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Studies on the Free-Living Generations of *Strongyloides planiceps* Rogers, 1943
II. Effect of Temperature on the Developmental Types

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In the first report of this series (Arizono, 1976), the effects of quantity of food and population density on the course of development of *Strongyloides planiceps* outside the host were described. These factors were closely connected with the determination of their further developmental course either to free-living females or to infective larvae. Namely, many free-living females and a few infective larvae were formed in low population density cultures given sufficient feces as food, but in high population density or in cultures given very small amount of feces, free-living females markedly decreased and reciprocally infective larvae increased. On the other hand, free-living males were formed in a constant ratio regardless of these two factors.

The present study was attempted to know the effect of temperature on the developmental course of *S. planiceps* outside the host. In addition, the question when the course either to free-living females or to infective larvae is determined, was also examined.

Materials and Methods

The strain of *S. planiceps* and method of collection of eggs used in this study were described in the first report (Arizono, 1976). For cultures, a known number of eggs (241-320 in experiment 1, 200 in experiment 2) was placed on filter papers which were previously coated with egg-free feces of 0.2 g amount, and incubated in the form of filter paper test tube method. When free-living adults and infective larvae grew in the cultures, they were recovered by the same manner as mentioned in the first report.

In experiment I, for studying the effect of temperature on the developmental course, the cultures were held under seven different temperatures (12, 16, 20, 24, 28, 32, and 36 C), then examined the composition of free-living males, free-living females and infective larvae formed in the cultures. Incubation periods were 24-30 hr at 28-36 C, 30-40 hr at 24 C, 3 days at 20 C, 4 days at 16 C and 9-10 days at 12 C, respectively. During the periods free-living adults and infective larvae were formed, but no second generations were produced.

Experiment II was carried out to know when their developmental course will be determined. For this purpose three groups of cultures were designed. In group 1, each of cultures was first held at 15 C for 6, 12, 24 or 48 hr then transferred to 28 C, and kept until free-living adults and infective larvae were formed. The cultures of group 2, on the contrary, were first placed at 28 C for 2.5, 5 or 8 hr then transferred to 15 C. In group 3, all cultures were held at first at 28 C for 2.5 hr. After that they were moved to 15 C and kept for 6, 12 or 24 hr then returned to 28 C again. The control cultures were kept at 28 C or 15 C throughout their whole development. In each experiment, 5 test tube cultures were

prepared. At the time of each transfer from 28 C to 15 C or vice versa, one of the cultures was usually offered for measurement of larvae to indicate the stage of development. Remaining 4 test tube cultures were examined on the composition of three types of development. On the other hand, 300–500 eggs in 5 ml of water in petri dishes were held at 28 C or 15 C, and counts of hatched and unhatched egg were made at different hours to know the time course of hatching. All cultures in experiment 2 were made with eggs from the same pool, and were carried out at the same time.

Results

Experiment I. In order to study the effect of temperature on the developmental types of *S. planiceps*, egg cultures were held at various temperatures between 12 and 36 C. Experiments were repeated 5 times on different days.

Recovery rates of each type of development are expressed in percentage to the number of eggs planted, and are shown in Table 1. At temperatures between 12 and 36 C, most of the total recovery rates (T) were more than 70%. Through these temperatures free-living males (M) were formed

Table 1 Comparison of three types of development of *S. planiceps* in cultures held at various temperatures

