Development of the smoky brown cockroach, *Periplaneta fuliginosa*, in relation to resistance to cold

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Abstract: A preliminary exposure of more than 28 days to $15^{\circ}\pm 1^{\circ}\text{C}$ and short day length could induce final instar nymphs of *P. fuliginosa* to tolerate a chilling temperature of $5.5^{\circ}\pm 0.5^{\circ}\text{C}$ for 90 days. The nymphs seem to enhance their resistance to cold without entering "true diapause". Second instar nymphs reared at $15^{\circ}\pm 1^{\circ}\text{C}$ seem to be more resistant to cold than those reared at $20^{\circ}\pm 1^{\circ}\text{C}$.

INTRODUCTION

It has been reported that older nymphs of the smoky brown cockroach, *Periplaneta fuliginosa*, survived for 90 days at $5.5^{\circ}\pm 0.5^{\circ}\text{C}$ that followed an exposure to $15^{\circ}\pm 1^{\circ}\text{C}$ for 28 days, suggesting that the nymphs can overwinter outdoors in Central Japan (Tsuji and Mizuno, 1973).

Many insect species in the temperate zone overwinter in a state of arrested development called "diapause", and the state of diapause is often characterized by enhanced resistance to unfavourable climatic conditions (Bon-nemaison, 1945; Bodenheimer, 1952; Andrewartha, 1952; Lees, 1955). The enhanced resistance seems to be a secondary adaptation which permits the diapause individuals to survive in an adverse environment (Lees, 1955; Tsuji, 1963).

Tsuji and Mizuno (1972, 1973) have already shown that *P. japonica* enters diapause at the second and final nymphal instar stages. However, we have not as yet sufficient data on *P. fuliginosa*. In the present study, experiments were carried out to investigate the nature of the enhancement of resistance to cold in nymphs of *P. fuliginosa* especially

in relation to diapause phenomena.

MATERIALS AND METHOD

The stock colony of *P. fuliginosa* was started from individuals captured in Tokyo, and has been maintained in the laboratory for more than 10 years at $27^{\circ}\pm 1^{\circ}\text{C}$. The food (Animal pellets "Oriental Rat Food MF and NMF") and containers used for the experiments were essentially the same as described in previous papers (Tsuji, 1965; Tsuji and Mizuno, 1972, 1973). Rearing and chilling conditions were also the same that used before (Tsuji and Mizuno, 1972, 1973) as follows.

27°C-L : $27^{\circ}\pm 1^{\circ}\text{C}$, 60 % to 80 % R.H., 16 hr illumination per day.

20°C-S : $20^{\circ}\pm 1^{\circ}\text{C}$, 50 % to 70 % R.H., 8 hr illumination per day.

15°C-S : $15^{\circ}\pm 1^{\circ}\text{C}$, uncontrolled R.H., 8 hr illumination per day.

Chilling: $5.5^{\circ}\pm 0.5^{\circ}\text{C}$, almost saturated R. H. if not specially described, usually in darkness.

Other details will be described for each experiment.

RESULTS AND DISCUSSION

I. Effect of an exposure of final instar nymphs to 15°C-S on the enhancement of their

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resistance to cold

Final instar nymphs soon after moulting were taken from a stock colony in which nymphs had been reared at 27°C-L for 90 days from their hatching. The nymphs were then reared at 15°C-S for different periods of time. After that, they were chilled at $5.5 \pm 0.5^\circ\text{C}$ also for different days. Survival of the nymphs was determined on the 7th day after returning them to 27°C-L.

The results are shown in Table 1. The preliminary exposure to 15°C-S for less than 18 days was not sufficient to induce the nymphs to tolerate the chilling treatment of

90 days, but the preliminary exposure for more than 28 days was clearly effective.

II. Effect of an intervening exposure to 15°C-S on further development of final instar nymphs at 27°C-L

In view of the fact that older nymphs of *P. fuliginosa* can enhance their resistance to cold when reared at 15°C-S (Table 1), one can venture a hypothesis that the nymphs enter diapause at this temperature. While, as described by Lees (1955), "many insects in a state of diapause die without developing or grow in a protracted and irregular manner

Table 1 Preliminary exposure of final instar nymphs to 15°C-S and enhancement of their resistance to cold ($5.5^\circ\text{C} \pm 0.5^\circ\text{C}$)

Temperature and days of preliminary exposure	Days of exposure to cold and % survival of nymphs					
	Males			Females		
	30	60	90	30	60	90
15°C-S 0	0			0		
3	0			0		
6	30	0		80	0	
9	100	0	0	88	0	0
18	100	67	0	100	0	0
28	67	57	60	100	88	40
60	100	70	60	100	80	70
27°C-L 1	0	0		0	0	
3	10	0		0	0	
9	10	0		20	0	

Six to seventeen nymphs were used in each treatment.

