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SURVIVAL AND REPRODUCTION OF A LABORATORY STRAIN OF BODY LICE (*PHTHIRAPTERA: PEDICULIDAE*) AT DIFFERENT AMBIENT TEMPERATURES

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Abstract In order to elucidate the role of temperature as a crucial survival factor for human body lice (*Pediculus humanus humanus*), we investigated the influence of different temperature regimes on survival of adult lice after their last blood meal as well as on oviposition and egg hatch rates. The Federal Environment Agency (FEA) maintains a laboratory colony of the human body louse *Pediculus humanus humanus* for efficacy testing of pediculicides, since the human body louse has proved to be an appropriate test organism for testing products for the control of its subspecies relative, the head louse *Pediculus humanus capitis.* The lice are reared on rabbits as substitute hosts. The laboratory lice are fed on Chinchilla bastard rabbits 4 to 5 times per week for ca. 15 minutes. Between daily feeding the lice are stored for routine cultivation at 32°C and 45-55% relative humidity and on non-feeding days at 25°C and 45-55% relative humidity. For investigation of survival and reproduction of lice, male and female adults were fed and then subsequently stored at ambient temperatures (T_A) between 16°C and 32°C at 45-55% relative humidity. The survival of unfed adults is negatively correlated with increasing temperature, with the longest survival (15 days) at an ambient temperature (T_A) of 16°C and the shortest survival at T_A 32°C (4-5 days). In contrast to this, oviposition rates as well as egg hatch rates were positively correlated with increase of temperature (1.15 eggs/d/female at T_A 22°C and 3.52 eggs/d/female at T_A 32 °C). The ambient temperature (T_A 25°C or 32°C) female lice experienced before and during oviposition had only a weak influence on egg hatch rates and oviposition rates. Experiments on influence of different temperature regimes revealed that eggs must at least be incubated for 5 days after oviposition at $T_A > 25$°C. Egg which were first incubated at T_A 25°C and then transferred to T_A 32°C had high hatch rates, indicating that the early development of eggs after oviposition is less temperature sensitive than later development.
Key words Human louse, temperature physiology, humidity, oviposition, hatch rates

INTRODUCTION

Human lice (*Pediculus humanus*) are obligate parasites on humans. It is under discussion if human body lice and human head lice are different species or two subspecies. In this paper, we follow Maunder (1983) who regards them as subspecies (body lice, *P. humanus humanus* and head lice, *P. humanus capitis*). The human body louse *Pediculus humanus humanus* is the main vector of epidemic typhus (louse-borne typhus), *Rickettsia prowazecki*, relapsing fever, *Borrelia recurrentis*, and trench fever, *Bartonella quintana*. In many countries, including all members of the EU, body lice are nowadays of only minor medical importance. Unlike its relative, human head lice are still an import public pest. Although principally human lice can be treated effectively with topical pediculicides, the frequent occurrence of resistances to some of them is a matter of concern (Downs et al., 1999; Kristensen et al., 2006). Thus efficacy testing of products and active substances against human lice is of increasing importance for public health. Human head lice (*Pediculus humanus capitis*) are difficult to rear in laboratory at greater numbers (Takano-Lee et al., 2003), but human body lice have been successfully adapted to rabbit hosts since the 1940s (Culpepper, 1948). Since differences in physiology of body and head lice are neglectable, body lice are an excellent test organism for efficacy testing of pediculicides (Burgess, 2004). To this end, high numbers of sensitive and vital body lice of defined age and nutritional status must be reared. The Federal Environment Agency (FEA) maintains the only laboratory body louse strain in Germany. The lab strain was established from lice from a strain at the USDA research laboratory in Orlando (Florida). Despite the possibility of rearing body lice in laboratories,

data on temperature physiology are scarce and the main body of literature is more than 60 years old (Leeson 1941a, 1941b). Body lice complete their whole life cycle on or in the close vicinity of their hosts, which are endotherms, and are thus likely to be adapted to specific temperature conditions crucial for louse survival and reproduction. In order to optimize our lice rearing conditions and improve our understanding of body louse temperature physiology, we have tested the influence of different temperature regimes on body lice. The emphasis of our study is on the influence of temperature on survival of adult lice after their last blood meal as well as on oviposition and egg hatch rates.

