INTRODUCTION

Since photoperiod regularly change with the season, they are most reliable as a seasonal signal for the life-cycle regulation of insects. However, photoperiodic response differs among different species or populations of the same species of insects. To understand the climatic adaptation of insects, the geographic variation in photoperiodic response controlling diapause and related phenomena has been intensively investigated in many insect species (Danilevsky, 1961; Tauber et al., 1986; Danks, 1987). Further, other conditions such as altitude, continentality of the climate and microhabitat conditions, food resources modify the photoperiodic response.

The *Aristolochia*-feeding butterfly *Atrophaneura alcinous*, which includes five subspecies in Japan, is distributed from the temperate region of northern Japan to the subtropical Ryukyu Islands. This butterfly emerges two or more times per year and enters diapause in the pupal stage. Kato (2000) demonstrated that pupal diapause of two populations (mountain and lowlands) from central Japan is induced by a short photoperiod, and that although their critical photoperiods are similar, their photoperiodic response curves are different. These populations differ in voltinism and host plant species: The mountain population has fewer generations per year than the lowland population; and the former uses *Ar. kaempheri* with thick and tough leaves, while the latter uses *Ar. debilis* with thin and tender leaves (Kato, 2001). Host-plant related occurrence of pupal diapause has also been reported for the North-American *Aristolochia*-feeding butterfly *Battus philenor* (Sims and Shapiro, 1983a, b).

Studies on the seasonal adaptation and diapause characteristics of butterflies living in Japan were comprehensively reviewed by Masaki and Yata (1988). However, more research is needed to fully understand the pupal diapause and life-cycle traits of *A. alcinous*, in which the influences of photoperiod and host plant on pupal diapause in local populations are still unclear. In the present study, the photoperiodic responses of populations differing not only in the latitude of their habitats, but also in altitude were determined taking account host plant use.

MATERIALS AND METHODS

**Insects.** Early stages and adults of *A. alcinous* were caught at the following locations (Fig. 1):
1) Ishigaki, Okinawa Prefecture, 24.3°N, about 40 m in altitude, 2) Kiire, Kagoshima Pref., 31.3°N, 720 m in altitude, 3) Kashihara, Nara Pref., 34.5°N, 50 m in altitude, 4) Yokosuka, Kanagawa Pref., 35.0°N, 150 m in altitude, 5) Fuchu, Tokyo Metro., 39.1°N, 150 m in altitude. The Ishigaki population is subtropical and the others are temperate. The Kiire and Yokosuka populations use *Ar. kaempheri* as the host plant, while the Kashihara, Fuchu and Yamagata populations use *Ar. debilis*. The Ishigaki population uses *Ar. liukiensi*, which is similar to the former plant in morphological characteristics such as leaf toughness and size.

**Photoperiodic treatments.** Field-caught females or laboratory-reared mated females were allowed to oviposit on the leaves of *Ar. debilis* in a temperature-controlled room (25°C). After hatching, the larvae of the *A. alcinous* populations were reared in plastic cups (11 cm in diameter and 6 cm in depth) under various photoperiods (from 12L–12D to 16L–8D) at 20°C. As larval food, fresh leaves of *Ar. debilis* were supplied.

**Determination of pupal diapause and critical photoperiod.** Newly eclosed pupae were kept at 16L–8D and 20°C, and adult eclosion was checked. The pupal color is not related to diapause in the subtropical populations, although it is closely associated with diapause in the temperate populations (Kato, 2000). Therefore, specimens that had not eclosed within 1 month after pupation were regarded as diapause pupae. The critical photoperiod was regarded as the photoperiod resulting in 50% diapause. When the shape of photoperiodic response curve was not typical, the intermediate photoperiod between the photoperiods giving the maximum and minimum percentages of diapause was taken as the critical period.

**RESULTS AND DISCUSSION**

The photoperiodic response curves of the *A. alcinous* populations are shown in Fig. 2. For the three populations (Kashihara, Fuchu, and Yokosuka) of central Japan, the photoperiodic response curves were of a typical long-day type and the critical photoperiods were approximately 14.5 h. For the northern population (Yamagata), the critical photoperiod was longer, approximately 15.3 h. In contrast, for the southern population (Kiire), the pupal diapause ratio was about 80% even under 14L–10D or longer photoperiods, and the critical photoperiod was about 13.5h. In the temperate population of this butterfly, as in other Japanese butterflies including *Papilio* species (Masaki and Yata, 1988; Ishii, 1989), the critical photoperiod increases as the latitude increases (Fig. 3). For the subtropical Ishigaki population, the critical photoperiod was as short as 12.4 h. This decrease in critical photoperiod is also seen in other subtropical butterflies (Masaki and Yata, 1988; Ishii, 1989; Hashimoto, 2002).

In the present study, the Kiire population, which inhabits mountain areas and uses *Ar. kaempheri*, showed a high rate of diapause under a long photoperiod. This phenomenon is similar to that of the Gotemba and other mountain populations in the Kanto region using the same host plant (Kato, 2000). The Yokosuka and Ishigaki populations use *Ar. kaempheri* and *Ar. riukiensis*, respectively. The leaves of both of these plants are thick and tough. However, both butterfly populations showed a typical response of long-day type photoperiod. Therefore, the quality of food is not the cause of the high incidence of pupal diapause under long days in the Kiire population. Previously, Kato (2000, 2001) inferred that both the cool temperatures in the mountain areas of the Kanto region and the host plant species are responsible for the unusual response. It is generally known that adaptation to the cool
weather at higher altitudes shifts the critical photoperiod for diapause induction towards a longer range (Tauber et al., 1986; Danks, 1987). In the nymphalid butterfly *Limenitis camilla*, for example, the critical photoperiod for larval diapause was longer in the mountain population than in the lowlands population (Hasegawa, 1987). In this butterfly, the host plant does not differ between the two populations. In the mountain populations of *A. alcinous*, the critical photoperiod, as defined in the above section (Materials and Methods), does not shift towards a longer period. In the mountain area of Gotemba, adult flight occurs two times per year (late spring and late summer), and its peak is much lower in the second flight than in the first one (Kato, 2001). The larvae from the first adults partly become diapause pupae without producing the second adults in mid-summer. *Ar. kaempheri* used as the host plant nearly completely stops developing new leaves in mid-summer. By contrast, in the lowlands of the Kanto region, the flight season is from spring to mid-autumn with four flight peaks (Kato, 2001). The diapause pupae begin to appear from early autumn, and the host plant (*Ar. debilis*) continues to grow till late autumn. This may also be true in the northern population that uses *Ar. debilis* (Yamagata). Based on these observations, a high incidence of diapause under long day exposure in the mountain populations might be regarded as adaptation to the host plant’s phenology associated with the cool mountain temperatures, rather than the difference in host species used.

Fig. 2. Photoperiodic response curves for pupal diapause induction in the populations of *A. alcinus* (*N*=18–32 for each point). A: Yamagata, B: Fuchu, C: Yokosuka, together with Gotemba* (Kato, 2000), D: Kashihara, E: Kiire, F: Ishigaki.

Fig. 3. The relationship between the distribution latitude and critical photoperiod for pupal diapause induction in the geographic populations of *A. alcinus*.
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REFERENCES


