

# 環 動 昆

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Vol. 15

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## 日本環境動物昆虫学会

2004

## Split Life Cycle and Differentiations in Diapause Characteristics in Three Host-habitat Strains of *Atrophaneura alcinous* (Lepidoptera : Papilionidae)

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(Received : February 5, 2004 ; Accepted : May 6, 2004)

### ジャコウアゲハの3つの食草・ハビタート型の生活史の分裂と休眠形質の変異

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ジャコウアゲハの生活史は、食草の質、温度、光周期、飢餓、込み合い等、気候要因と生物的要因に依存する。休眠誘導だけでなく休眠の期間も、光周期やその他の休眠誘導に影響した様々な因子 (個体飼育、餌植物の質、温度、飢餓など) に依存した。変異は個体群間だけでなく、同じ個体群の中の個体間にもみられ、そのゆえに個体群の中で生活史の分裂を起こさせた。兵庫県神戸地域の個体群は、生息域、食草 (木本と草本の4種がある)、行動、色彩、休眠反応、発育速度、サイズと温度耐性で3つの型に分けられた。これらのデータから、開放的な生息域では単純な生活史が一般的であるが、森林内に棲むものは多型的で、休眠が生活史の様々な相でおこることが判った。この生活史の分裂は、食草中の毒物質を体内に蓄積することによる捕食の回避で個体群密度が高くなり、餌を食い尽くしてしまうという事態に対する危険分散のための適応だと考えられた。安定的な森林内の生息圏では餌の回復を待つという時間的逃避が、生息圏自体が不安定な開放的な場所では、移動をして世代を重ねるr-戦略型がカタストロフィーを回避するのに有効であるのだろう。

The life cycle of the birthwort swallowtail butterfly, *Atrophaneura alcinous* depended on both climatic and biotic factors such as quality of food plant, temperature, photoperiod, starvation and crowding. Not only the induction of diapause, but also the period of diapause depended on photoperiods. The period of diapause depended on various other factors that affected diapause incidence, such as isolation, food quality,

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temperature and food deprivation. The variability was observed not only among populations but also among individuals within a population, causing the life cycle to split within the population. Three populations of *A. alcinous* from Kobe (Hyogo Prefecture, Japan) were characterized by the habitat, food plant, behavior, coloration, diapause response, growth rate, body size and temperature tolerance. i.e., woody species vs. weedy species. The data suggests that an open-land population has a simple life cycle, producing more annual generations, whereas a wood-land population is polymorphic, diapausing at different phases of its life cycle. The split life cycle was attributed to adaptation to avoid occasional exhaustion of food plants due to overcrowding caused by the escape from the predation by sequestered toxic substances derived from the host plant. Stable wood-land habitat may favor temporal escape strategy from population catastrophe by diapause, whereas unstable open-land habitat may favor migrant r-strategists to spatially escape such a catastrophe.

**Key words:** Life cycle, Diapause, *Atrophaneura*, Swallowtail, *Aristolochia*

## Introduction

The birthwort, *Aristolochia* contains four distinct species; two, *A. kaempferi* and *A. onoei*, are woody forms growing in more or less shady environment, and the others, *A. debilis* and *A. contorta*, are weeds covering open-land. The two groups are distinct not only in the habitat but also morphology and demographic characteristics.

*Atrophaneura alcinous*, a swallowtail butterfly, feeds on both types of birthwort species. Since the host plant contains noxious substances that help the butterfly to build up a high population density by avoiding bird predation, they sometimes completely defoliate the host, which leads to the starvation for the insect. Since the noxious substance is also a feeding stimulant for the insect and sequestered in the insect body, the larvae frequent the cannibalism when starved (Nishida and Fukami, 1989a; 1989b). The two types of habitats were distinct in bird predation, heavy in the woods compared to the open-land, and in the amount of solar heat radiation

and desiccation during summer. Thus, butterfly populations occurring in the two types of environment may build different demographic characters in their life cycle. The top of the Rokko Mountains is covered by *A. onoei* var *arimaensis* which is gradually replaced by *A. debilis* toward the mountain foot. The swallowtail, *A. alcinous* is common in the mountains and the outskirts.

This study compared the developmental and life cycle traits in three populations of *A. alcinous* from distinct habitats in and around the Rokkos.

## Materials and Methods

### Insects and the habitats

The Aina stock (AN): Eggs laid by more than ten pairs of spring morph adults collected in May 1987 in Aina, Yamadacho, Kobe (34° 42' N 135° 18' E), were supplied for the subsequent experiments. This stock feeds on *A. o.* var *arimaensis* growing on the north and west slopes of the Rokko and Tanjo Mountains along the Arima-Takatsuki Land Gap (fault).

This population may be characterized as the "hill-side population". The host plants form small cohorts scattered around the area where small populations of the swallowtail succeed generations. The butterfly populations probably form a continuous large population since the birthwort cohorts are not distinctly separated from each other. Field observation made in 1987 confirmed a large number of flights in May-June, a small number of flights in July-August and almost no flights in September, implying that this population had basically a bivoltine life cycle in this habitat.

Adjacent to this habitat (Teradami, Hazeya-cho, Nishi-ku, Kobe), *A. debilis* grows, on which another population of the swallowtail infests. The two types of the butterfly populations were distinct in their flight patterns; the AN butterflies flew along the shady hedge of the woods bordering the vegetable field, whereas those infesting *A. debilis* flew slowly as if drifting above the sunny field. The latter population seemed to have a bivoltine or trivoltine life cycle.

