

# Host Plant Quality Influences Diapause Induction of *Byasa alcinous* (Lepidoptera: Papilionidae)

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**ABSTRACT** Using host plant quality as a diapause-inducing stimulus may be adaptive for herbivorous insects that use host plants whose phenology is unpredictable based on photoperiod or temperature. We examined the effects of leaf toughness of the woody vine *Aristolochia kaempferi* Willd. on larval performance and pupal diapause induction of the swallowtail butterfly *Byasa alcinous* Klug (Lepidoptera: Papilionidae). We also conducted field observations to assess whether human disturbance affects leaf phenology of *A. kaempferi*. Laboratory experiments showed that tough leaves decreased the survival rate of young larvae and lengthened the larval period, although pupal weight was not affected by leaf toughness. The incidence of pupal diapause was greater with tough leaves than with soft leaves and increased with larval duration. In the field, leaf age composition in *A. kaempferi* was greatly influenced by human mowing, which led to changes in leaf quality. These results suggest that *B. alcinous* has a plastic diapause strategy using host plant quality as a diapause-inducing stimulus, which may have evolved in frequently disturbed environments.

**KEY WORDS** facultative diapause, monophagous herbivore, phenotypic plasticity, seasonal change in host quality

Host plant quality is a key determinant of the fecundity of herbivorous insects (Awmack and Leather 2002). Diet quality varies not only between species (Jordano and Gomariz 1994, Ohsaki and Sato 1994) but also within species (Sipura and Tahvanainen 2000, Wheeler 2001). One ubiquitous intraspecific change in diet quality is seasonal (Feeny 1970, Calvo and Molina 2005), with unfavorable periods for insects. Many insects can tolerate unfavorable seasons by entering diapause, which is induced by various environmental cues that allow insects to predict seasonal environmental changes (Tauber et al. 1986). Photoperiod and temperature mainly act as diapause-inducing stimuli, but diet quality also regulates diapause in some insects and mites (Hare 1983, Tauber et al. 1986, Hunter and McNeil 1997, Goehring and Oberhauser 2002, Ishihara and Ohgushi 2006, Ito and Saito 2006). Nonseasonal environmental changes are difficult to predict using photoperiod, especially the regrowth of new plant tissues after disturbances, including flooding, hurricanes, and mammalian browsing (Martinsen et al. 1998, Spiller and Agrawal 2003, Nakamura et al. 2005, Bailey and Whitham 2006), because they are likely to occur aseasonally. In insects for which variation in host quality can be caused by disturbances, it seems adaptive to use leaf quality as a diapause-inducing stimulus because regrowth provides occasional

resources favorable for herbivorous insects. Thus, which environmental cues insects use should depend strongly on the extent to which the change in diet quality is predictable.

*Byasa alcinous* Klug (Lepidoptera: Papilionidae) is a swallowtail butterfly distributed in east Asia. Larvae feed only on *Aristolochia* spp., which produce aristolochic acids (Fukuda et al. 1982). In Honshu, central Japan, short daylength induces pupal diapause, but the response to long daylength varies among populations (Kato 2000). Lowland populations do not enter diapause under long-day conditions, whereas mountain populations have a strong tendency to diapause (Kato 2000). The host plant of lowland populations is *Aristolochia debilis* Siebold et Zucc., a perennial herb that produces new leaves successively from spring to late autumn. In contrast, the host plant of mountain populations is *Aristolochia kaempferi* Willd., a woody vine that produces new leaves only from spring to early summer; thus, the leaves of *A. kaempferi* are tough and thick from midsummer onward. Consequently, the presence of diapause under long-day conditions is considered an adaptation to seasonal decline in host plant quality (Kato 2001). However, Kato (2005) recently found a population that does not enter diapause under long-day conditions, despite using *A. kaempferi* as its host, and suggested that there is altitudinal variation in the phenology of *A. kaempferi*. If nonseasonal factors, including disturbances, improve the seasonal

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decline in host plant quality, the absence of diapause under long-day conditions may represent one aspect of phenotypic plasticity, not a fixed strategy adapted to local phenology.