Experiment No.	Number of eggs planted	Number* of test tubes	Form of worm	Mean % recovery of worms						
				12	16	20	24	28	32	36°C
1	288	3	T	NE**	85.8	77.5	87.9	83.8	74.6	NE
			M	—	18.8	18.5	18.4	20.6	16.0	—
			F	—	0.7	9.7	37.8	48.4	47.4	—
			f	—	66.3	49.3	31.7	14.8	11.2	—
2	248	4	T	NE	92.1	88.3	87.7	72.3	84.6	70.3
			M	—	19.1	15.7	17.3	13.0	14.6	11.2
			F	—	0.4	22.9	46.5	41.6	57.8	57.9
			f	—	72.6	49.7	23.9	17.7	12.2	1.2
3	320	5	T	67.7	84.1	87.5	89.0	87.4	80.5	2.4
			M	10.3	24.1	23.8	24.6	23.9	24.6	0.3
			F	0.0	0.9	11.1	25.0	17.4	25.7	1.5
			f	57.4	59.1	52.6	39.4	46.1	30.2	0.6
4	241	4	T	73.4	69.8	82.7	NE	86.1	67.4	NE
			M	11.4	19.6	22.5	—	25.6	21.1	—
			F	0.0	6.1	36.3	—	41.2	30.7	—
			f	62.0	44.1	23.9	—	19.3	15.6	—
5	241	4	T	91.4	88.4	84.7	82.9	79.4	84.0	84.9
			M	4.1	5.9	7.6	6.0	5.5	5.8	5.5
			F	0.0	52.4	64.8	75.8	73.1	77.3	79.0
			f	87.3	30.1	12.3	1.1	0.8	0.9	0.4

* Number of test tube cultures observed at each temperature. **NE : Not examined. T : Total recovery rate. M : Recovery rate of free-living males. F : Recovery rate of free-living females. f : Recovery rate of infective larvae. M, F and f are each expressed as percentage of the number of eggs used.