The Gakushu-no-mori stock (GM): The stock was established from spring morph adults collected in May, 1987 in the Gakushu-no-mori Study Forest in the Kobe Municipal Arboretum. The habitat is located less than 10 km away from the AN collection site. Both habitats are separated by Suzurandai residence area. This population also feeds upon *A. o. var arimaensis* which forms a biggest patch together with the east slope population 10 km away from it. The butterfly population harbored in this patch was probably not more than 100 adults. The life cycle pattern was basically bivoltine; the first peak of adult flights occurring in mid-late May and the second peak occurring late July-early August. The autumnal flight (September) did occur but was rare. The life cycle pattern of this stock may, however, be complex in the field, since occasionally

smaller spring morph adults fly together with larger summer morph in July-August season. The adults flew along shady paths but occasionally appeared in the modestly bright valley. This population may be characterized as the "wood-land" population.

The Yodogawa stock (YD): The stock was established from the adults collected in the Yodogawa riverside by Dr. R. Nishida of Kyoto University, Pesticide Research Institute. This population infests a weedy species, *A. debilis* and the life cycle pattern was estimated as trivoltine or tetravoltine. This may be characterized as the "open-land" population. The adults fly about sunny riverside.

### Rearing

Larvae were fed with fresh leaves of *A. o. var arimaensis* throughout the experiment. The experiment was made twice in 1987, May-June and June-August. Since they occasionally had prolonged pupal stage even under long-day conditions, two crowding conditions were experimentally set up. The "mass culture (MC)" condition kept four larvae in a plastic container of 11 cm across and 6 cm tall ("shallow cup") during the first three instars which were thereafter transferred to another type of container of 11 cm across and 9.5 cm tall ("deep cup") and kept individually or with one "room-mate." The "single culture (SC)" condition kept one individual in the same container condition throughout its development as the "mass culture." The "starvation (SV)" condition deprived the larvae, kept in the MS, of the food plant every other day until 10 days after hatching. The incidence of larval molting in MC was counted by the renewal of old skin individually marked by a felt pen on the dorsum.

Pupae were kept in the same condition as they were reared as larvae, though the AN pupae of the second generation raised at LD 13:11, 25 °C were divided into two groups which were exposed to LD

16:8, a long-day and to LD 13:11, a short-day to examine the effect of photoperiods on pupal period. One group of long-day grown GN pupae, which entered diapause, were chilled on a schedule as follows: 10 °C for a month, 5 °C for a month and 10 °C for a month. The other group was kept where they had grown. Starved pupae grown under a long-day also passed this chilling schedule. After returned to 25 °C, they were kept under LD 16:8.

## Results

### Larval period

Table 1 showed the larval periods of the three populations when raised at LD 16:8, 25 °C, compiling both male and female data, since the sex ratio (females / males plus females) was not heavily biased, i. e., 0.535 for AN, 0.444 for GM and 0.535 for YD. The larval period was usually longer in females; 16.85 ± 1.39 (*N*=20) vs 15.70 ± 1.66 days (*N*=23) in AN and 18.50 ± 1.02 (*N*=60) vs 17.09 ± 1.51 days (*N*=69) in YD for the first generation

and 25.58 ± 3.80 (*N*=12) vs 21.14 ± 1.350 days (*N*=7) in AN and 26.50 ± 2.190 (*N*=24) vs 28.40 ± 4.37 days (*N*=15) in GM in the second generation.

The first generation grew basically in a diapause-free manner, whereas the second generation entered diapause in high percentages even under a long-day condition, which is reflected also on the large difference in larval period between the generations. The larval period was affected by the developmental program which later instituted diapause. The SC larvae took more time to pupate (Table 1), which "wasted" some 169.8 degree-days. The strain difference was not pronounced (Aspin-Welch test, *p* > 0.05), though a slight elongation was observed in the GM stock, which occurred in the second generation also: 23.95 ± 3.78 (*N*=19) in AN vs 27.23 ± 3.29 days (*N*=39) in GM.

The larval period was extended considerably by starvation, namely 27.23 ± 3.29 (*N*=39) in MC whereas 32.58 ± 2.56 days (*N*=50) in SV. The difference was highly significant (Aspin-Welch test, *p*

**Table 1** Larval periods of three populations of *A. alcinous* raised at LD 16:8, 25 °C, fed with *A. onoei* var *arimaensis*. Both males and females were combined. Means in days and (SDs)

Stock	Generation density <sup>1)</sup>	Instar						N
		I	II	III	IV	V	I-V	
AN	I M	3.37 (1.04)	1.54 (0.67)	2.45 (0.51)	3.21 (0.60)	5.54 (0.77)	16.23 (1.63)	43
	II M	—	—	—	—	—	23.95 (3.78)	19
GM	I M	3.00 (0)	1.86 (0.36)	2.18 (0.39)	4.00 (0.61)	7.04 (1.06)	18.04 (1.37)	27
	I S	—	—	—	—	—	22.37 (4.98)	12
YD	I M	2.19 (0.44)	2.42 (0.59)	3.15 (0.64)	3.60 (0.64)	6.51 (0.90)	17.74 (1.48)	129
	II M	—	—	—	—	—	27.23 (3.29)	39

1) M: mass culture, S: single culture, I: first, II: second generation.

<0.01)

Figure 1 illustrates the difference between the larvae destined to diapause and those to non-diapause. Larval development was severely retarded at 30 °C; the retardation was estimated as 8.73 days (181.7 degree-days) from the “highest velocity” extrapolated from the 20 °C and 25 °C data, provided that the development was ‘normal’ at both temperatures. The threshold temperature for “normal” development was estimated as 10.54 °C and the required heat units for larval development as 310.2 degree-days from the “normal” development at 20 and 25 °C.

Although diapause-destined larvae grew more slowly, photoperiodic effect on the larval period *per se* was not detected, since nondiapause individuals took  $21.46 \pm 1.13$ , and  $21.50 \pm 1.73$  days to pupate at LD 16:8 and 14:10, respectively and diapause individuals took  $27.38 \pm 3.42$ ,  $26.25 \pm 2.30$  and  $26.50 \pm 1.68$  days at LD 16:8, 14:10 and 13:11, respectively.