MATERIAL AND METHODS

Body Lice

The body lice used in our experiments are derived from a body louse strain that is maintained for about 40 years at the former Institute for Water-, Soil- and Air-Hygiene of the Federal Environment Agency of Germany. The lice strain is adapted to rabbits as hosts (Culpepper, 1948). For routine lice rearing, the lice are fed on rabbits (race: Chinchilla bastards) 4 to 5 times per week for 15 minutes. Between feeding the lice are stored in glass petri dishes on a cloth of cotton corduroy (5 cm x 5 cm) at 32°C on feeding days and at an ambient temperature (T_A) of 25°C on non-feeding days. When fed four times a week, the lice developed to adults after 11 (10-12) feeding episodes at age of 20-21 days after hatch from egg (Figure 1). The lice deposit their eggs on the corduroy cloth. Eggs remain in an incubator at T_A 32°C and until larvae hatch after 8 (6-9) days. Earlier own studies had shown that optimum oviposition and hatch rates are achieved with lice which are fed with 4 blood meals on 4 subsequent days, and are kept at 32°C with an average oviposition rate of 3.8 eggs/d/female (min. 3.4; max. 4.4) and an average hatch rate of 96.1% (min. 92.6%; max. 98.8%).

All lice used in our experiments are progeny of the described laboratory louse strain. The rearing conditions are modified according to our experimental needs: the main parameter which was changed is ambient temperature, and in one case the feeding schedule (see below). As in standard laboratory rearing, all lice groups were kept in glass petri dishes with closed (but not sealed) lids together with a piece of corduroy cloth and kept in incubators (T_A 25°C and 32°C: Memmert Type BE 30 and Type BE 400, Memmert Elektronik, Schwabach, Germany; T_A 16°C to 22°C: Memmert Type JCP 400 and Type JCP 500, Memmert Elektronik, Schwabach, Germany, and Binder Type KB 115, Binder GmbH, Tuttlingen, Germany). At all temperatures and temperature regimes in our experiments, relative humidity in the incubators was constant at a range from 45-55% RH. (Hygrometer 70100 F 222, G. Lufft Mess-und Regeltechnik, Fellbach, Germany).

Figure 1 SURVIVAL AND REPRODUCTION RATES OF A LABORATORY STRAIN OF BODY LICE (*PEDICULUS HUMANUS HUMANUS*) AT DIFFERENT AMBIENT TEMPERATURES
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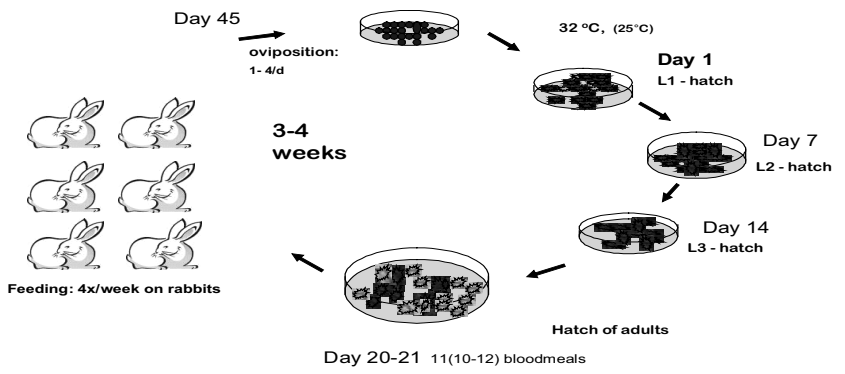

Figure 1. Developmental cycle of the Rabbit-adapted strain of *Pediculus humanus humanus* in the laboratory.

Influence of Temperature on Adult Survival After Last Blood Meal

All adults used for this experiment had 4 blood meals after their last ecdysis on 4 subsequent days. During this period, they were kept at T_A 32°C. After their last blood meal, the adults were kept at T_A 16°C (3 experiments with groups of 45 ♂/45 ♀ each), 18°C (3 experiments with groups of 60 ♂/60 ♀ each and 3 experiments with groups of 120 ♀ each), 22°C (3 experiments with groups of 60 ♂/60 ♀ each), 25°C (3 experiments with groups of 45 ♂/45 ♀ each and 3 experiments with groups of 60 ♂/60 ♀ each), 28°C (3 experiments with groups of 60 ♂/60 ♀ each) and 32°C (3 experiments with groups of 45 ♂/45♀ each and 3 experiments with groups of 60 ♂/60 ♀ each), respectively. The first observation of number of surviving lice was made 24 h after the beginning of the experiment. This observation is denoted as day 1.