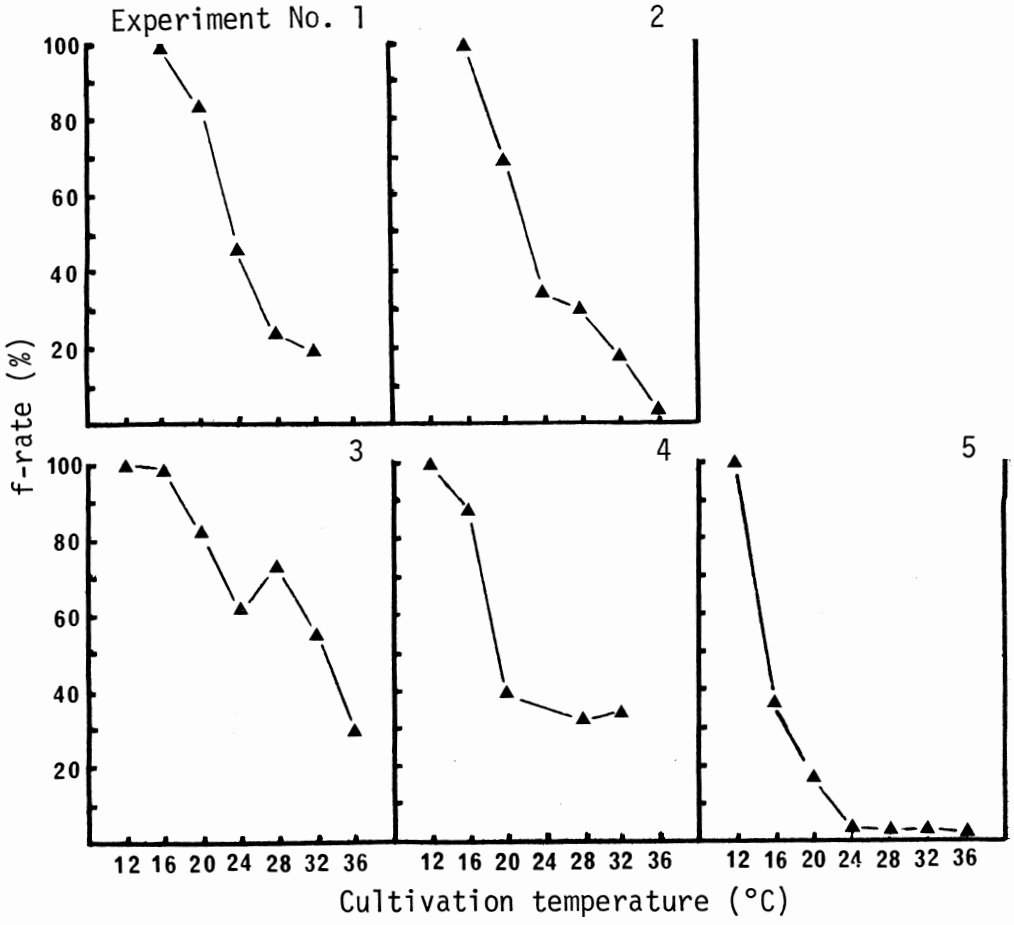

Fig. 1 Relationship of f-rates with cultivation temperatures.
f-rate $=\frac{f}{F+f}\times 100$ (f : infective larvae, F : free-living females)

at almost constant ratio, although in experiment 3 the rate was exceptionally low at 36 C. These results suggest that the course to free-living males was not affected by temperature.

On the other hand, free-living females and infective larvae were both distinctly influenced by temperature. Recovery rates of free-living females (F) which were minimum at 12 or 16 C, increased gradually as temperature raised, and reached maximum at 28–36 C. In contrast with this, the rates of infective larvae (f) which were maximum at 12 or 16 C, decreased gradually according to increase of temperature. However, sums of the rates of free-living females and infective larvae were nearly constant in each experment. Therefore, it was supposed that free-living females and infective larvae were formed reciprocally and the ratio was dependent on the temperature.

The relationship between free-living females and infective larvae was shown by f-rate (f/F+f×100) in Fig. 1. This index means that higher f-rate shows more infective larvae instead of free-living females. At low temperatures 12 or 16 C the f-rates showed nearly 100% in all experiments, and they decreased gradually according to the rise of temperature. They were mostly less than

30% at high temperatures 28 to 36 C.

Experiment II. The following experiment was carried out to know when the differentiation either to free-living females or to infective larvae will be determined. As shown in Fig. 1, the f-rates of cultures held at 12–16 C were high, but they were low at 28–36 C. Accordingly, if culture is transferred from 15 C to 28 C before determination of developmental course, the f-rate will be low similarly to culture held only at 28 C, but it will be high if transferred after determination. On that account, three groups of cultures were designed as shown in Fig. 2.

Through this experiment, total recovery rates were usually more than 70%, and compositions of free-living males were almost constant as between 43.6 and 52.5%. The f-rates are shown in Fig. 2 together with the body length of larvae at the time of transfer.

The cultures of group I were at first held at 15 C (----→) for 6, 12, 24 or 48 hr then transferred to 28 C (——→). Hatching rate of eggs in 15 C was 69.4% after 6 hr and 100% after 12 hr (Table 2).

The f-rates of cultures which were transferred from 15 C incubation for 24 or 48 hr to 28 C were as high as 86.9 or 95.5%, which were similar to that of 15 C control culture (C-2, 97.3%). This means that the larvae were not influenced by 28 C factor but influenced by 15 C during 24 or 48 hr in this condition. On the other hand, in cultures held at 15 C for 6 or 12 hr then transferred to 28 C, the f-rates were as low as 24.5 or 34.3%. These are definitely lower than 15 C control culture and close to f-rate of 28 C control culture (C-1, 17.0%). Therefore, most of the larvae or eggs at the time of 6 or 12 hr incubation in 15 C were not affected by 15 C yet, and thought to be undetermined on the developmental course.

In group II, cultures were at first held at 28 C for 2.5, 5 or 8 hr thereafter transferred to 15 C. Under 28 C incubation, 88.6% of eggs hatched out after 2.5 hr, and 100% after 3.5 hr (Table 2). When cultures were transferred to 15 C after 2.5 hr in 28 C, f-rate showed 75.3%, which was close to 15 C control culture (C-2). Accordingly, the developmental course of the larvae or eggs at this time were not determined yet. However, when cultures were transferred to 15 C after 5 or 8 hr in 28 C, f-rate showed 18.8 or 17.9%. These results suggest that most of the larvae of 5 or 8 hr incubation were already fixed on their future developmental course by 28 C.

