Studies on the Self-regulation of Life Cycle in *Anomala cuprea* HOPE (Coleoptera : Scarabaeidae). III. The Effects of Larval Density and Delayed Hatching on the Life Cycle¹

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The effects of larval density and delayed hatching on the life cycle of *Anomala cuprea* were investigated under field conditions and the vertical distribution of larvae was examined in relation to the soil temperature. The mean developmental velocity tended to diminish with the increase of larval density. After hibernation the population was separated into two groups, one emerged as adults from June to July and the other in the following June. When larvae hatched from eggs in mid September, they remained in the first instar or developed only to the second instar before hibernation. The first instar larvae suffered from high mortality during hibernation. The individuals that had survived hibernation entered diapause in the summer and pupated the following year. The period of adult emergence in all the experimental populations was the same as in the natural population.

The vertical distribution of larvae in the soil changed seasonally. They stayed near the surface from summer to autumn and burrowed deeper in winter. The larvae with a yellowish body colour stayed in deeper sites except from spring to early summer. The significance of this variation in distribution is discussed. From these results it was concluded that the life cycle of this species was regulated to synchronize the seasonal occurrence of adult emergence.

INTRODUCTION

In southern Japan, *Anomala cuprea* HOPE usually has a one-year life cycle. The adults survive for one or two months, laying eggs into the soil from June to August, and the preimaginal instars live underground. The variation in developmental stage of larvae is very large at the time of hibernation. But the adults emerge from the ground within a short period from June to July.

The larvae develop under the strong influence of temperature. FUJIYAMA and TAKAHASHI (1973a, b; 1977) examined the larval development under different temperature conditions in the laboratory. They pointed out that temperature regulates the adult emergence through its effect on the larval diapause. However, the detailed

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analysis of this regulation under field conditions has not been done despite the fact that the soil temperature greatly varies with the depth where the larvae live. We examined the life cycle by making large variations in larval development under semi-natural conditions. NISHIGAKI (1974, 1975) in *Anomala cuprea* and WIGHTMAN (1974a) in *Costelytra zealandica* showed that the larval development and survival vary with the larval density. But they did not consider the influence of density on the seasonal life cycles. In the present study the vertical distribution of larvae in the soil and the change of the soil temperature were also examined.

The results suggested that the regulatory mechanism of life cycle synchronizes the adult emergence despite the great variations in larval development.

MATERIALS AND METHODS

Adults were collected by a light trap at the Nara Agricultural Training Center at the beginning of August in 1972. Eggs were deposited in moist saw dust and incubated at 20°C or 15°C. The larvae which had hatched within a few days were used for experiments. All field experiments were conducted at the Nara Agricultural Training Center.

Experiment 1 (effects of larval density)

Reticulate plastic tubes of 10 cm in diameter and 110 cm in length were buried vertically, the top 10 cm being exposed above the ground. The tubes were lined with plastic mesh bags and filled with sandy loam soil to the height of 70 cm, and a mixture of soil and cut rice straw to the top, which was a little higher than the ground level. First instar larvae were put in the upper parts (within a depth of 30 cm from the surface) of the tube. The larval density in each tube was 1, 2, 5, 10 or 20 (density-1, -2, -5, -10 or -20). There were five replicates for each density. When two or more larvae were kept in a tube, they were separately placed to prevent cannibalism. The experiment started on August 25, 1972. At the time of observation, the bags were pulled out from the tubes, and the number of survivors, their instars and positions in the tube were recorded at every 10 cm. Then the larvae were returned to the original positions together with a sufficient amount of cut rice straw at the top layer as food. The observations were carried out monthly except in winter. When prepupae or pupae were found, they were removed because they would die due to the destruction of their earthen cells.

Experiment 2 (effects of delayed egg hatching)

This experiment was set up on the 15th of September, 1972. The larval density was 3 in each replicate. All other methods were the same as in Exp. 1.