Oviposition Rate and Hatch Rate at Different Temperatures

For the determination of temperature dependence of oviposition and hatch rate, we used females which were kept and reared at the described optimum conditions (4 blood meals on 4 subsequent days) at T_A 32°C. After their last blood meal, female lice were transferred to T_A 22°C, 25°C, 28°C and 32°C, respectively, for oviposition. Number of deposited eggs was first counted after 24 h. All eggs which were deposited were controlled daily for hatch of larvae and number of larvae was counted. A total of 1,260 female adult body lice were tested, with 20 to 40 lice per temperature and at least 3 experiments for each temperature

Influence of Temperature Before and During Oviposition on Rate and Time of Hatch

In this experiment series, we investigated the effects of T_A on development of eggs in the ovary of females and during oviposition. Adults were fed with 4 to 6 blood meals after their last ecdysis. After the last of these blood meals, they were kept at T_A 25°C and 32°C, respectively. After 3 days at these experimental temperatures, they were again fed on rabbits and kept for oviposition at T_A 25°C and 32°C, respectively. The eggs were incubated then either at T_A 25°C or 32°C in four combinations (Table 1), and hatch rate and time between oviposition and hatch was determined.

Generally, more eggs were obtained for our experiments at T_A 32°C than at T_A 25°C. At T_A 25°C, at total of 791 eggs were deposited and observed. Due to the higher oviposition rate at T_A 32°C, a total of 2,135 eggs could be obtained. In contrast to the experiment described in the next paragraph, the oviposition rate was not evaluated in this experiment, since the eggs were directly taken from our standard laboratory culture and number of females was not counted. For oviposition rates at various T_A, see description of the experiments in previous paragraph.

Influence of Temperature After Oviposition on Hatch Rate and Time of Hatch

In this experiment series, changes of T_A were made at two different stages of egg development in order to test effects on early embryo development (first 3 or 5 days of egg development after deposition) and effects on later embryo development. Adults were fed with 4 blood meals on 4 subsequent days. After oviposition at T_A 32°C, the adults were removed and eggs were incubated at six different temperature regimes. Table 2 gives an overview of the temperature regimes. In experiments III and V, the incubation temperature of the eggs was changed after 3 days, and in experiments IV and VI after 5 days. Each experiment was conducted simultaneously with 4 groups of female lice; group size amounted to 30 lice in experiments I and IV ant to 40 lice in experiments II, III, V and VI.

Criteria For Lice Selection, Control and Examination

Lice were selected with a stereo-microscope. The lice were selected for their nutritional status (optical examination of the amount of blood inside the gut). Eggs were examined daily for larval hatch. After hatching, the remaining material was examined with a stereo-microscope and classified in the following categories: hatched larvae; larvae stuck in egg while hatching; sterile eggs; eggs with unstructured content (early embryonic development: differentiated yolk balls in embryo, body contours become visible and start of segmentation); eggs with structured content (later embryonic development: formation of tagmata, legs, antennae, eye spot). In our results, eggs with early and later development are subsumed. In all experiments, less than 1% of all eggs were sterile. Values for on hatch rate and duration of embryonic development are given as medians.

Table 1. Effect of ambient temperature on egg development in the ovaries of females and during oviposition. Hatch rate is given as median with minimum to maximum range in brackets. First day of hatch is defined as the day when the first larva was observed, main hatch day is defined as the day where < 80 % of larvae had hatched. Oviposition day is counted as day 1.

Experiment	I	II	III	IV
T_A before and during oviposition	25°C	25°C	32°C	32°C
T_A after oviposition	25°C	32°C	25°C	32°C
Hatch rate in % *Median* *(Range)*	0 (-)	80.2 (79.1 — 80.6)	0 (-)	89.0 (88.6 — 89.4)
First day of hatch	-	8	-	7
Main hatch day	-	9	-	8

Table 2. Effect of ambient temperature on later embryonic development. Hatch rate is given as median with minimum to maximum range in brackets. First day of hatch is defined as the day when the first larva was observed; main hatch days are defined as the days where < 80 % of larvae had hatched. Oviposition day is counted as day 1.