To determine whether *B. alcinous* shows a facultative diapause in response to host plant quality, we examined the effects of *A. kaempferi* leaf toughness, an indicator of food quality, on larval duration, pupal weight, survival rate, and diapause induction of *B. alcinous*, by using a population likely to experience disturbances. We also examined the effects of human disturbance on *A. kaempferi* leaf quality in the field.

### Materials and Methods

**Study Insect.** *B. alcinous* is distributed in Taiwan, China, Korea, and Japan (Fukuda et al. 1982). Kato (2001) showed the following general life cycle in Honshu, Japan. Adults emerge in April to May and reproduce. In mountain populations, the new generation mostly diapauses at the pupal stage, and pupae eclose in the spring after hibernation. In contrast, in lowland populations, the first generation ecloses in a month without diapause and reproduces once or more before late autumn.

We collected insects in the Tokyo University Forest in Chiba (35° 12' N, 140° 9' E, 150–300-m elevation; Honshu, Japan), with evergreen broad-leaved forests and plantations of *Cryptomeria japonica* D. Don and *Chamaecyparis obtusa* (Siebold & Zucc.). The host plant *A. kaempferi* is found on the forest floor and at forest edges. Because no other plant species belonging to the genus *Aristolochia* were found at this site, *A. kaempferi* is the sole host plant for *B. alcinous*. Kato (2000) categorized butterflies collected 10 km away from this site as a "mountain population" that diapauses under long-day conditions (Kato 2000). However, because we sometimes observed adults and larvae in late July and August (S.T., unpublished data), at least some individuals do not enter diapause in the summer.

Captive adult females were allowed to oviposit on *A. debilis* or *A. kaempferi* in transparent plastic cages. Larvae from three females were used for the experiment. Two of the adult females (A and B) were collected at 150-m elevation on 31 May 2006, and another (C) was collected at 300-m elevation on 27 July 2006.

**Food Plant.** All larvae were fed *A. kaempferi* in this experiment. Almost all leaves were collected at Hachioji, Tokyo (35° 37' N, 139° 14' E, 300–550-m elevation; Honshu, Japan). Leaves were put in plastic bags immediately after collection and kept in a refrigerator until leaf toughness was measured. Leaf toughness was measured with a penetrometer (Sands and Brancatini 1991). First, a leaf was sandwiched between two transparent plastic plates with a 3-mm-diameter hole and held horizontally. The tissue between major leaf veins was then pierced slowly by an iron rod (2.6 mm in diameter) with a flat end. The maximum force used to pierce the leaf was measured with a digital force gauge (RX-5, Aikoh Engineering, Osaka, Japan) attached to the iron rod. Although leaf toughness is a continuous variable, it was impossible to give leaves with exactly the constant toughness to each

larvae throughout the experimental period. Accordingly, we fed two levels of leaves with different toughness, which roughly corresponded to the levels of young leaves after disturbance and mature intact leaves, respectively. Leaves with a penetration force <1.5 N were categorized as soft, and those higher than 2.0 N were categorized as tough. We have chosen leaf toughness as a leaf quality in this experiment because leaf toughness is known to correlate with other characters (e.g., Reich et al. 1992, Suzuki 1998). Actually, leaf toughness and C:N ratio of *A. kaempferi* was positively correlated ( $r = 0.46$ ,  $n = 22$ ,  $P = 0.02$ ), and C:N ratio differed significantly between tough and soft leaves ( $F = 6.31$ ;  $df = 1, 13$ ;  $P = 0.03$ ; one-way analysis of variance [ANOVA]); the mean  $\pm$  SE of tough and soft leaves was  $18.70 \pm 1.31$  and  $14.62 \pm 0.88$ , respectively.