In summary, 1) males generally had shorter larval period than females, 2) the AN stock had shorter larval period than the GM stock, 3) larvae destined to diapause at pupae had longer larval period than larvae destined to non-diapause, 4) first

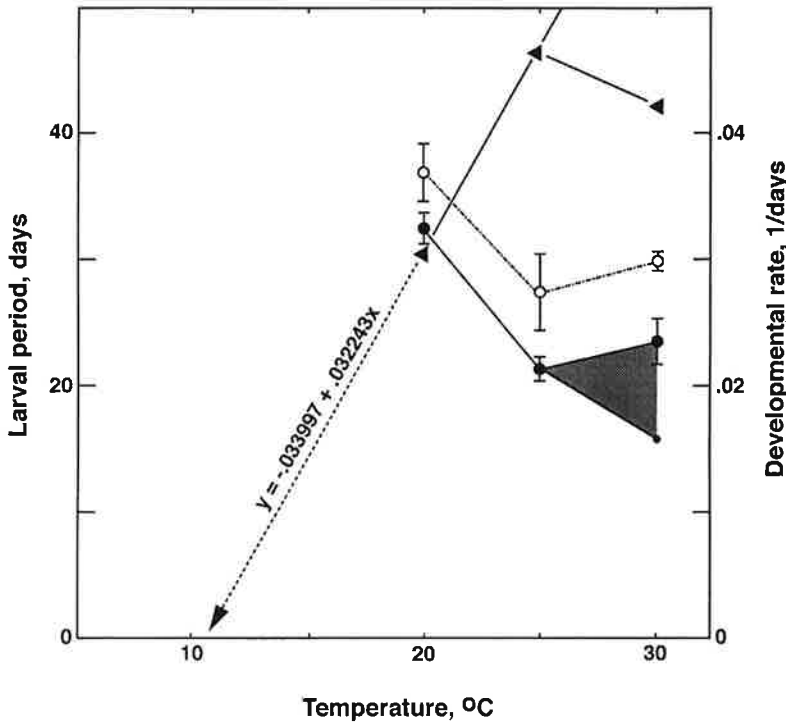


Fig. 1 Larval period (d) (closed circles) and developmental quotient (1/d) (triangles) of the second generation AN larvae of *A. alcinous* destined to nondiapause, when they were reared at LD 16:8 and larvae destined to diapause (open circles). Vertical lines indicate the standard deviation. (The shaded is heat units wasted for high temperature-induced developmental delay). The wasted heat units were nearly 50%. Small closed circle was a projected larval period at 30 °C, if no delay was caused, which was extrapolated from the 20 and 25 °C data points.

generation larvae had shorter larval life than second generation larvae together with smaller variance, 5) temperature higher than 30 °C slowed down the larval development, 6) the threshold temperature for larval development was estimated as 10.54 °C and heat units required for larval development as 310.2 degree-days unless the development was affected by diapause program, 7) photoperiod did not affect the rate of larval development when diapause was not programmed, and 9) starvation and isolation prolonged the larval period.

#### Pupal period and diapause

Pupal period depended on 1) the ambient temperature (Fig. 2), 2) the sex (Fig. 3), 3) the stock slightly (Table 2), and most strongly 4) the diapause program but not on the generation (Table

3) nor rearing density (Table 2). The pupal period may be determined by combination of these factors but when all data from LD 16:8, 25 °C were compiled, the mean periods of male pupae in the three stocks ranged between 11.6 and 12.4 days whereas those of females between 12.0 and 13.3 days, shorter in males than in females (Table 2). Figure. 2 also illustrates this difference in a temperature range between 20 and 30 °C.

The threshold temperature ( $t_0$ ) for pupal development was estimated in the AN stock as 7.47 °C, from the regression line for the rate of development at the three temperatures, and heat units required for pupal development as 234.8 degree-days ( $t_0 = 7.47$  °C). However, 30 °C seemed to be too high for pupal development. Therefore this point was

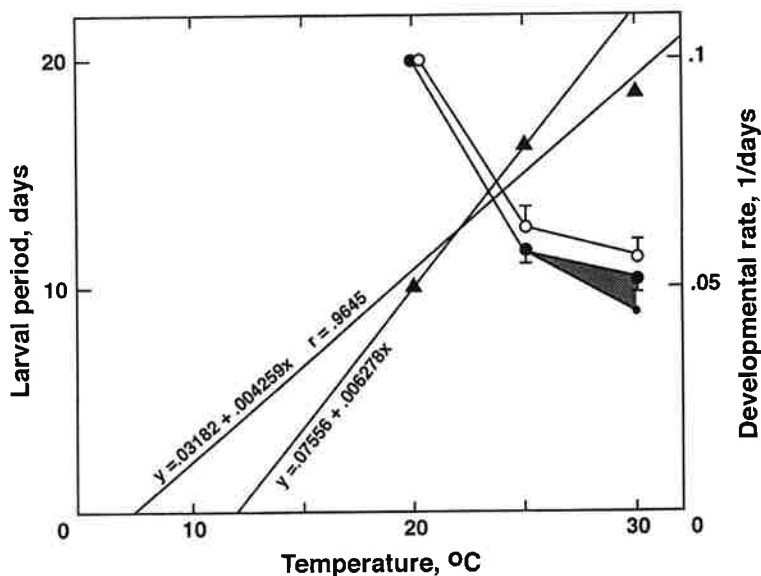


Fig. 2 Pupal period (d) and developmental quotient (1/d) (triangles) of the 2nd generation AN pupae of *A. alcinous* at LD 16:8. The regression was made either based on 20 and 25 °C points (dotted line) assuming that the 30 °C point suppressed normal development or based on 20, 25 and 30 °C points assuming that the 30 °C data point is normal. The shaded is a possible high temperature retardation of development. Closed circles are mean values for males, whereas open circles for females. The small closed is the extrapolated value from linear regression based on 20 °C and 25 °C data.

