae and become frozen to hibernate. Nevertheless, the main life strategy of these mites appears to be based on the rapid nymphal development and, on the other hand, on the extreme viability of adult mites, which are apparently able to produce eggs, not mentioning spermatophores, during at least two summer seasons. Increase of temperature in spring seems to stimulate further development of eggs and synchronize the whole life cycle [Jameson, 1967]. Taking into account this consideration, it is possible to assume, that any room temperature cannot whatever serve optimum, as previous authors [Hsu, Chen, 1960a, b; Neal, Barnett, 1961; Jameson, 1967, 1968; Everett et al., 1973; Cunningham et al., 1975; Simonova, 1977, 1983; etc.] have thought, but only shortens life cycle and makes it more intensive.

Appearance of the large amount of sterile eggs looks somewhat strange, especially in the presence of an active male in the same Petri dish, that was also reported in the work of Simonova [1977, 1983]. It looks particular strange in the case of relatively small number of eggs deposited in all by

an individual female during the whole lifetime in comparison with some other arthropods and mites. One cannot except that just the relatively high ambient temperature may provoke the formation of eggs unable to develop subsequently, especially if some eggs look good on the time of their deposition. In any case, production of eggs by rather many trombiculid species appears to be extremely low and it seems nearly impossible that in spring such a large amount of larvae feed in the ears of bank voles, in the regions where the only one species is seen to dominate. Correspondingly to low production of eggs, mites deposit them separately that have been observed in many works made so far [Michener, 1946a, b; Wharton, 1946; Mehta, 1948; Wharton, Fuller, 1952; Sasa, Miura, 1953; Hsu, 1959; Shirasaka, Sasa, 1967], although sometimes there were found batches of freshly laid eggs [Melvin, 1946; Simonova, 1977]. Evidently due to sparse population of mites, they elaborate a special generation mode such as parthenogenesis that has been experimentally recorded and confirmed for several species [Ito et al., 1957; Ito, 1967; Kaufmann, Traub, 1966]. Conversely, the phenomenon of hermaphroditism, which was also shown for trombiculids morphologically [Shatrov, 2000] and supposedly to have a wide distribution, has not been shown previously.

No special behavioral reactions of females directed to their own eggs were clearly observed in the present study. Nevertheless, some investigators have reported the mode of behavior when female roll its egg on the substrate to hide it [Wharton, 1946; Jayewickreme, Niles, 1947; Everett et al., 1973]. On the other hand, cannibalism on their own eggs was described for adult mites of many species [Wharton, Carver, 1946; Cockings, 1948; Lipovsky, 1951, 1953, 1954; Neal, Barnett, 1961; Shoshina, 1964; Audy, Lavoipierre, 1966; Shirasaka, Sasa, 1967; Simonova, 1977]. Some species of trombiculids demonstrate a particular preference in consumption of eggs of various arthropod species, including mites [Minter, 1957; Nadchatram, 1968; Wharton, Carver, 1946; etc.] that supposedly define a potential capability for them to be cannibalistic. It is evident, however, that in the case of *H.zachvatkini* the collembolan eggs are highly preferable that leads to the obvious exceeding feeding and, consequently, to deterioration of mite physiology and, as a result, to gut defecation, the nearest consequence of which may be a premature death. A reason that the schizeckenosy may be considered as a natural substitution of normal defecation [Mitchell, Nadchatram, 1969] sounds to be incorrect and groundless from the positions of physiological and behavioral considerations [Shatrov, 1993b; 2000]. It is clear that in nature, soil-inhabiting mites neither get so plentiful meal nor eat too much. It is also nearly inconceivable that deutonymphs and adult mites could increase their sizes after feeding that was never observed in my work with trombiculids for more than twenty years, but, on the contrary, have been reported in some previous research [Jayewickreme, Niles, 1946; Wharton, Carver, 1946; Sasa, Miura, 1953]. In short, the life of mites in nature appears mostly to be cold and hungry, although we still not know much about their natural life [see also, Daniel, 1961].

For feeding of trombiculid larvae in mass cultures white mice are frequently used [Nadchatram, 1968; Kulkarni, Mahadev, 1973; Kulkarni, 1988; Southcott, Frances, 1991; etc.], which are placed in containers over the water. In this method, the loss of a few larvae is not a problem, and the final output of successfully fed larva is apparently high. On the contrary, if each larva is greatly valuable, feeding within isolated capsule appears to be preferable and allows preserving all larvae, which have started feeding. Guinea pigs seem to be unusual hosts for trombiculids from the Old World, but due to their extremely wide host-parasite specificity it is not a serious obstacle for their successful feeding. It is interesting to note that Richards [1948] used guinea pigs to attract *Neotrombicula autumnalis* larvae in nature in England.

Concerning the life strategy of other terrestrial representatives of Parasitengona, there were some comprehensive works published recently [Wendt et al., 1992; Wohltmann, 1996, 2000; Wohltmann, Wendt, 1996; etc.], carefully examining, in particular, the life cycles and life strategies as well as phenology of mites of the families Microtrombidiidae, Johnstonianidae and Erythraeidae. These works revealed a great similarity in some details of realization of the life strategy events among trombiculid mites and the mites from these families, in particular. It should be noted, however, that trombidiid mites, especially those of the families Trombidiidae and Microtrombidiidae, collected in late spring deposit eggs immediately, from which larvae hatch in mass some time later [Newell, Tevis, 1960; Shatrov, unpublished data]. Thus, these mites do not demonstrate obvious egg diapauses because, supposedly, this period of their life cycle is naturally realized on the soil surface and mediated by the

relatively high positive temperatures. The most evident difference from all other Parasitengona is that the Trombiculidae is the only family, where larvae parasitize vertebrates, and the only family, where the deutonymphs and adult mites disappear deep in the soil, and seemingly evolutionary irretrievably. Just in these abilities a riddle of their life should be searched.

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