Experiment Nr.	I	II	III	IV	V	VI
T_A before and during oviposition	32°C	32°C	32°C	32°C	32°C	32°C
T_A at first 3 days of egg development	32°C	25°C	32°C	X	25°C	X
T_A at first 5 days of egg development	32°C	25°C	X	32°C	X	25°C
T_A at remaining time of egg development	32°C	25°C	25°C	25°C	32°C	32°C
Hatch rate in % *Median* *(Range)*	95.6 (94.2 — 98.1)	0 (-)	41.9 (38.0 - 42.9)	92.0 (87.6 — 96.8)	92.1 (88.6— 93.9)	81.4 (68.9 — 85.4)
First day of hatch	7	-	14	13	11	13
Main hatch days	8		15 - 18	14 - 15	12	14

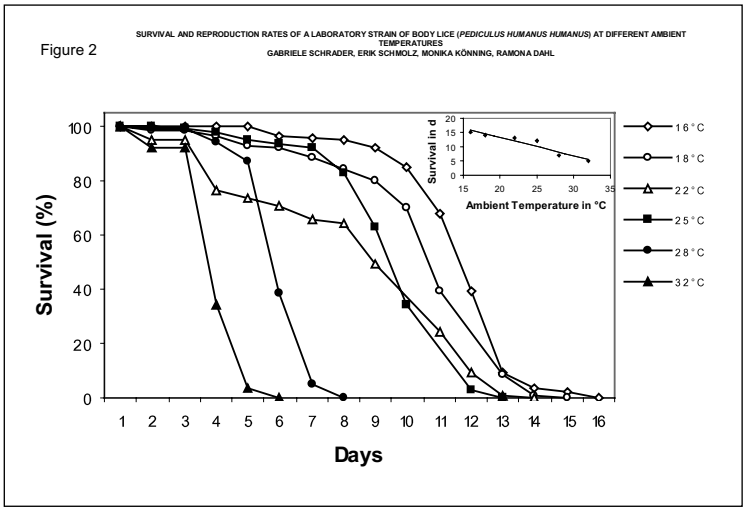

Figure 2. Survival of starving adult body lice. Lice were fed with 4 blood meals on Rabbits after last ecdysis and then incubated at different ambient temperatures. Small insert figure shows the correlation of maximum survival with ambient temperature. Linear regression equation is $y = -0.6376x + 25.984$, $r^2 = 0.9097$.

RESULTS

Influence of Temperature on Adult Survival After Last Blood Meal

The survival of adults is negatively correlated with increasing temperature (Figure 2). At T_A 16°C, no surviving lice (both sexes) were found at day 15. After 12 days no surviving lice (both sexes) were found at T_A 25°C, whereas at T_A 32°C all females were found dead at day 4 and all males at day 5.

Oviposition Rate and Hatch Rate at Different Temperatures

In contrast to adult survival, oviposition rates as well as egg hatch rates were positively correlated with increasing temperature. Lice deposited 1.15 eggs/d/female (min.1.1; max. 1.2) at T_A 22°C, 1.27 eggs/d/female (min. 0.5; max. 2.0) at T_A 25°C and 2.4 eggs/d/female (min. 2.3, max. 2.65) at T_A 28°C. The highest oviposition rate was observed at T_A 32°C with 3.52 eggs/d/female (min. 3.13; max. 4.0). No larval hatches from eggs were observed at T_A 22°C and 25°C. 89.9% of larvae from eggs deposited at T_A 28°C hatched successfully (min. 84.9; max. 93.7). As with oviposition, the highest hatch rate was observed at T_A 32°C with 95.2% (min: 92.5; max. 98.1).

Influence of Temperature Before and During Oviposition on Rate And Time of Hatch

The ambient temperature female lice experienced before and during oviposition had only a weak influence on egg hatch rates and oviposition rates. Table 1 summarizes the results. The highest hatch rate was observed at T_A 32°C during the whole period of development (experiment IV). Eggs from females which experienced T_A 25°C three days before and during oviposition (experiment II) had a slightly lower hatch rate compared to 32°C. The same holds good for the time needed for egg development. Low temperatures of T_A 25°C after oviposition (experiments I and III) were fatal for all eggs (hatch rate 0 %), regardless of the temperature the females experienced before oviposition.