excluded and 159.3 degree-days ( $t_0 = 12.3^\circ\text{C}$ ) were estimated base only on the 20 and 25 °C data (Fig. 2). At least several days seem to be wasted at tem-

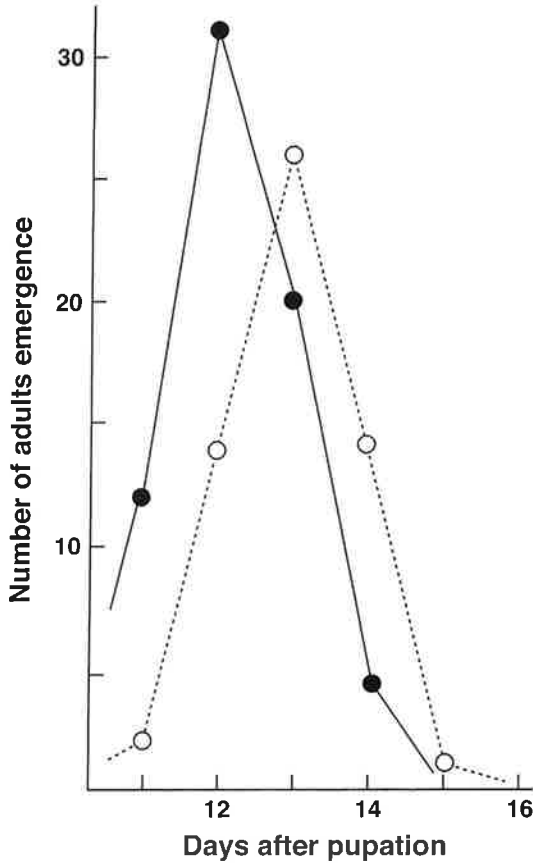


Fig. 3 Difference in pupal period between the two sexes of *A. alcinous* when they were kept at 25 °C, LD 16 : 8. The second generation of YD stock was used. Males (closed circles), whereas females (open circles).

peratures higher than 30 °C (shaded area in Fig. 2) This waste could be an incipient summer diapause.

Pupal period was shortest in the AN stock, being  $12.02 \pm 0.67$  days ( $N=43$ ), whereas those of the GM and YD stocks were  $12.50 \pm 0.51$  ( $N=24$ ), and  $12.57 \pm 0.90$  ( $N=124$ ) days, respectively. The single culture resulted in a slight elongation in pupal period (Table 2).

The diapause incidence illustrated the distinctness of the three stocks most clearly. For example the GM stock produced diapause pupae even when reared under a long-day condition; 25 % of the SC larvae ( $N=12$ ) and 14.7 % of the MC larvae ( $N=34$ ) in the first generation, whereas no diapause was induced in the other stocks in this condition. In the second generation, however, the AN stock also produced significant numbers of diapause pupae at LD 16 : 8, 25 °C, whereas 100 % ( $N=39$ ) GM pupae entered diapause. The diapause pupae were determined as surviving pupae for more than 80 days without emerging. Figure 4 illustrates the effect of ambient temperature (A) and photoperiod (B). High temperature and long day conditions tended to suppress diapause. The switch, however, was not sharp and substantial number of pupae entered diapause even at LD 16 : 8, and substantial number of pupae emerged promptly without diapause at LD 14 : 10. The regression line of Fig. 4 (left) estimated that 50 % diapause should be obtained at 24.1 °C, 100 % diapause at 14.8 °C, and 0 % at 33.2 °C. The

Table 2 Pupal periods in three populations of *A. alcinous* for diapause-free development of the 1st generation reared at LD 16 : 8

Stock culture <sup>1)</sup>		Males	(N)	Females	(N)
AN	M	$11.87 \pm 0.69$	23	$12.20 \pm 0.62$	20
GM	M	$12.40 \pm 0.52$	10	$12.57 \pm 0.51$	14
	S	$12.83 \pm 0.58$	3	$13.33 \pm 0.58$	3
YD	M	$12.24 \pm 0.82$	69	$12.97 \pm 0.84$	57

1) M: mass culture, S: single culture.



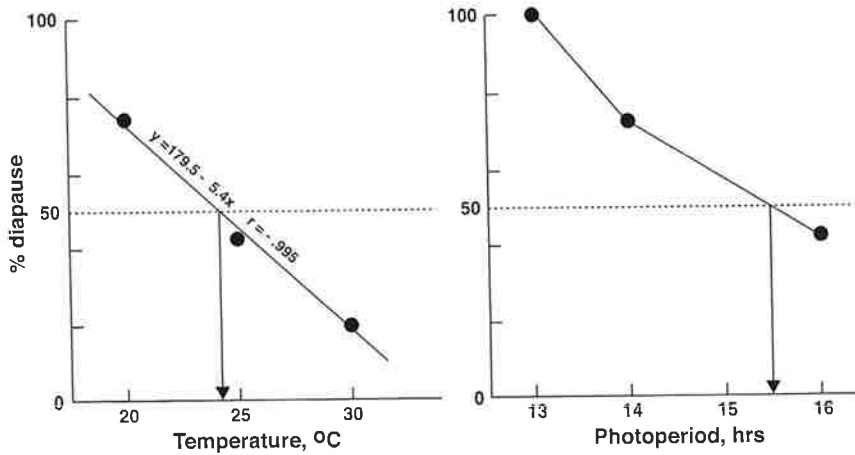


Fig. 4 Photoperiodic regulation of diapause in the second generation AN stock of *A. alcinous* at 25 °C (right) and temperature effect on the determination by LD 16 : 8 (left). Lines drawn to x-axis show critical photoperiod and temperature.

critical photoperiod at 25 °C was estimated as 15 hr 28 min (Fig. 4, right). The reverse response, however, appeared at 20 °C, diapause incidence 73.7 % at LD 16 : 8 ( $N=19$ ), whereas 38.9 % at LD 14 : 10 ( $N=18$ ).