**Experiment.** Larvae from each female were divided into two groups; one group was given soft leaves and the other group was given tough leaves until pupation. All leaves given had been measured and categorized. The experimental period was 8 June–31 August for larvae from female A, 8 June–17 August from female B, and 5 August–4 November 2006 for larvae from female C. Larvae were reared in 900-ml transparent plastic cups with moist paper under a constant temperature of 20°C and a photoperiod 16:8 (L:D) h. Under these conditions, most of the individuals belonging to mountain populations enter diapause, whereas lowland populations do not (Kato 2000). The petiole of each leaf was wrapped with moist paper to avoid wilting. Leaves were exchanged for new leaves before wilting or before being fully consumed. One to six larvae were reared in each cup depending on the instar, and all larvae were reared individually after the fourth instar. The number of larvae reared in a cup did not differ between treatments. Larval duration, pupal weight, and pupal diapause were recorded for each larva. Leaf toughness may be a deterrent, especially for younger larvae; thus, the number of larvae that died and survived until the third instar also was recorded for each treatment. Larval duration was defined as the period from hatching to pupation. Pupal weight was measured with an electric balance 1 or 2 d after pupation. At the same time, each pupa was identified as diapausing or nondiapausing by color (Kato 2000); diapausing pupae are light brown, whereas nondiapausing pupae are bright yellow. When this discrimination was difficult, pupae that eclosed within 1 mo under constant conditions (20°C and a photoperiod of 16:8 [L:D] h) were regarded as nondiapausing.

**Effects of Disturbance.** To examine the effects of human disturbance on *A. kaempferi* leaf quality, we examined leaf age composition in disturbed and undisturbed habitats. We counted leaves on 8 August 2006 at Tateyama, Chiba (34° 58' N, 139° 51' E, 20–40-m elevation; Honshu, Japan), in evergreen forests adjacent to paddy fields. At the forest edge facing a road adjacent to paddy fields, farmers mowed shrubs, grasses, and herbs at least three times a year. After the mowing, the height of vegetation except trees was <1 m, with apical buds of plants often being cut off. In the forest interior, mowing was never seen. We counted

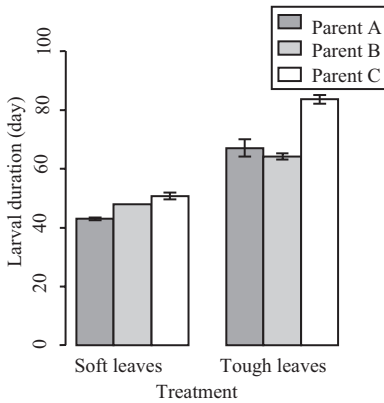


Fig. 1. Effect of leaf toughness on larval duration. Data represent mean  $\pm$  SE.

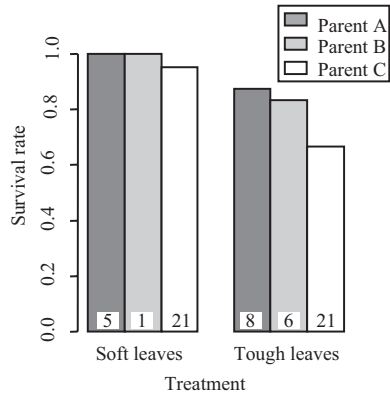


Fig. 3. Effect of leaf toughness on survival rate of young larvae. Sample sizes are given in each bar.

all leaves in each habitat and recorded leaf age. Leaf age was categorized by color and shoot condition; light green leaves with developing shoots were regarded as young leaves, whereas dark green leaves with developed shoots were mature leaves. These two states were easily distinguishable. The difference in leaf age was mostly caused by human mowing because intact *A. kaempferi* normally stopped growing in this season (Kato 2001). Plants over 1 m in height were excluded because tall plants may not be affected by human disturbance; no new leaves were observed for plants over 1 m in height in either habitat (S.T., unpublished data). Ten young and three mature leaves were collected from the field and their toughness was measured with a penetrometer as described above.

