Life-Cycle Synchronization in *Chrysolina aurichalcea*
(Coleoptera: Chrysomelidae) to Its Host *Artemisia princeps*:
Effects of Host-Leaf Age on Survival

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To investigate the effect of host-leaf age on the survival of *Chrysolina aurichalcea*, larvae and adults were reared on *Artemisia princeps* leaves of three different growing ages (young, mature and senescent). For adult longevity and fecundity, mature leaves were the most suitable despite of the beetles' preference for young leaves. When larvae were fed with senescent leaves, their growth rate decreased and mortality increased. On the other hand, 1st instar larvae did not show a clear preference towards young leaves, and those released under planted hosts could not develop into the next instar when the plant was taller than 10 cm. Therefore, the need to synchronize the egg hatching to host sprout emergence in early spring may have evolved into a unique life cycle of autumn breeding.

Key words: insect-plant relationship, feeding preference, *Chrysolina aurichalcea*, life-cycle synchronization, life-cycle evolution

INTRODUCTION

Chrysolina aurichalcea (MANNERHEIM) is a univoltine beetle which feeds mainly on mugwort, *Artemisia princeps*. In central Japan larvae hatch in early spring and feed on sprouts found near the ground. Adults emerge early in summer and enter reproductive diapause after voraciously feeding on hosts. In autumn, the diapause is terminated by the short-day photoperiod (FUJIYAMA, 1986). Adults feed on mature leaves and soon begin oviposition on withered host leaves. The species overwinters during the egg stage. On the other hand, *A. princeps* is a perennial herb which puts its shoots forth early in spring, flowers in autumn and loses its above-ground vegetation in winter.

Many chrysomelids in Japan overwinter in adult stage (44 species), while *C. aurichalcea* and two other species characteristically hibernate in egg stage. Why do these few species overwinter in the egg stage rather than as adults? As *C. aurichalcea* is a stenophagous insect, its life cycle is strongly influenced by seasonal changes in quality and quantity of its host plant. Among many other factors that determine the seasonal cycle, here we have investigated its ecological performance in different food conditions. From the perspective of life-cycle synchronization, sprout emergence of the host plant is the primary factor influencing the development of this special type of hibernation.

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MATERIALS AND METHODS

Population examined. Adult *C. aurichalcea* were collected in autumn at Utsukushigahara Heights (alt. 2,000 m), Nagano, central Japan. Eggs laid by the adults were kept at 3°C for 5–9 months in order to terminate their diapause. These eggs were then transferred to a 20°C chamber and newly hatched larvae were used for experiments. To obtain 4th instar larvae and adults, these larvae were reared on young *A. princeps* leaves at 20°C.

Since the insect shows rather high activity at low temperatures, part of the experiments described below was carried out at 15°C, a temperature normally regarded as somewhat low for many other insects.

Rearing experiment of larvae with leaves of various ages. The procedure for rearing larvae followed FUJIYAMA and MIYACHI (1985). *A. princeps* leaves of various ages, the stems of which were wrapped in moistened absorbent cotton, were placed in plastic cups (11 cm dia. $\times$ 4 cm depth) with filter papers spread on the bottom. The leaves were changed with new ones every 3 or 4 days. Ten larvae were reared in each cup at 20°C, 16L–8D, and their instar and survival were recorded every day. Mature 4th instar larvae were transferred to containers with moistened sawdust for pupation. Adults were weighed within a day after emergence. The experiment was replicated 10 times at each leaf age. Ages of the leaves used were 0–2 weeks (young), 1 month (mature), and 3–4 months (senescent) (see also Table 1). Since this experiment was performed in early spring, 3-month- to 4-month-old leaves were considered senescent, although 5-month- to 6-month-old leaves were used for this designation in other experiments. Leaves of different ages could be collected simultaneously from different altitudes (e.g. alt. 600–2,000 m).