The cultures of group III, as shown in Fig. 2, were all held at 28 C for the first 2.5 hr, during which period the larvae or eggs were not affected by 28 C as described above. After that, cultures were transferred to 15 C, and 6, 12 or 24 hr later, or in other words 8.5, 14.5 or 26.5 hr after beginning of cultivation, they were returned again to 28 C. Some cultures were continuously kept at 15 C without returning to 28 C. The f-rate was 26.2% when cultures were returned to 28 C after 6 hr incubation in 15 C. Since this f-rate is close to that of 28 C control (C-1), the larvae for 6 hr in 15 C were not affected by this temperature, but susceptible to the latest 28 C. However, when cultures were returned to 28 C after 12 or 24 hr at 15 C, f-rate increased to 59.6 or 69.0%, which were rather similar to those not returned to 28 C (75.3%).

Table 2 Hatching rate of eggs of *S. planiceps* at various times of incubation at 15C and 28C

Hours	Hatching rate (%)	
	15C	28C
1.5	NE*	78.0
2.5	NE	88.6
3.0	35.9	NE
3.5	NE	100.0
6.0	69.4	NE
9.0	88.7	NE
12.0	100.0	NE

* Not examined

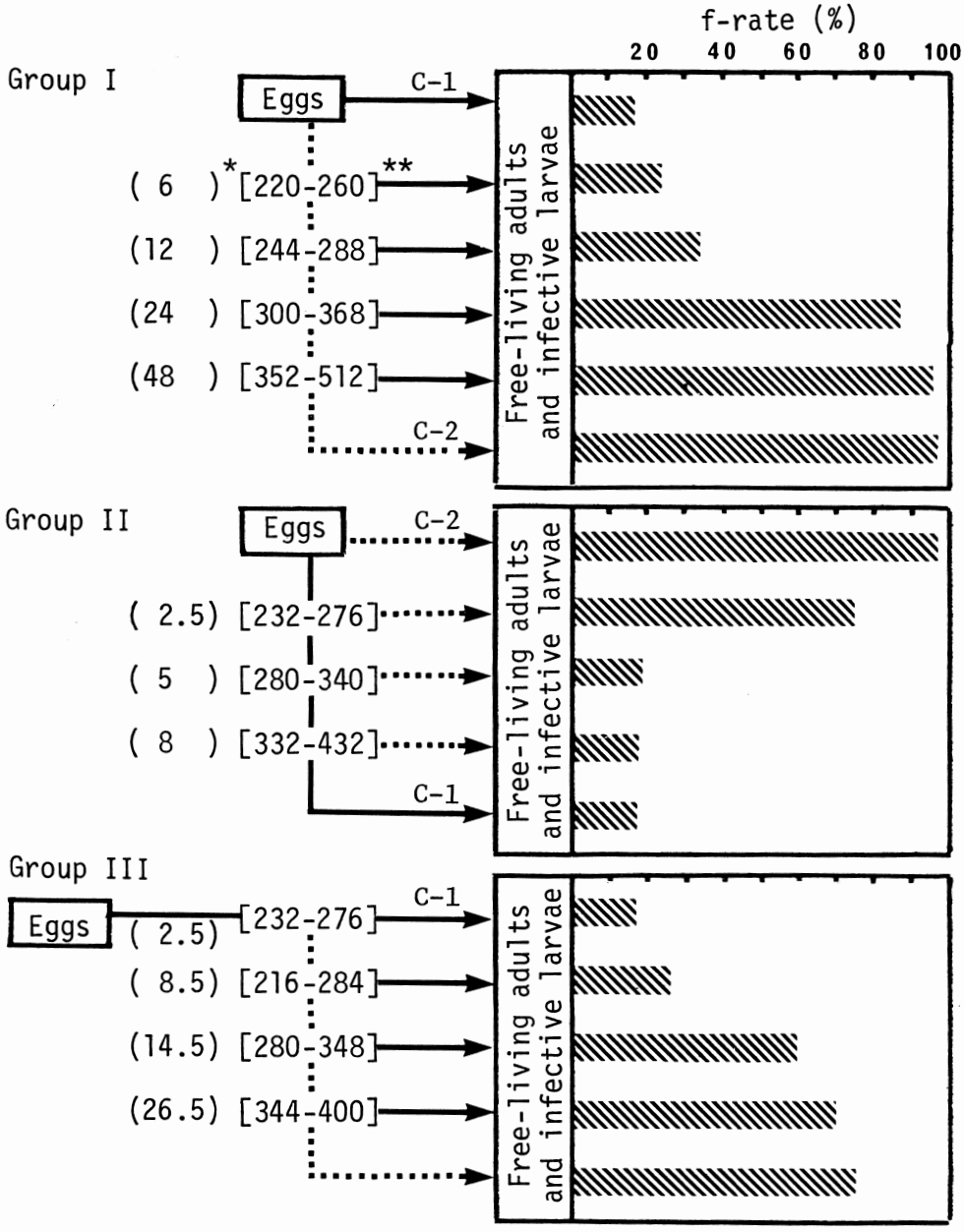

Fig. 2 Diagram showing the f-rate in cultures of *S. planiceps* held at 28 C (——) or 15 C (----) and transferred from 28 C to 15 C or vice versa after various hours. f-rate in each division represent a mean for the f-rates of 4 test tube cultures.

* Hours after beginning of cultivation in parentheses. ** Length of larvae in micron at the time of transfer in brackets. C-1: Control cultures held only at 28 C. C-2: Control cultures held only at 15 C.

Therefore, developmental course of the larvae which passed 12 or 24 hr at 15 C was determined by this condition and not susceptible to the latest condition 28 C.

Through the results of these three groups of cultures, the newly hatched larvae which usually appeared during 6 to 12 hours at 15 C or 2.5 hr at 28 C, was apparently not yet determined on their future developmental course. However, the larvae incubated at 15 C for 24 hr (in group I) or at 28 C for 5 hr (in group II) were already determined their developmental course. In cultures of group III, the course was not determined by 2.5 hr at 28 C but determined by subsequent 12 hr at 15 C.