**Data Analysis.** The effects of leaf treatment on larval and pupal performance were analyzed with a generalized linear mixed effect model. Treatment was a fixed factor and female parent was a random factor. The response variables were larval duration, pupal weight, early larval survival rate, and the incidence of pupal diapause. The first two variables were assumed to have normal distributions with identity links. The latter two variables were assumed to have binomial distributions (dead or

alive, diapausing or nondiapausing) with logit links and Laplace approximations. In the soft-leaf treatment, female C produced both diapausing and nondiapausing pupae, so the relationship between larval duration and the incidence of pupal diapause was analyzed by logistic regression. The effect of human disturbance on leaf age composition was analyzed with a Fisher exact test. The difference in leaf toughness between young and mature leaves was tested by one-way ANOVA. All statistics were analyzed using R 2.3.1 (R Development Core Team 2006) for Windows.

Results

**Larval Performance and Diapause Induction.** Larval duration differed significantly between leaf toughness treatments ( $F = 40.33$ ;  $df = 1, 2$ ;  $P = 0.02$ ; Fig. 1), with larvae feeding on tough leaves having a longer larval period. Pupal weight did not differ between treatments ( $F = 1.68$ ;  $df = 1, 2$ ;  $P = 0.32$ ; Fig. 2). Survival rate until the third instar was lower in larvae feeding on tough leaves than in larvae feeding on soft leaves ( $\chi^2 = 4.84$ ,  $df = 1$ ,  $P = 0.03$ ; Fig. 3). The incidence of pupal diapause differed significantly between the treatments ( $\chi^2 = 7.91$ ,  $df = 1$ ,  $P = 0.005$ ; Fig. 4).

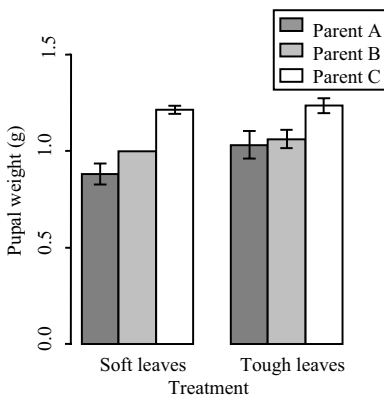


Fig. 2. Effect of leaf toughness on pupal weight. Data represent mean  $\pm$  SE.

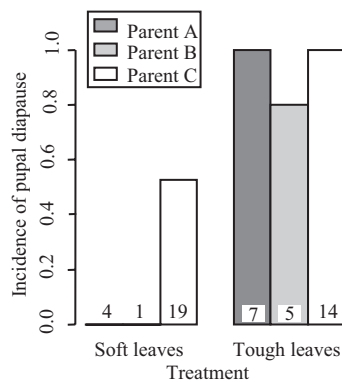


Fig. 4. Effect of leaf toughness on incidence of pupal diapause. Sample sizes are given in each bar.

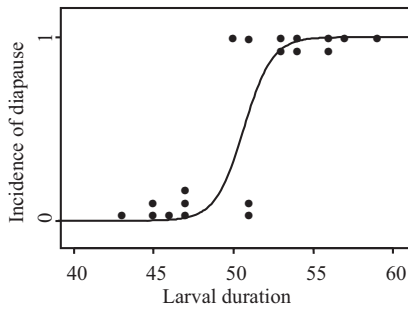


Fig. 5. Relationship between larval duration and incidence of pupal diapause, larvae from parent C in soft-leaf treatment. Each dot represents individuals.

All but one pupa entered diapause in the tough-leaf treatment. In the soft-leaf treatment, larvae from female C had almost equal probabilities of becoming diapausing or nondiapausing pupae. We found a positive correlation between larval duration and the incidence of pupal diapause ( $\chi^2 = 18.87$ ,  $df = 1$ ,  $P < 0.001$ ; Fig. 5), and diapause incidence drastically changed at a larval duration of  $\approx 50$  d.