Preference for leaves of various ages. In a plastic box (17 $\times$ 23 $\times$ 8 cm), a small lump of young leaves (0–2 weeks) and of senescent leaves (5–6 months) were placed 3 cm apart (Fig. 2A). Five 1st instar larvae, a 4th instar larva, or an adult were released on either lump at room temperature (15–18°C). First instar larvae that moved to another lump of leaves were counted 1.5 h after the release. For 4th instar larvae and adults, the type of leaves they stayed to feed on for more than 10 min was recorded. Before the experiments, the 1st instar larvae and adults were starved for 1 day, and 4th instar larvae for 14–16 h. The experiment was replicated 11 times for 1st instar larvae, 28 times for 4th instar larvae, and 30 times for the adults.

Vertical mobility of 1st instar larvae on host plant stems. Mugworts of various heights (2, 10, 20, 30, 50 cm) collected in Matsumoto and at different altitudes of Utsukushigahara were planted in plastic trays (36 $\times$ 60 $\times$ 16.5 cm). In a tray, 11 plants of 2 cm ht. (ca. 10 cm intervals in 3 rows), or 6 each of other heights (20 cm intervals in 2 rows) were arranged. After the plants took root, trays were placed indoors (13L–11D, ca. 23°C). At the root of each mugwort, three 1st instar larvae were released and their survival was monitored every day for 1 week. The experiments were repeated twice for each tray.

Fecundity in adults reared with leaves of various ages. A pair of newly emerged adults were kept at 15°C, 11L–13D, in a plastic container (10 cm dia., 5 cm depth) prepared similarly as for the larval experiment. A piece of gauze (5 $\times$ 5 cm) was placed in each container as an oviposition site. Adults were divided into four groups (12–21 pairs each) and were fed with leaves of different ages: young, mature, senescent or flowering

stems. Survival was checked and eggs laid were removed to be counted every day.

RESULTS

Leaf age and larval development

Both larval growth (Table 1) and larval survival rate (Fig. 1) were significantly affected by the age of leaves offered. Developmental period shortened significantly when younger leaves were fed (U -test: $p < 0.001$). The survival rate during 1st instar stage, and throughout the entire larval stage, lowered when senescent leaves were given (χ^2 -test: $p < 0.001$, Fig. 1). Regardless of the food condition, an apparent decrease in the survival rate was observed at 4th instar stage, is presumably due to prepupation failure in the moistened sawdust. Rearing larvae on senescent leaves brought about small adults, while no weight difference was found between adults feeding on young and mature leaves (Table 1).

Table 1. Developmental period of *Chrysolina aurichalcea* larvae and fresh body weight of emerged adults reared on *Artemisia princeps* leaves of different ages

Leaf age	Initial number of individual	Developmental period $\pm$ S.E. (day)	Body weight of an adult ^a $\pm$ S.E. (mg)	
			Female	Male
0 weeks (young)	99	38.3 $\pm$ 0.5 ^b (61)	53.4 $\pm$ 1.2 (27)	40.1 $\pm$ 0.7 (29)
1 month (mature)	100	41.4 $\pm$ 0.6 ^b (59)	53.9 $\pm$ 1.0 (32)	40.1 $\pm$ 0.7 (20)
3-4 months (senescent)	60	51.6 $\pm$ 1.1 ^b (17)	36.5 $\pm$ 1.2 ^c (6)	29.8 $\pm$ 1.0 ^c (10)

Number of individuals are in parentheses.

0 weeks: top part of 5-10 cm plant. 1 month: lower leaves of 20-30 cm plant. 3-4 months: lower leaves of 60-80 cm plant.

^a Body weights were measured within 24 h of adult emergence.

^b Values are significantly different from each other (U -test: $p < 0.001$).

^c Each value is significantly different from other treatments (U -test: $p < 0.001$).

Fig. 1. Comparison in survival curves of *Chrysolina aurichalcea* larvae fed with host leaves of different ages: young (●), mature (▲), and senescent (■). Classes of leaf age are defined in Table 1.