The intensity of diapause varied between the two generations. All diapause pupae ( $N=8$ ) of the GN stock obtained in the first generation surpassed 180 days, when kept at LD 16 : 8, 25 °C, whereas those of the second generation ( $N=21$ ) started emerging before 90 days after pupation and only two individuals stayed in diapause beyond 200 days in the same condition. No diapause pupae were obtained at LD 16 : 8, 25 °C with the AN stock in the first generation, whereas diapause pupae appeared in the second generation ( $N=5$ ) from which adults emerged between 99 and 144 days. Diapause pupae grown under LD 14 : 10 stayed longer in diapause, namely 80 % in diapause for more than 200 days.

To investigate if photoperiods during diapause affected the diapause period, the two groups of diapause pupae grown under LD 13 : 11 were kept either under LD 16 : 8 or 13 : 11 at 25 °C. The long-

day induced emergence between day 102 and day 181, whereas the short-day induced the first emergence on day 131 and 50 % stayed in diapause for more than 200 days. Photoperiods affected diapause period during pupal stage also at 20 °C. All diapause AN pupae completed pupal development within 200 days when diapause was induced and the pupae were subsequently maintained at LD 16 : 8, whereas 40 % stayed in diapause for more than 200 days when diapause was induced and the pupae were subsequently maintained at LD 14 : 10. The effect of temperature during diapause was minimal, since the modal days of emergence fell between 120 and 140 days at the three temperatures examined.

The chilling as scheduled in Materials and Methods greatly accelerated diapause development, since 21 GM diapause pupae emerged on day 85 when maintained at 25 °C, whereas the first emergence occurred 14 days after transfer to 25 °C following the 30 day chilling schedule and 83 % ( $N=12$ ) completed emergence within 70 days following the chilling.

The starvation intensified diapause, since 33.3 %

diapause GM pupae ( $N=30$ ) which were deprived of food every other day for the first 10 days during feeding, stayed in diapause for more than 70 days after they passed the same chilling schedule whereas only 8.3% "normally fed" pupae stayed in diapause for more than 70 days.

Figure 5 illustrates the survival of the three stocks. The GM stock showed the highest mortality especially at pupal stage. The mortality was then investigated at three temperature conditions with the AN stock (Fig. 5). Although larval development was retarded at 30 °C as shown in Fig. 1, larvae survived this temperature but with high mortality during pupal development.

Size and weight of pupae showed an opposite trend between the generations. The smallest pupae of all three stocks were obtained in the GM stock in the first generation,  $26.53 \pm 1.19$  mm long and  $13.79 \pm 0.95$  mm wide vs  $27.09 \pm 0.84$  mm long and  $13.81 \pm 0.70$  mm wide in the AN stock, and  $27.41 \pm 0.96$  mm long and  $14.41 \pm 0.70$  mm wide in the

YD stock.  $872 \pm 115$  mg pupae ( $N=19$ ) were obtained in the AN stock in the second generation, whereas  $991 \pm 93$  mg pupae ( $N=39$ ) in the GM stock. Unexpectedly, starved larvae ( $N=50$ ) became heavier pupae, namely,  $1027 \pm 121$  mg. The temperature effect on the pupal weight was small, since pupae resulted in  $886 \pm 138$  ( $N=20$ ),  $872 \pm 115$  ( $N=19$ ) and  $870 \pm 124$  ( $N=19$ ) mg at 30, 25 and 20 °C, respectively, and so was photoperiodic effect, since  $872 \pm 115$  ( $N=19$ ),  $858 \pm 130$  ( $N=14$ ) and  $901 \pm 98$  ( $N=22$ ) mg pupae were obtained at 250 C, LD 16 : 8, 14 : 10 and 13 : 11, respectively.

SC larvae became slightly smaller pupae;  $25.73 \pm 2.15$  mm long and  $13.09 \pm 1.14$  mm wide ( $N=12$ ) vs.  $26.53 \pm 1.19$  mm long and  $13.79 \pm 0.95$  wide in the MC ( $N=34$ ).

## Discussion

The life cycle of *A. alcinous* was regulated mainly by photoperiodic induction of pupal diapause which was further fine-tuned by various factors such as

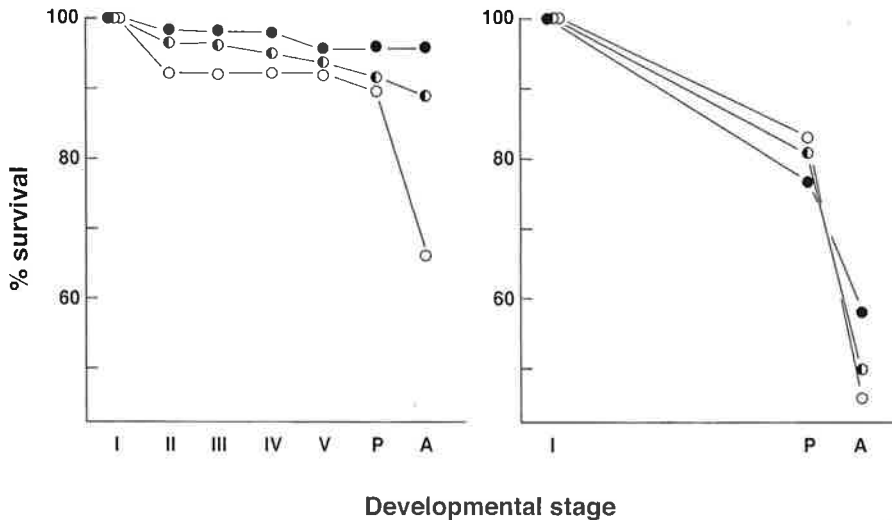


Fig. 5 The survivorship curve of three stocks of *A. alcinous* in the first generation (left). Closed circles, AN; semiclosed, YD and open, GM and the effect of ambient temperature on the survival of the second generation AN stock (right). Closed circles, 20 °C; semiclosed; 25 °C and open, 30 °C. I, II, III, IV, and V indicate larval instars, P, pupa, and A, adult stage.