Soil temperature. The temperature was measured with an electric thermograph at five points, i.e. 1 m above the ground (air temperature), the soil surface (0 cm), 10 cm, 30 cm and 100 cm below the soil surface. Additional measurements were made at 50 cm below the surface from July 18, 1973.

Classification of developmental stage. The course of development from hatching to adult was divided into seven stages: the first instar, the second instars, the feeding stage of the third instar, the yellow stage of the third instar (or "the yellow stage"), prepupa, pupa and immature adult. The feeding-stage larvae of the third instar are immature

and creamy white or yellowish brown in body colour with their gut filled with food. The yellow stage larvae are mature and yellow in body colour with their gut empty. The immature adult stage designates the period from the adult eclosion in the soil until the exodus from the soil.

RESULTS

Experiment 1

Survivorship curves of larvae in various initial densities are shown in Figs. 1 and 2. A large number of larvae died in the early period of the experiment regardless of the initial densities. In the winter, a small number of individuals died (Table 1) and the death was presumably due to their susceptibility to low temperature (FUJIYAMA and TAKAHASHI, 1977). Fig. 2 shows further that the mortality increased with the increase of larval density but the numbers of individuals reaching the yellow stage increased with the increase of density. In this density range, therefore, there was no optimum density that maximized the number of final instar larvae as reported by NISHIGAKI (1974).

Fig. 1. Survivorship curves in preimaginal stages of *Anomala cuprea* in different initial densities (Exp. 1). The numerals beside curves show the initial densities.

Fig. 2. Survivorship curves in percentage under different densities. The numerals beside curves show the initial densities.

Table 1. SURVIVAL RATE(%) IN EACH DEVELOPMENTAL STAGE IN HIBERNATION

Developmental stage	Experiment 1			Experiment 2			Total		
	A/B ^a	%	95% confidence interval of the average (%) ^b	A/B	%	95% confidence interval of the average (%)	A/B	%	95% confidence interval of the average (%)
First instar	—	—	—	1/9	11	0.6—44.3	1/9	11	0.6—44.3
Second instar	10/13	77	48.0—93.4	8/15	53	29.4—80.9	18/28	64	42.4—80.8
Feeding stage in third instar	42/56	75	63.7—86.3	—	—	—	42/56	75	63.7—86.3
Yellow stage	2/2	100	22.4—100	—	—	—	2/2	100	22.4—100 ^c

^a The survival rate was calculated by the ratio of the number of individuals on April 29th(A) to the number of individuals on November 23rd(B) but for the yellow stage it was calculated by the ratio of that on April 29th(A) to that on December 17th(B).

^b The values of 95% confidence interval of average to small numbers (less than 30 individuals) were shown in CROW (1956).

^c The value of survival rate of another experiment at the same time and place was 14/16, i.e. 87.5% (64.8%—97.7%).

The larval development at each density is shown in Fig. 3. On December 17, the overwintering larvae in density-1 were either in the yellow stage or in the feeding stage of the third instar but some larvae in the higher densities were still in the second instar. On April 28, some prepupae were found at densities-1 and 2, but none at the higher densities. These results indicated that the larvae developed faster at the low densities than at the high. In May and June pupae or prepupae were found in every density. But neither pupae nor prepupae were found from July to April, although the surviving larvae had reached the yellow stage by the end of July. They pupated

Fig. 3. The effect of larval density on development (Exp. 1). 1: first instar. 2: second instar. 3: feeding stage in third instar. Y: yellow stage in third instar. P: prepupa or pupa. The dates show the census dates.

in the following May. Consequently, their pupation was delayed for one year compared with those which had reached the yellow stage before the first winter.

Experiment 2.

The survivorship curve of the larvae reared in the field from Sept. 15 is shown in Fig. 4. Some dead larvae were found from the beginning of the experiment to April 29. As shown in Table 1, the mortality during hibernation was very high in the first instar (89%) but lower in the second instar (47%). The seasonal change in distribution of developmental stages is shown in Fig. 5. No larvae developed to the third instar in the year of hatching. In the winter of 1972, all larvae remained at the first or second instar. They developed to the feeding and yellow stages of the third instar by June and by the end of August, respectively. The yellow stage lasted until the second winter, and pupae appeared in May, 1974.