Preferential feeding between young and senescent leaves

Figure 2 shows that more than 90% of 1st instar larvae stayed on the leaves onto which they were released. The larvae bit the leaves as soon as they were released. Most of those on senescent leaves, however, wandered within the leaf lump. This was seldom observed on young leaves. Only 4% of larvae on young leaves and 5% on senescent leaves moved beyond the original leaf lump. Fourth instar larvae also showed no clear preference, though some larvae moved onto young leaves. Adults, however, walked actively to search for a suitable food source and showed a strong preference for young leaves. Although adults have much higher mobility than larvae, even newly hatched larvae were locomotive at a speed of 15.6 ± 2.5 mm/min at 15°C.

Effect of plant height on survival of 1st instar larvae

Figure 3 depicts the relationship between plant height and the percentage of larvae that survived to develop into the 2nd instar. As the plant became higher,

Fig. 2. Preferential feeding by *Chrysolina aurichalcea* between young and senescent leaves of *Artemisia princeps*. A: a device for the experiment. Young and senescent leaves were simultaneously offered to five 1st instar larvae, one 4th instar larva, or one adult in a plastic case (17×23×8 cm). The insects were released on one type of leaves at the beginning of the test. B: results of the experiment. Open columns represent percentage of individuals initially placed on young leaves, shaded ones that on senescent leaves. An open column below the zero line or a shaded column above it indicates the percentage of individuals that moved to another pile of leaves.

Fig. 3. Effect of host plant height on the survival rate of 1st instar larvae of *Chrysolina aurichalcea* (●) released at the root of the plant. The average height of the lowest leaf on the vertically standing stem is also shown (■).

Table 2. Effect of *A. princeps* leaves of different ages on survival period, oviposition period, and fecundity of *C. aurichalcea*

Leaf age	Survival period $\pm$ S.E. ^a (day)	Ovipositional period $\pm$ S.E. ^b (day)	Fecundity $\pm$ S.E. ^c
0-2 weeks (young)	100.6 $\pm$ 5.8 (21)	43.5 $\pm$ 5.5 (21)	251.5 $\pm$ 35.9 (21)
1 month (mature)	109.9 $\pm$ 5.0 (18)	60.6 $\pm$ 5.1 (18)	441.9 $\pm$ 47.9 (18)
5-6 months (senescent)	90.7 $\pm$ 3.9 (12)	41.0 $\pm$ 3.2 (12)	174.2 $\pm$ 28.9 (12)
Flowering stem	100.1 $\pm$ 4.8 (12)	53.2 $\pm$ 5.0 (12)	329.5 $\pm$ 39.0 (12)

Number of females are in parentheses.

^a No significant difference (ANOVA: $p > 0.05$, $df=3$, $F=1.59$).

^b Significantly different at $p < 0.05$ (ANOVA, $df=3$, $F=2.97$).

^c Significantly different at $p < 0.001$ (ANOVA, $df=3$, $F=7.14$).

the percentage of the successfully developed larvae decreased remarkably. When larvae were released under plants taller than 10 cm, most of them vainly wandered around the roots searching for leaves. The lower leaves that some of these larvae climbed up to were usually senescent, and therefore did not allow the larvae to develop. Thus almost all larvae released under the plants taller than 10 cm failed to reach the 2nd instar. This result clearly indicates that 1st instar larvae require the very young mugwort to be found near the ground surface.

Relationship between fecundity and leaf age

The reproductive characteristics of adults fed on leaves of three different ages are shown in Table 2. There were significant differences in ovipositional period ($p < 0.05$, $df=3$, $F=2.97$) and in fecundity ($p < 0.001$, $df=3$, $F=7.14$), but not in survival period ($p > 0.05$, $df=3$, $F=1.59$). As for the former two, mature leaves and flowering stems produced higher values. These results coincide with the fact that the adults reproduce in autumn when such food resources are most abundant.