Although the times required for the determination of developmental course were different in three groups of experiment as mentioned above, the body length of larvae at the above time was similar in three groups, namely 300–368 μ in the first, 280–340 μ in the second, and 280–348 μ in the third group of cultures. These larvae showed no morphological differentiation between free-living females and infective larvae. On the other hand, this stage of larvae sometimes showed separation of old cuticle at the head region, and very rarely the cast-off cuticle was observed. So that the larvae might be at the stage of first molting. From these observations it can be said that the developmental course of the larvae of about 300–350 μ long which are at the stage of first molting, is already determined and unable to change the course. In more advanced stage, genital primordium began to grow and sexual differentiation became evident as the spicule primordia was observed in the rectal region of males. The larvae which would develop into infective larvae showed no growth of genital primordium. On the other hand, the early first stage larvae of about 220–280 μ long were not determined yet on their future development either to free-living females or to infective larvae.

Discussion

Nishigori (1928) reported that the temperature influenced the developmental types of *S. stercoralis*. That is, at moderate temperatures (25–35 C) many free-living adults were produced, while at lower or higher temperatures, many infective larvae appeared. On the other hand, Tanabe (1938) did not find the effect of temperature on *S. ratti* at al.

In the present study with *S. planiceps*, effect of temperature on the developmental course either to free-living females or to infective larvae was clearly observed. At lower temperatures such as 12 or 16 C, many infective larvae were produced, and at high temperatures 28–36 C, many free-living females were produced instead of infective larvae. As the total recovery rates were usually more than 70% and sums of free-living females and infective larvae were nearly constant, the free-living females and infective larvae were thought to originate from larvae of the same origin. On the contrary, free-living males were formed at a constant ratio at any temperature.