Influence of Temperature After Oviposition on Hatch Rate and Time Of Hatch

Since our results indicated that the effect of temperature on females before and during oviposition has only little effect on egg development, we tested whether specific periods during egg development after oviposition are temperature sensitive. All females were incubated and deposited their eggs at an optimum temperature of T_A 32°C. After oviposition, the eggs were incubated at high (32°C) or low (25°C) temperatures for 3 or 5 days. In order to differentiate between temperature effects on early or later embryonic development, after these 3 or 5 days the eggs were either transferred to the corresponding lower or higher temperature or incubated at the same temperature until completion (or definitive failure) of development. Table 2 gives an overview of results and temperature regimes used. Like in our previous experiment on maternal effects, nearly all eggs (95.6%) hatched when incubated constantly at 32°C (experiment I) and all eggs failed to develop into larvae at constant T_A 25°C (experiment II).

When incubated at low temperatures for the first 3 or 5 days of their development after oviposition (experiments V and VI), the eggs had still a high hatch rate, which was lower when eggs were incubated for 5 days at T_A 25°C compared to 3 days (92.1% vs. 81.4%), with a corresponding longer time needed for development. Hatch rates were considerably lower (41.9%) when the eggs were incubated for 3 days at T_A 32°C and transferred to 25°C for the rest of their development (experiment III). Interestingly, an abnormal high rate of larvae which tried to hatch but did not complete hatching was observed in this experiment with 14 % larvae stuck during hatch. In all other experiments, the rate of incomplete hatches was < 1 %.

In contrast to this finding, a comparatively high hatch rate (92.0%) was observed in eggs that were kept for two days longer (5 days) at 32°C before being transferred to a lower temperature (experiment IV). This leads us to the conclusion that the temperature at days 4 and 5 after oviposition is most temperature sensitive during egg development. This finding is supported by the fact that eggs which are incubated for 5 days at 25°C show a lower hatch rate even when transferred to 32°C afterwards, whereas only 3 days at 25°C and thus days 4 and 5 at 32°C result in the same hatch rate than eggs which were kept for 5 days (including days 4 and 5) at 32°C and then transferred to a lower temperature.

DISCUSSION

Our experiments could show that adult survival is negatively correlated with increasing temperature. Adult lice survived for 15 days without food at T_A 16°C and only for 4 days (females) to 5 days (males) at T_A 32°C. For higher temperatures, these results are in the same range as found in previous studies: Leeson (1941b) found survival in adult body lice after their last blood meal to be 2 to 3 days at T_A 35°C and 3 days at T_A 30°C. However, at T_A 15°C, adults survived for only 9 days and at T_A 24°C for only 5 days, which is both much shorter than in our study. Humidity was higher in Leesons experiments (90% RH) compared to our study (45% to 55% RH). Maunder (1983) notes that high humidity can be disadvantageous for body lice since their copious faeces, which is normally dry, becomes then damp and sticky. Lice can become coated with their own excrements and get stuck on the clothes were they live. Humidity regimes can therefore be an explanation for differences in our results, together with the fact that Leeson worked with different strains of body lice he did not further define and which were fed on humans. The strain used in our study can be principally also fed with human blood, resulting in normal reproduction rates, but differences may exist between the strains (Habedank et al., 1999). Generally, since most insects are ectotherms, metabolic rates are positively correlated with increasing T_A, resulting in a higher energy expenditure leading to shorter survival under starvation conditions (Schmolz and Lamprecht, 2004). Ambient temperatures of 30°C to 35°C are normally associated for body lice with a habitation in the close vicinity of their endothermic hosts, were blood meals can be taken up to satisfy their energy demands. In the absence of a host, ambient temperatures are likely to fall below 30°C or even 20°C. Adult lice were able to survive for two weeks at T_A 16°C in our experiments, which is a time span where the chances of meeting a new host are sufficiently high. Moreover, longer periods of survival can not be expected. Phtirapterans in general complete their life cycle exclusively on their hosts, and are not adapted for survival of longer periods without them.