DISCUSSION

Most species of Chrysomelidae in temperate regions hibernate during adult stage and lay eggs in spring. Their larvae feed on young leaves of their host plants. *C. aurichalcea* has adopted an unusual special type of hibernation: overwintering in egg stage. The adaptative alternation of the seasonal cycle in *C. aurichalcea* could have close relation with the availability of the host, both in quality and quantity, as is known in *Pyrrhalta humeralis*. This chrysomelid beetle also overwinters in egg stage to synchronize its larval development with host leaf development (SHINKAJI et al., 1979).

We have shown that the larvae of *C. aurichalcea*, fed on young leaves of its host plant, grew faster and survived more than those fed on senescent leaves (Table 1 and Fig. 1). More importantly, the 1st instar larvae revealed an incompetence both in distinguishing the host leaf age (Fig. 2) and in climbing up the stems higher than

10 cm to search for suitable food (Fig. 3). However, in nature 1st instar larvae appear in early spring when host sprouts are exclusively found on the ground since *Artemisia* sprouts before other shrubs (FUJIYAMA et al., 1981). The larvae will readily be able to find young mugwort under such field conditions. This is possible only in early spring, hence, hatching early in the season is crucial for *C. aurichalcea*.

In general, adult insect females show higher fecundity and survival when they are fed with younger leaves (DE WILDE et al., 1969; PHILLIPS, 1976; RAUPP and DENNO, 1983). There are a few exceptions; leaf age of *Pinus banksiana* has no effect on the fecundity of *Neodiprion swainei* (ALL and BENJAMIN, 1975), and SCHROEDER (1986) reported a lepidopteran larva which has a physiological ability to utilize mature leaves of the host plant. The effect of leaf age on the fecundity of *C. aurichalcea* observed in the present study is similar to the latter; mature leaves were the most suitable (Table 2). Under natural conditions, adults of the beetle begin oviposition in autumn when emerging leaves are no longer found. A related species of *Artemisia* contains toxic terpenes that increase with age (HALLIGAN, 1975). *A. princeps* may also contain similar substances, especially in mature and senescent leaves. A physiological adaptation to utilize mature leaves of *A. princeps*, therefore, must have occurred in adult *C. aurichalcea*.

All the evidence presented here indicates how the life cycle of *C. aurichalcea* is totally adjusted to the seasonal changes in the host plant. Food availability for 1st instar larvae is the key factor in the present life cycle, characterized by egg hibernation. In natural conditions, newly emerged leaves of mugwort are available throughout the plant's growth season (spring, summer) at the top of the plant, if insects can find and reach them. The poor host-searching ability of 1st instar larvae, however, precludes the use of such leaves. Supposing that *C. aurichalcea* breeds in the spring, the time lag from sprout emergence to egg hatching would inevitably amount to more than 1 month, due to the length of the preovipositional and egg periods. A young mugwort would grow more than 15 cm during that period, and suitable host leaves would become unavailable for 1st instar larvae. Therefore, autumn breeding to synchronize egg hatching with host sprout emergence in the following spring is essential for *C. aurichalcea*.

In southern Japan, where winter is relatively mild, newly developing leaves of mugwort are found on the ground throughout winter. In such regions, the beetles breed even in winter, and the age-structure of the winter population is rather diverse, consisting of eggs, larvae, and adults (FUJIYAMA, 1986). Furthermore, even females from northern Japan were able to continue oviposition during winter, when they were kept at 15°C and continuously offered fresh leaves (FUJIYAMA, unpublished data). These facts indicate that breeding adults usually die in autumn from shortage of food rather than a physiological life span. Ancestral populations of *C. aurichalcea* may therefore be of a winter-spring breeding type. The breeding season could have shifted under warmer and more generous environmental conditions, and the poor host-searching ability of the 1st instar larvae would have favored earlier breeding. Autumn breeding of the beetle was thus realized, followed by the adaptation of egg and adult diapause under cooler and more severe environmental conditions. This is a possible explanation for the unique life cycle of *C. aurichalcea* characterized by autumn breeding and egg hibernation.

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