Fig. 1 Accumulated adult emergence curves for nymphs reared at 27°C-L with or without an intervening exposure of 60 days to 15°C-S
— : Nymphs exposed on the 100th day.
-- : Nymphs not exposed.

when exposed to temperatures that would be expected to favour morphogenesis”.

To investigate this possibility in *P. fuliginosa*, adult emergence from the nymphs which had been reared at 27°C-L, with an intervening exposure to 15°C-S for 60 days at the final instar stage, was examined. A slight but no extreme retardation of adult emergence was found in the results (Fig. 1).

III. Changes of dry matter and fat content of final instar nymphs at 15°C-S

The state of diapause is usually characterized by increased reserves or an excess of dry matter (Sacharov, 1930; Buxton, 1930; Mellanby, 1938; Squire, 1940; Levenbook, 1951; Fukaya, 1951; Koidsumi, 1951; Ushatinskaya, 1952; cited in Lees 1955; Ikemoto, 1958; Tsuji, 1963). So the changes of percentage dry matter and fat content of 8th (final) instar nymphs of *P. fuliginosa* at 15°C-S were determined.

Final instar nymphs soon after moulting were obtained from a stock colony in which the nymphs had been reared at 27°C-L for 88 days from their hatching. The nymphs were then transferred to and reared at 15°C-S. Determinations of body weight, dry weight, and fat content of the nymphs were carried out after 0, 60, 90, 120, and 150 days rearing at 15°C-S. On each determination, several individuals were taken and individually weighed before and after complete drying at 105°C, as well as before and after fat extraction in a Soxhlet equipment with ether. The results in females are shown in Fig. 2.

The determinations were also made in fully grown 7th instar nymphs, 8th (final) instar nymphs of known age, and young adults which were reared at 27°C-L. The results in females are shown in Fig. 3.

Figure 2 indicates that the dry matter and fat content of final instar nymphs increased with increased body weight at 15°C-S, though the nymphs grew very slowly at this temperature. However, this is not characteristic in the nymphs reared at 15°C-S, because the increased dry matter and fat content were also observed in the final instar nymphs reared at 27°C-L (Fig. 3). Figure 3 also shows that the dry matter and

Fig. 2 Changes of body weight, dry matter, and fat content of final instar nymphs at 19°C-S (Females)

Fig. 3 Changes of body weight, dry matter, and fat content of older nymphs at 27°C-L (Females)

fat content decrease when the nymphs moult.

The results in males were similar to those in females.

IV. Adult emergence from final instar nymphs at 15°C-S

One hundred final instar nymphs (50 ♂ and 50 ♀) soon after moulting were taken from a stock colony which had been reared at 27°C-L for 90 days from their hatching, and were transferred to and reared at 15°C-S. No adult was found after 200 days of the 15°C-S rearing, but 49 adults (28 ♂ and 21 ♀) and 36 nymphs (17 ♂ and 19 ♀) were found on the 400th day. All the adults emerging were with abnormal wings (Fig. 4). Similar results have been reported before (Tsuji and Mizuno, 1972).

Fig. 4 Adults with abnormal wings emerging at 15°C-S

V. Development of young instar nymphs at 15°C-S

When reared at 20°C-S, nymphs of *P. fuliginosa* prolonged the duration of their second instar stage (Tsuji and Mizuno, 1972), but they showed no sufficient enhancement of resistance to cold (Tsuji and Mizuno, 1973). In order to examine the development of young nymphs at 15°C-S, measurement of head capsules was carried out with nymphs which had hatched at 27°C-L within 2 days and were reared at 15°C-S. The results are shown in Fig. 5.

At 15°C-S, there was no instar-specific retardation of development and the rate of development was evenly decreased in all instars observed here. The head widths of

Fig. 5 Results of head-width measurements and accumulated moulting curves for younger nymphs at 15°C-S
■ □ : Distribution of measurements.

the nymphs older than the 2nd instar were smaller than those of corresponding instars at 27°C-L (Tsuji and Mizuno, 1972), suggesting the existence of additional nymphal moults at lower temperatures.

VI. Resistance to cold in 1st and 2nd instar nymphs reared at 15°C-S

No first instar nymphs survived for 60 days at $5.5 \pm 0.5^\circ\text{C}$ even when previously reared for 40 days at 15°C-S (Tsuji and Mizuno, 1973).