Seasonal changes in vertical distribution of the larvae in the soil.

Figs. 6 and 7 show the seasonal changes in vertical distribution of larvae in Exp.

Fig. 4. Survivorship curve of larvae in Exp. 2. The egg hatching was artificially delayed until the 15th of September by a low temperature (15°C).

Fig. 5. Preimaginal development in Exp. 2.

Fig. 6. Seasonal change of vertical distribution of various larval stages in the soil (Exp. 1). For the symbols see Fig. 3.

Fig. 7. Seasonal change of vertical distribution of larvae of Exp. 2 in the soil.

Fig. 8. Seasonal change of the soil temperature at various depths. ●—●: 0 cm, ○—○: —10 cm, ●—●: —30 cm, ○—○: —100 cm.

1 and 2, respectively. The mean depth of larvae in the soil changed seasonally. The larvae lived in upper parts of the soil until October. They began to move downward in November and hibernated. In the early spring they moved to upper parts for feeding and grew to yellow larvae. During hibernation, the second- and third-instar larvae burrowed deeper than the first instar larvae did (Figs. 6 and 7). The yellow larvae stayed in deep positions except in the spring when they moved to upper parts for pupation. This seasonal change of distribution was examined in relation to the soil temperatures. The temperature was higher at the upper parts than at the lower from April to August (Fig. 8). The trend was reversed from October to February. In September and March, the temperatures at different depths were almost the same. The larvae moved to the depth with a higher temperature, except for the younger larvae in October (Figs. 6, 7 and 8).

In the middle of autumn, they remained in the shallower parts in the soil than the annual mean depth (ca. 26 cm) in spite of the higher temperature in the deeper soil. As the temperature at any depth was higher than the developmental zero of this species, the results can be explained on the basis of NAKASHIMA's findings (1952). He showed that the second instar larvae of *A. rufocuprea* preferred a low temperature when they were placed in a temperature gradient, but that when they were kept in the same gradient with the food only available at the warmest end, many larvae moved to the food. Therefore, in our case, the active larvae might have responded to the food rather than the temperature gradient, because the food (rice straw) was available only near the upper end.

DISCUSSION

In this experiment, prepupae and pupae were all removed at the time of observation. Therefore, the time of adult emergence was estimated on the basis of heat requirements for the development of prepupa and pupa (FUJIYAMA and TAKAHASHI, 1973a). In Fig. 9 the adult emergence observed in a persimmon orchard near the experimental field is shown for comparison. In this orchard 33 out of 41 larvae were

Fig. 9. Estimated periods of adult emergence from the prepupal or pupal development in the field rearings in 1973. The total number of adults observed in the census area of a persimmon orchard (control) was 394. The numerals show the initial density of rearing.

in the yellow stage, 7 in the feeding stage, of the third instar and one in the second instar on December 17, 1972.

Fig. 9 shows that the time of adult emergence tends to be delayed with the increase in larval density. But all individuals that pupated in the same year emerged as adults from June to July. The individuals which did not pupate in the second year pupated in May of the third year. They would become adults and appear on the ground in a period of one month from the beginning of June. This approximately coincided with the adult emergence observed in the orchard and also in other places by FUKAZAWA et al. (1971).

The adults of *A. cuprea* appear from June to July in the Kansai district (south of 35°N) and lay eggs from late June to early September. The eggs hatch about 20 days after deposition in the field. Consequently, various larval stages can be seen in autumn. In the present experiments the variation in larval development was at least in part due to the effect of larval density (Exp. 1) and the delayed hatching (Exp. 2). In November, 1972, the variation in developmental stage among the experimental plots was very large, ranging from the first instar to the yellow stage of the third instar. Adult emergence from the soil was, however, restricted to the period from June to July in both the experimental and natural populations (Fig. 9). These results agreed with the prediction from the laboratory data (FUJIYAMA and TAKAHASHI, 1973b).