temperature, food quality, crowding and starvation. Such complex fine-tuning of life cycle characterized the three populations used here as an open-land (YD), a hillside (AN) and a woodland (GM) population. Although the open-land and woodland habitats were not widely separated, gene flow seemed to be restricted to some extent, since wing patterns of adults, pupal coloration and color of pupating thread are slightly differentiated (Kozuki *et al.*, 1992) as well as behaviour, diapause and other developmental characteristics. For example, the critical photoperiod for diapause induction was estimated in this study as 15 hr 28 min in GM, whereas it was 13 hr 30 min at 25 °C in Osaka, Kyoto and Fukui open-land populations (Ishii, 1985).

Based on such data, **Table 3** characterizes the three populations. Birthwort growing on riverbed is frequently cut or burned or uprooted. The habitat is, therefore, ephemeral. The butterfly occupying such a niche tends to increase the generation number by reduced diapause tendency and enhanced migratory ability. On the other hand, the woodland habitat is stable and the woody species can recover easily from various kinds of disturbances. The plant reproduction depends more on a vegetative manner rather

**Table 3** Difference in pupal period (nondiapause) in the AN stock of *A. alcinous* between generations and sexes

Sex	Generation			
	I	(N)	II	(N)
Males	11.87 ± 0.69	23	11.67 ± 0.58	3
Females	12.20 ± 0.62	20	12.75 ± 0.96	4

than spreading seeds, which somehow limits the ability of wide colonization.

Although aristrochic acid (Nishida and Fukami, 1989a; 1989b) helps the insect to escape from predation, it may impose a biochemical burden on the insect itself, since the larval development was pronouncedly retarded in the second generation. The situation in the second generation may not be optimal in the woodland. The plant quality may decline and the crowding may prevail. Larvae may consume the birthwort completely. Long-day induced diapause in the first generation of the GM stock may, thus, be adaptive. If the stock built up a large population in later generations, it may be of great risk since together with cooler environment and limited patch size of the host, predation, food senescence, and cannibalism may capitulate the

**Table 4** Characterization of three habitat populations of *A. alcinous*

Character <sup>1)</sup>	Stock		
	YD	AN	GM
Habitat	riverside	hillside	woods
Host plant	<i>A. debilis</i>	<i>A. onoei</i>	<i>A. o. a. var arimaensis</i>
Larval develop.	intermed	Fast	slow
Pupal period (nondiapause)	long	short	long
% diapause I	0	0	~ 15
under long days II	< 5	~ 40	100
Estimated voltinism	3 ~ 4	2	1 ~ 2
Pupal size I	large	normal	small
II	—	small	large

1) I: first generation, II: second generation.

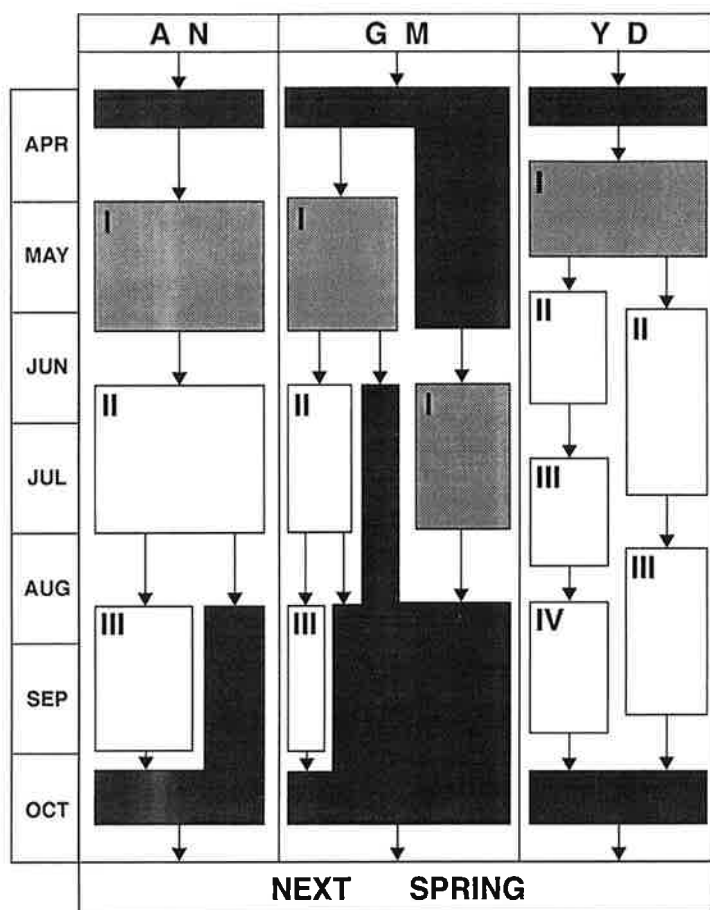


Fig. 6 Diagram showing projected life cycle patterns of three stocks of *A. alcinous* in the field based on laboratory rearing and field observation. Darkly shaded box represents diapause pupae, whereas lightly shaded, spring form adults. Light represents summer form adults. Roman letters are the generations for the year.

chance of survival.

Figure 6 illustrates the projected life cycles of the three populations based on the field observation and laboratory rearing. The GM population may split the life cycle and produce four kinds of diapause pupae before winter. The life cycle is not regulated solely by photoperiods, since the photoperiodic regulation was reverse in the second generation. SV and SC cultures also affected diapause incidence and the intensity. Such a mode of diapause regulation

may provide not only a bet-hedging but also an opportunistic fine-tuning mechanism.