Up to the present, three environmental factors participating in the development of *S. planiceps* were studied, namely, amount of food (feces) and population density in the first report (Arizono, 1976), and temperature in the present study. All of these factors similarly affected the determination of developmental course either to free-living females or to infective larvae, but not concerned in the course to free-living males. Therefore, it can be said, as stated by Little (1962) in the study of *S. fülleborni*, that there are two kinds of eggs which are produced by parasitic females, one is potential male and the other potential female. From the latter are produced either free-living females or infective larvae. The fact that the infective larvae is potential female, is well coincident with the fact that only females are found in parasitic generation.

In regard to the effect of temperature, three working mechanisms will be presumed as follows; by affecting the feeding activity

of worms, by affecting the nature of feces as food, or by direct effect on the physiological milieu of larvae. Although no evidence is provided, the first or second mechanism seems to be more easily acceptable. Because the effect of amount of food, the effect of population density, and the effect of temperature are all well understood by one induced factor, nutrition.

Premvati (1958) stated, without experimental evidence, that the type of development will be decided when the eggs hatch out and spend as the first stage larvae before the first molt. In the present study, using the temperature effect as determinant factor, the larvae less than about 280 μ long remained undetermined. On the other hand, developmental course of the larvae of about 300–350 μ long had been already determined and was not able to change the course. As the first molting perhaps occured among larvae of 300–350 μ long, these results support the hypothesis of Premvati (1958).

The determination of future developmental types in the larval stage is well studied in some insects and is not a rare phenomenon. For instance, in honey bee, *Apis mellifera*, the larvae until three days old are not determined physiologically on the developmental course either to queen or worker (Weaver, 1966). To clarify the biological importance of determination of developmental types of *S. planiceps* at the larval stage, further ecological studies will be needed.

Summary

In order to study the effect of temperature on the developmental course of *S. planiceps*, cultures (241–320 eggs in 0.2 g of feces) were held at various temperatures between 12 and 36 C. At high temperatures 28–36 C, many free-living females with a few infective larvae were formed. On the contrary, at low temperatures 12–16 C, free-living females became a few in number, and conversely many infective larvae were formed. Thus the temperature was proved as one of the factors which concerned the determination of developmental course either to free-living females or to infective larvae. On the other hand, free-living males were formed always in a constant ratio irrespective of temperature, and thought to be genetically determined.

The question when the course to free-living females or infective larvae will be determined, was studied experimentally at various temperatures. It became evident that the early first stage larvae of about 220–280 μ long were not determined yet on their future developmental course, but they were susceptible to environmental condition. While, developmental course of larvae of about 300–350 μ long, which were at the stage of first molting, was already fixed. Therefore, the course to free-living females or infective larvae was considered to be determined in the first stage larvae.

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Strongyloides planiceps の自由生活世代の研究
II. 温度が発育型に及ぼす影響について

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Strongyloides planiceps の宿主体外における自由世代雄成虫，同雌成虫および感染幼虫の三者への発育分化に及ぼす温度の影響を検討した．0.2 g の便に対して241-320 個の虫卵を置いた試験管濾紙培養を 12 ないし 16C の低温で培養すると，自由世代雄成虫を除いて大部分が感染幼虫となるが，28 ないし 36C の高温下では感染幼虫にかわつて多数の自由世代雌成虫が形成された．一方自由世代雄成虫は 12C から 36C の各温度段階で一定の出現比率を示し，その発育方向は少くとも虫卵の時期には決定されていると考えられる．従つて温度は，第1報で報告した食物（便）の量および個体密度と並んで，自由世代雌成虫または感染幼虫の二者への分化を選択決定する一要因である事が明らかとなつた．

さらに，この自由世代雌成虫と感染幼虫の二者への分化が発育のどの過程で決定されるかを知るため，温度を決定要因とした培養実験を試みた．その結果，孵化したばかりの幼虫は，発育方向が未決定で，温度効果に感受性を有したが，300-350 μ の第1回脱皮期の幼虫は，形態的には未分化であるにもかかわらず，その発育方向が決定されている事が明らかとなつた．従つて，自由世代雌成虫または感染幼虫への発育方向は，第1期幼虫の時期に決定されるものと考えられた．