**Effects of Human Disturbance on Host Quality.** In the disturbed habitat (i.e., forest edge), both young ( $n = 57$ ) and mature ( $n = 21$ ) leaves were present, whereas only mature leaves ( $n = 177$ ) were found in the undisturbed habitat (i.e., forest interior). The leaf age composition differed significantly between disturbed and undisturbed habitats ( $P < 0.001$ ). Leaf toughness differed significantly between young and mature leaves in the disturbed habitat ( $F = 7.40$ ;  $df = 1, 11$ ;  $P = 0.02$ ); the mean  $\pm$  SE of mature and young leaves was  $2.17 \pm 0.22$  and  $1.43 \pm 0.13$  N, respectively.

## Discussion

**Larval Performance.** Tough leaves decreased the survival rate of young larvae and elongated the larval stage (Figs. 1 and 3), suggesting negative effects of leaf toughness on the fitness components of *B. alcinous*. However the pupal weight did not differ significantly between treatments (Fig. 2). This suggests that there is no significant difference in the reproductive output in adult stage, considering a general correlation between adult weight and its fecundity in many insects (Honěk 1993). The difference in larval survival found here seems to be magnified in the field, because a long larval duration may additionally reduce survivorship through the cumulative effects of predation risk (Hägström and Larsson 1995), parasitism (Benrey and Denno 1997), or chilling (Fordyce and Shapiro 2003). Hence, tough leaves may lead to much higher mortality than soft leaves. Because leaf toughness was positively correlated with C:N ratio, the leaf quality including toughness and/or C:N ratio may have affected fitness components of *B. alcinous*.

**Diapause Induction.** It is noteworthy that larvae from the same female increased their incidence of pupal diapause in the tough-leaf treatment but not in

the soft-leaf treatment (Fig. 4). This is direct evidence for a plastic diapause response to leaf quality. The positive relationship between larval duration and diapause induction (Fig. 5) may imply that leaf quality acts indirectly via slower larval development. Regardless, leaf quality is a diapause-inducing stimulus for *B. alcinous*. Temperature also influences the incidence of diapause under long-day conditions (Kato 2000). In the population we used, host plant quality experienced in the larval stage also influenced diapause induction. More extensive studies are required to ascertain whether a plastic response to host plant quality is common in other regions.

**Adaptive Significance of a Plastic Diapause Strategy.** Under what conditions does a plastic diapause strategy become adaptive? Although dietary quality is an important factor determining fitness in many herbivorous insects (Awmack and Leather 2002), only a few studies have shown that diapause is induced by plant quality rather than by photoperiod or temperature (e.g., Tauber et al. 1986). The plastic diapause based on host quality observed in *B. alcinous* is probably due to the difficulty in predicting host plant quality using only photoperiod and temperature. We showed that leaf age composition in *A. kaempferi* differed between habitats with and without human mowing. This difference was mostly caused by regrowth after human mowing, leading to changes in leaf quality. In addition, we observed no differences in leaf age composition between the forest edge and interior in a site without human mowing (S.T., unpublished data). These results indicate that the phenology of *A. kaempferi* is not fixed but changes in response to occasional disturbance. Climatic changes between years also may cause a variable phenology, but regrowth after disturbance is more difficult to predict because it is likely to be independent of climatic changes, including temperature. Therefore, using host plant quality as a diapause-inducing stimulus may be adaptive for *B. alcinous* populations using *A. kaempferi* subject to disturbance.

Induction of reproductive diapause using food quality as a stimulus has been reported in some herbivores including the Colorado potato beetle (Hare 1983), willow leaf beetle (Ishihara and Ohgushi 2006), and Kanzawa spider mite (Ito and Saito 2006). Plastic reproductive diapause is adaptive through later reproduction of the focal individual and through larval survivorship in the next generation. However, in our study system, plastic diapause does not improve fitness through reproduction of the focal individual, but it improves the survivorship of its offspring, because *B. alcinous* uses different resources in the larval and adult stages. In this sense, the plastic diapause strategy found in *B. alcinous* seems to be unique.

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