In contrast to survival, oviposition as well as egg hatch rates were positively correlated with increasing T_A. At T_A 32°C, nearly three times more eggs were deposited compared to T_A 22°C, and eggs failed to develop successfully at T_A 25°C and below. Leeson (1941a) found a hatch rate of 79% at T_A 32°C and 65% RH, and 97% at T_A 29°C and 60% RH. Although the relative humidity was slightly higher in his experiments, the main difference compared to our results is an inverse correlation of hatch rate to T_A with a higher hatch rate at 29°C than at 32°C, whereas in our experiments, more larvae hatched at T_A 32°C (95%) than at T_A 28°C (90%). The lowest T_A at which Leeson observed hatches was 24°C and 90% RH (hatch rate 11%). Machel and Krynski (1976) published a study on biological data of a laboratoratory strain of body lice (Weigl strain) and found no larvae hatching at T_A 25°C, high hatch rates at 28°C and 32°C and a negative effect of high temperature (T_A 37°C with a hatch rate of 55%). Although some differences exist between these data sets, all values are in the same range and indicate that the temperature threshold were eggs do not develop is $T_A \leq 25$°C at moderate humidity and a little lower at high humidity.

Since T_A 25°C seems to be a critical value for successful egg development, we investigated if low temperatures have a negative impact at specific points of time during development. Our experiments revealed that the temperature females experienced before and during oviposition had only a weak influence on egg hatch rates and oviposition rates. Eggs from females which were incubated at 25°C had a hatch rate in about the same range than eggs from females at T_A 32°C provided that the eggs were stored at T_A 32°C after oviposition. Thus, we assume that low temperature has no effect on eggs when they are in the ovary of the mother, whereas even eggs from females kept at high temperatures can not develop after oviposition when then incubated at low temperatures.

For further differentiation of this effect, we investigated the effects of ambient temperature on early and later embryonic development. To this end we examined hatch rates and time needed for successful development after oviposition at various temperature regimes. Eggs which were incubated at low temperatures during the first 3 or 5 days of their development had a good hatching success provided they were transferred to a higher temperature for the rest of their development. Hatch rates were lower the longer the eggs were incubated at lower T_A. In reverse, more of half of the eggs which were incubated for only 3 days at a high temperature and were then transferred to a low temperature failed to develop in contrast to eggs which were incubated first for 5 days at a high temperature and then at a low T_A developed with a high hatch rate. Thus, we were able to demonstrate that high temperatures are most critical for egg development after 3 days

and later, and that early embryonic development is less cold sensitive than later embryonic development. At day 4 to 5 of egg development after oviposition, the larva inside the egg begins to suck egg fluid and larger amounts of air bubbles into its gut. This process stretches and extents the larva so that it reaches the bottom of the egg shell. As a consequence, the larva is able to press itself against the egg lid, a process which is important in preparation for hatch (Sikes and Wigglesworth, 1931). At low temperatures, air and fluid sucking is probably hindered, since body movements are generally impeded at low T_A in ectotherms. This would be an explanation for the unusual high proportion of larvae which got stuck during hatch when exposed only for the first 3 days to T_A 32°C and then, in a crucial phase of hatch preparation, are transferred to T_A 25°C. In reverse, eggs which are deposited at T_A 25°C delay their embryonic development. When then transferred to a higher T_A after 3 or 5 days, their decisive phase of development falls in a point of time with optimum temperatures.

Our experiments could show that a moderate humidity of 45% to 55% RH. is sufficient for successful egg development and allows a comparatively long survival of starving adults. Leeson (1941a) found that optimum egg hatch rates were achieved at humidity from 50% RH. to 75% RH. at a T_A range of 37°C to 29°C. These values are somewhat higher than the humidity in our experiments, but since we measured humidity in the incubators and the lice and eggs were kept in glass petri dishes with closed lids (where humidity is difficult to determine), we assume that the actual microclimate had a slightly higher humidity than the values we actually measured. Humidity in a shirt of a resting person at ambient temperatures from 23°C to 37°C are in a range from 23% RH to 70% RH, and body lice have been shown to prefer low or medium humidity (Culpepper, 1946).

Rearing laboratory strains of rabbit-adapted body lice is an important means for efficacy testing of pediculicides, which is of increasing importance since recently human head lice frequently exhibit resistances against active substances commonly used (Kristensen et al., 2006). To provide meaningful test results, provision with standardized test material (i.e. a laboratory culture of lice) is essential to receive comparable efficacy data. Moreover, lice from a standardized laboratory strain offer an opportunity to receive new basic data for the temperature biology of body lice.

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