In the present study, nymphs hatching at 27°C-L within 24 hr and being reared at 15°C-S for 155 days were chilled. Some were 2nd instar nymphs and others were still 1st instar ones on the 155th day. The nymphs were chilled in a glass container (9cm diameter, 6cm depth) covered with a glass or wire-net lid. In some containers, a piece of food and/or a small bottle containing absorbent cotton with water was placed, and in others not.

The results are shown in Table 2. The 2nd instar nymphs could tolerate the chilling treatment for 60 days only when kept with water, whereas the 1st instar nymphs

Table 2 Resistance to cold ($5.5^{\circ}\pm 0.5^{\circ}\text{C}$) in first and second instar nymphs reared at 15°C-S

Stage chilled	Lid of test container	Addition of:		% survival of nymphs
		Water	Food	
First instar	Glass	+	—	0
	Glass	—	+	0
	Glass	—	—	0
	Wire net	+	+	0
	Wire net	—	—	0
Second instar	Glass	+	—	90
	Glass	—	+	0
	Glass	—	—	0
	Wire net	+	+	60
	Wire net	—	—	0

Ten nymphs were used in each treatment.

could not at all. As 2nd instar nymphs reared at 20°C-S could not tolerate the chilling treatment (Tsuji and Mizuno, 1973), those reared at 15°C-S seem to be more resistant to cold.

CONCLUSIONS AND SUMMARY

A preliminary exposure of more than 28 days to 15°C-S could induce final instar nymphs of *P. fuliginosa* to tolerate a chilling temperature ($5.5^{\circ}\pm 0.5^{\circ}\text{C}$) for 90 days (Table 1, and Tsuji and Mizuno, 1973). The exposure to 15°C-S caused no extreme retardation of their development when returned to 27°C-L (Fig. 1). No 15°C-S -specific increase of fat content and dry matter was found in final instar nymphs (Figs. 2 and 3). Even at 15°C-S , final instar nymphs were able to become adults, though the adults emerging had abnormal wings (Fig. 4). Thus the nymphs seem to enhance their resistance to cold without entering "true diapause".

The rate of development in younger instar stages was evenly decreased at 15°C-S (Fig. 5), and 2nd instar nymphs showed no such instar-specific retardation of development as observed at 20°C-S (Tsuji and Mizuno, 1972). However, 2nd instar nymphs reared at 15°C-S seem to be more resistant to cold (Table 2) than those reared at 20°C-S (Tsuji and Mizuno, 1973).

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摘 要

耐低温性に 関連したクロゴキブリの発育

昆虫には越冬前に特定のステージで発育停止、すなわち休眠に入り、耐寒性を増加させ、そのステージに特異的な水分含量低下、粗脂肪含量の増大を示すものが多い。

クロゴキブリの大型幼虫は、あらかじめ 15°C 短日（8 時間照明）条件下で飼育すると、5.5°C 低温に 90 日以上耐えられる。

そこで、脱皮後間もない終令幼虫を 15°C 短日下で 150 日間飼育すると、その間脱皮は行われず、体重の増大に伴い、含水率の低下、粗脂肪含有率の増大がみられた。しかしこの変化は 15°C 短日下に特異的なものではなく、27°C 長日（16 時間照明）条件下でも同様の変化

がみられた。27°C では短期間で脱皮が起ったがその際には含水率は上昇し、粗脂肪含有率は低下した。一方、15°C 短日 60 日間飼育の後 27°C 長日に戻した場合、極端な羽化のおくれは認められなかった。又 15°C 短日に放置すれば、発育はおそいが 400 日で半数以上が羽化した。したがって、クロゴキブリ終令幼虫の 15°C 短日下での予備飼育による耐低温性の獲得は、休眠を伴っていないと思われる。

1 令幼虫から 15°C 短日下で飼育を行うと、ゆっくりではあるが次々と脱皮発育し、650 日で約 40 % が 8 令（残りは 6~7 令）に達した。この 8 令幼虫は 27°C 飼育の 8 令（27°C では終令）より小型で、低温下では適温下より脱皮回数が多くなることが暗示された。又 15°C 短日下では、20°C 短日下でみられたような 2 令のみに特異的な令期間の延長はみられなかった。しかし、20°C 短日下で得られた 2 令幼虫が 5.5°C に 60 日間耐えられなかったのに対し、15°C 短日下で得られた 2 令幼虫は 60~90 % 生存し、耐低温性が強まっていることがうかがわれた。