In our experiments all individuals underwent two-year life cycles when they hatched in mid September, whereas only a few of those that had hatched in August did so. It is, therefore, certain that the prolonged period of oviposition has an important effect on producing the semivoltine life cycle in southern Japan (south of 35°N). Judging from previous experiments (FUJIYAMA and TAKAHASHI, 1973a, b, 1977), the occurrence of a one- or a two-year life cycle depends on whether the larva reaches the end of second instar before the first winter or not. In Sakurai City (Nara Pref.) and Hamakita City (Shizuoka Pref.), more than 90% of the population was univoltine (FUJISHITA, 1972; our unpublished observations), while about 30% was semivoltine in Kōnosu City (Saitama Pref.) (YUASA and ENDO, 1938). In the northern parts of

Honshu such as Kanto and Tohoku, the total amount of the effective day-degrees for larval development per year is smaller than in southern Japan, and the proportion of semivoltine individuals in the population would be higher than those in the southern parts. The first and second instar larvae, especially the former, stayed in upper layers of the soil in winter compared with the other instars (Figs. 6 and 7). These first instar larvae mostly died during hibernation, presumably owing to their susceptibility to low temperature (FUJIYAMA and TAKAHASHI, 1977). This mortality reduces the number of individuals which would be expected to be semivoltine.

There are many reports on the seasonal larval movement of Scarabaeidae in the soil (*e.g.* SAWADA, 1949; NAKASHIMA, 1952; SAWA and TAMURA, 1953; AINO *et al.*, 1956; GRANOVSKY, 1956, *etc.*). Our results generally agree with SAWADA's. Namely the larvae stayed near the soil surface from spring to autumn and hibernated in deeper places. The diapausing yellow larvae, however, stayed at deeper position as shown in Figs. 6 and 7.

The micro-habitat temperature for yellow larvae in autumn was moderately high (10–25°C) and its daily change was very small, *e.g.* less than 1°C at the depth of 50 cm from the soil surface. Diapause in the yellow stage is induced when the larvae are exposed to only moderately high temperatures (15–30°C) from the end of second instar to the feeding stage of the third instar but it is not induced when they are exposed to low temperatures for sufficient period (*e.g.* 50 days at 10°C) in those stages (FUJIYAMA and TAKAHASHI, 1973a, b; 1977). The temperature in autumn induced diapause in the yellow stage, and the larvae became prepupae only after hibernation. The feeding larvae of the third instar were active until late October or early November, and they began to move upwards at the end of March and to feed in early April. By chilling the feeding larvae of the third instar, the diapause in the yellow stage is averted (FUJIYAMA and TAKAHASHI, 1973b). Therefore, those larvae that had hibernated at the feeding stage of third instar developed into prepupae without intervention of diapause. Thus, they did not show any developmental delay in the yellow larval stage. Similar regulatory mechanisms of life cycle in Scarabaeidae have been suggested in *Costelytra zealandica* (WIGHTMAN, 1974b) and *Anomala rufocuprea* (FUJIYAMA, 1975).

The second instar larvae are cold-hardy in winter. They grew to the yellow stage until July of the next year, after which they entered diapause, despite the fact that there was still a sufficient amount of heat for further development. The diapausing yellow larvae stayed at deeper positions in the soil where the environmental conditions were relatively stable. This might have ensured the yellow larvae to be in diapause until the end of autumn. The larvae terminated diapause during winter, and became prepupae in the spring of the third year (1974). They pupated nearly at the same time as those which had reached the yellow stage in the first autumn did. Thus the seasonal change in vertical distribution of the larvae also plays an important role in the synchronization of adult emergence.

The period of adult emergence from the soil is restricted to a short period owing to these factors. This synchronization of adult emergence ensures higher opportunities for copulation and reproduction at suitable temperatures (25.7–28.5°C) as shown by ONO (1957).

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