The termination of diapause is also complex. The diapause period was affected by photoperiod, temperature, food quality and starvation. It is puzzling, unless considering a population hazard, why a population from more stable habitat diversified their life cycle. Alternative reason may be that their habitat is more or less isolated and communication between the habitat patches is limited. In such situations,

diapause is a favored escape mechanism over migration. Montine populations in various insects are in many cases sedentary where the habitat patches are isolated, or even tend to lose hind or both wings or otherwise lead to microptery.

The habitat differentiation is probably real in the case of *A. alicinous*. Frequently differentiated habitats are inhabited by closely related but different species with different life cycle characteristics (Ohsaki, 1979a; 1979b; 1980) but the YD and GM populations probably belong to the same species since the former normally grow on the natural host of the latter. Nonetheless, both environmental and biological parameters for the life cycles of the two populations may be grossly different. The genetic dissection for these populations are therefore of immense interest.

#### Acknowledgments

We thank Dr. R. Nishida of Kyoto University for providing YD, Mr. S. Miyake of Kobe Municipal Arboretum for letting us use the arboretum for our field observation and for supplying host plants, and Ms. Reiko Tomita for excellent artworks.

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## プラスチック・コンテナを用いたカワネズミ *Chimarrogale platycephala* (Temminck) の生息調査法

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(受領: 2004年4月3日; 受理: 2004年5月24日)

**Use of Plastic Containers as a New Strategy to Survey the Distribution of Japanese Water Shrew *Chimarrogale platycephala* (Temminck).** Akio Ichikawa<sup>1)</sup>, Hiroshi Nakamura<sup>2)</sup> and Toshio Yoshida<sup>2)</sup> (<sup>1)</sup>The United Graduate School of Agricultural Science, Gifu University, Minami-minowa, Nagano 399-4598, Japan, <sup>2)</sup>Faculty of Agriculture, Shinshu University, Minami-minowa, Nagano 399-4598, Japan). *Jpn. J. Environ. Entomol. Zool.* **15** : 169-177 (2004).

This study aimed to establish a new nonlethal strategy to confirm the distribution range of Japanese water shrew. The survey was conducted in southern Nagano Prefecture during from September 2001 to November 2002 except for the December to March period. Distribution was judged by the feeding marks that remained in the containers in the survey period. Characteristics of feeding marks of water shrews are that the fish bait is halved without its backbone, or is pulled out of the container with visible feeding marks. The survey was affected by periods of interruption caused by rainfall, but was not affected by watercourse width. The survey period found to be sufficient to determine an absence of water shrew was 17 days.

**Key words:** Japanese water shrew, Plastic container, Distribution, Feeding mark, Survey period

12月から3月を除く2001年9月から2002年11月にかけて、長野県の木曾川流域および天竜川流域において、プラスチック・コンテナを用いたカワネズミの生息調査法を確立することを目的に、食痕による出現種の推定、プラスチック・コンテナ設置期間について調査を行なった。カワネズミの食痕はコンテナ内部に、餌である生魚の頭部から背骨を残さずに、半分ほど紛失する状態で残るものや、そのような食

痕がついた状態でコンテナの出入り口から糸ごと外に引き出されるといった特徴がみられた。プラスチック・コンテナ設置期間中の降雨などによる中断によって設置期間は長期化する傾向がみられ、流路幅は設置期間を変化させることはなかった。非生息地点と判定されるまでに最低限必要なコンテナ設置期間を推定した結果、17日間であった。

## はじめに

カワネズミ *Chimarroga platycephala* (Temminck) はモグラ目トガリネズミ科 (Insectivora; Mammalia) に属し、水生適応した哺乳類の一種である。本種は主に山地溪流に生息し、魚類や水生昆虫を捕食する高次消費者であることから、溪流環境の指標種と位置付けることができる。これまでに、本種の狩り行動 (今泉・北垣, 1997) や、青森県における生息状況 (小原, 1999)、生息環境や活動 (阿部, 2003) に関する報告があるものの、その他多くの報告は断片的なものであり (例えば藤原, 1959; 湯川, 1968)、未知な部分が多い動物である。本種は水辺に依存した生活史を送るため、砂防工事に伴う河川改修などの環境改変によって個体数は減少する傾向がみられ、いくつかの都道府県の県版RDBで絶滅の危険性が高い種として扱われている (例えば長野県生活環境部環境自然保護課, 2004, 準絶滅危惧種; 新潟県環境生活部環境企画課, 2000, 準絶滅危惧種など)。そのため、本種を保全していくためには、生活史の解明や生息状況の詳細な把握が必要である。

これまで本種の生息状況を調査する方法としては、ハジキワナやシャーマントラップ、網付円筒、サンショウウオ捕獲用のワナ (ムジリ)、金網ワナを用いた捕獲が報告されている (今泉ほか, 1990; 阿部, 1992; 小原, 1999; 阿部, 2003)。しかし、シャーマントラップ、金網ワナなどの生け捕り用のトラップを用いた場合でも、およそ2時間おきの頻繁な見回りをしないと、本種は死亡することが多い (小原, 1999)。そのため、これまでの環境アセスメントなどにおける哺乳類の生息状

況調査ではほとんどの場合、本種は捕殺されるのが現状である。本種の希少性を考えれば、殺傷することなく簡便に本種の生息状況を評価することの出来る手法の開発は急務であるといえる。

北垣 (1996) は、河川内に設置したプラスチック・コンテナで魚を飼育し、本種を誘引して生態観察を行なった。筆者らは、プラスチック・コンテナ内に魚を糸で石にくくりつけ、本種が魚に残す食痕を観察できるように改良を加え、生息調査を行なった。

本研究では、捕殺せずに生息状況を評価する手法の確立を目的とし、プラスチック・コンテナ内に残る食痕による出現種の推定、プラスチック・コンテナ設置期間について調査を行なった。

## 調査地および調査方法

### 1. 調査地

本研究では流路幅が最大でも10m未満の小河川を調査河川とし、木曾川流域および天竜川流域の長野県南部に位置する河川 (木曾川流域1河川、天竜川流域10河川) において調査を行なった。延べ調査地点は61地点であった。なお、同一地点で季節を違えて複数回生息調査を行なった場合、各回の調査は独立のものとし、調査回数を調査地点数に換算した。

### 2. 調査方法

#### 1) 食痕による出現種の推定

プラスチック・コンテナ (31.0×23.6×16.0 cm; BeBox MC-9L アイリスオーヤマ(株); 以下コンテナ) はメッシュ状になっており、内部が水が貫流するようになっている。コンテナには持ち運びのため、側面に3.5cm×7.0cmの穴 (以下、

出入り口とする)が開いており、事前の観察により、カワネズミが出入り口を通過してコンテナを自由に出入りするのを確認した。コンテナ内部に重石を置き、その上に餌(体長100mm程度のアジ Carangidae)の尾付近をたこ糸でくくり、さらにそれを小石にくくりつけて置いた。餌は水中に沈めずに流水に浸すように設置し、餌の乾燥を防いだ(Fig. 1)。コンテナは、流路の兩岸際に1対、出入り口が水面上に出るように設置した。本種以外の生物による餌の持ち去りを防ぐため、コンテナには蓋をした。

食痕の確認および餌の交換のため、1-3日おきにコンテナの見回りを行なった。なんらかの食痕を確認した場合、出現する動物をセンサーカメラ(センサー部;赤外線式,カメラ部;K-MINI コニカ(株))により確認し、翌日に食痕の特徴を記録した。

調査は12月から3月を除き、2001年9月から2002年11月にかけて実施した。

## 2) プラスチック・コンテナ設置期間

カワネズミの生息地点は、本種の食痕を確認し、

さらにセンサーカメラによって出現を確認できた地点と定義した。これに対し非生息地点は、プラスチック・コンテナ設置期間(以下コンテナ設置期間)内で、食痕が全く確認されない、あるいは他種による食痕のみであった地点と定義した。本種によるものと思われる食痕を確認したものの、その後出現を確認できない場合は、非生息地点とした。生息地点におけるコンテナ設置期間は、コンテナを設置し、はじめて本種の食痕を確認するまでの延べ日数から、降雨などによりコンテナの設置を中断した日数を差し引いた日数とした。非生息地点におけるコンテナ設置期間は、最低2週間、さらに可能な限りコンテナ設置を継続し、コンテナを設置した延べ日数から中断した日数を差し引いた日数とした。

コンテナ設置期間中の中断日数やコンテナ設置地点の流路幅は、餌の誘因性や本種がコンテナを発見するまでの時間に影響を及ぼす可能性がある。そこで、全調査地点について、中断日数を、中断なし、1~3日間、4~10日間に区分して、それぞれのコンテナ設置期間を比較した。次に、メジャー



Fig. 1 Stones were used inside the container as a weight and the bait was tied up with a kite string.



を用いて計測した流路幅値を用いて、本種の生息地点および非生息地点それぞれの流路幅値を2群としたMann-WhitneyのU検定 ( $p < 0.05$ ) を行なった。さらに本種の生息地点のみについて、流路幅とコンテナ設置期間を2変量として、Spearmanの順位相関係数の検定 ( $p < 0.05$ ) を行なった。

本種の生息地点のみについて、非生息地点と判定されるまでに最低限必要なコンテナ設置期間を推定するため、従属変数を累積生息地点率（ある時点での累積生息地点数/全生息地点数 $\times 100$ ）、独立変数をコンテナ設置期間として対数曲線を用いて回帰した。

## 結 果

### 1. 食痕による出現種の推定

調査を行なった延べ61地点のうち、カワネズミの生息地点は31地点、非生息地点は30地点であった。

コンテナに出現した動物を確認した翌日に食痕の特徴を記録した結果、本種の食痕は、コンテナ内部に餌である生魚の頭部から背骨を残さずに半分ほど紛失する状態で残るものや、そのような食痕がついた状態でコンテナの出入り口から糸ごと外に引き出されるといった特徴がみられた (Fig. 2)。

コンテナに侵入し、食痕を残す他の野生動物ではドブネズミ (*Rattus norvegicus* (Berkenhout))、小型ネズミ類（多くはアカネズミ *Apodemus speciosus* (Temminck))、イタチ (*Mustela itatsi* Temminck)、カワガラス (*Cinclus pallasii* Temminck)、サワガニ (*Geothelphusa dehaani* (White)) が確認された (Table 1)。アカネズミなど、本種よりも体サイズが一回り小さいネズミ類の食痕は餌の背骨が残る点に加え、頻繁にコンテナ内に糞が確認されることで本種のもとの区別することが出来た (Fig. 3)。また、ドブネズミのような大型のネズミ類やイタチの食痕は餌がまったく残らず、また多くは餌をくりつけていた結び目も残らなかった。サワガニによる食痕は、本種による食痕と類



Fig. 2 The feeding mark of Japanese water shrew which tore a bait off. This figure was not the survey method which used plastic containers but placed baits beside the watercourse. By marking for tracking, the tail of the individual became white.

似した特徴を示したものもあったが、餌である生魚の眼球をきれいにくりぬく特徴を示した (Fig. 4)。カワガラスでは餌につつかれたような穴が開く特徴がみられた。また、出現する動物とそれぞれの食痕の特徴を明らかにして以降、その後の調査で食痕から出現する種を予想して自動撮影を行なった結果、食痕による識別と実際に出現した種の不一致はみられなかった。

### 2. プラスチック・コンテナ設置期間

生息地点のうち、中断日数が0日の地点では、平均  $2.1 \pm 1.9$  (S. D.) 日でカワネズミの食痕を