INTRODUCTION

The cherimoya, *Annona cherimola* Mill. (Magnoliales: Annonaceae), is a subtropical orchard tree. Although it is self-compatible, autogamy is generally impossible because of protogynous dichogamy (Sanewski, 1991). This is a relatively archaic plant that does not use bees as pollinators. Thus, hand pollination is used to obtain an adequate fruit set, but this takes time and money (Gazit et al., 1982; Richardson and Anderson, 1996). Several studies have been conducted to identify suitable pollinators of cherimoya and its relative, atemoya (*A. cherimola*/H. squamosa), to reduce orchard labor. Nitidulid beetles are the main visitors of *Annona* spp. flowers world widely (Gazit et al., 1982; George et al., 1989; Nagel et al., 1989; Nadel and Peña, 1994), and secondary visitors include staphylinid beetles and *Orius* spp. (Hemiptera: Anthocoridae) (Caleca et al., 1996, 1998; Palmeri and Longo, 1997).

These fruit trees were introduced to the Japanese main island, Honshu, two decades ago. They are cultivated in greenhouses to avoid frost damage. Such facilities are favorable for the use of mass-released insect pollinators, since the greenhouse can prevent the insects from escaping. We have been studying visitors to cherimoya flowers in Japanese orchards for the purpose of finding suitable pollinators during the flowering season (Tsukada et al., 2005b). *Annona* spp. floral differentiation occurs year-round in their natural distribution area. However, in Honshu, pruning in early spring results in full-blooming around June. We found several individuals of *Carpophilus marginellus* Motschulsky (Coleoptera: Nitidulidae) on the flowers, among other species such as *Mimemodes monstrosus* (Reitter) (Rhizophagidae) and *Haptoncus ocularis* (Fairmaire) (Nitidulidae) during the flowering season.

We have already reported the developmental characteristics of *H. ocularis* as a candidate pollinator (Tsukada et al., 2005a). However, high mortality at high temperatures limits its use in greenhouses during summer (Higuchi et al., unpublished). This prompted us to investigate another candidate pollinator. Among the flower visitors in Japan, the number of *C. marginellus* was second...
largest, after *M. monstrosus* (Tsukada et al., 2005b). Both males and females enter the female-stage flowers and leave them during the male stage with much pollen on their bodies (unpublished data). Therefore, we assume that they pollinate the plant. This sap beetle has a body length of ca. 3 mm (Hisamatsu, 1985), and is distributed widely in warm regions from East Africa to Japan (Kirejtshuk, 1998). Females lay their eggs on rotting fruits such as orange and pineapple. Larvae develop there, and pass through three instars before pupation. Mature last-instar larvae wander about before entering the soil, where they pupate. Larvae also develop on ripened *Annona* fruits. However, *Annona* fruits usually ripen after harvest, so *C. marginellus* is not likely to damage the fruit in orchards. Also, even though some flower visitors, including nitidulid beetles, are known to injure plant ovaries, which causes aesthetic damage to some orchard fruits, this seems not to occur on the cherimoya (personal observation). Thus, the use of this species as a pollinator is feasible. On the other hand, *C. marginellus*, as well as other *Carpophilus* species, can be pests of dried fruits and cereals, but adequate pest management has not been established, because of the lack of published data on their biology.

To investigate the use of beetles as pollinators of the cherimoya, especially by mass rearing and release in greenhouse orchards, researchers must know their developmental characteristics. Among many abiotic environmental factors, circumstantial temperature is one of the most crucial factors that regulate the development of insects. Except at extremely high or low temperatures, insects develop faster and attain smaller adult size at higher temperatures (Atkinson, 1994). Data of developmental rate and adult size offers basic information for the mass rearing of insects. In this study, we experimentally elucidated the thermal requirements and adult size of *C. marginellus*. Our results offer basic information both for the use of *C. marginellus* as a pollinator for cherimoya and other *Annona* plants, and for management of it as a pest of dried fruits.

**MATERIALS AND METHODS**

**Insect stock culture.** The insects originated from feral individuals caught in the flowers of an experimental orchard of Mie University, central Japan. They were reared in plastic containers measuring 26 cm × 18 cm × 8 cm (height). Three holes of 4-cm diameter were made in the lid of each container and covered with a fine gauze, which allowed sufficient ventilation but prevented insects from escaping. Cut pineapple was periodically supplied as food, and the bottom of the container was lined with autoclave-sterilized soil for pupation. Water was sprayed as needed on the soil. The beetles were easily reared at 25°C in a 16-h light/8-h dark (16L8D) photoperiod.

**Development from egg to adult.** The procedure for the rearing of insects was similar to that used for *H. ocularis* (Tsukada et al., 2005a). The females laid their eggs into the pineapple, but because finding eggs there was extremely difficult, we used the following procedure to obtain eggs. First, 52 pairs of male and female were prepared. Each was allowed to oviposit into a cut pineapple in a 30-ml vial in a chamber kept at 18, 20, 25, or 30°C under a 16L8D photoperiod. The opening of the vial was plugged by cotton to allow moderate ventilation. The insects were then removed within a day and placed into a new vial with another piece of pineapple for the next oviposition. This procedure was repeated until sufficient larvae were obtained. Each vial with the pineapple piece was kept at the same temperature, and the pieces were examined every day. When larvae were found, they were isolated and each was gently transferred to a 10-ml vial with a ca. 5-g pineapple piece as food and a piece of wet tissue paper as a pupation site. Usually, there was no need to add another piece of pineapple, but we added one when the food appeared insufficient. The tissue paper was ca. 0.4 g in weight and contained ca. 1.2 ml of water, a suitable volume for the pupation of *C. marginellus* (unpublished data). The vials were examined every day, and pupation date and adult emergence date were recorded. The dates of oviposition and appearance of new larvae (regarded as egg hatch) were also recorded. The sex of each individual was determined after adult eclosion by observation of the tip of the abdomen under a binocular microscope.

**Adult size.** The emerged adults were individually dried, and head width (distance between anterior ends of the compound eye) was measured using a micrometer under a binocular microscope.
Statistical procedures. The parameters of thermal requirements (i.e., thermal constant [K] and thermal threshold [T₀]) were obtained from the regression of 1/D against T, where D is days required to complete a stage and T is temperature (°C). These regressions were estimated from the raw data, not from the means of each temperature. The effect of temperature on mortality at larval and pupal stages was analyzed using a log-linear model. Adult size was analyzed using a GLM two-way ANOVA followed by a Tukey-Kramer multiple comparison test. These and other statistical tests were performed with the NCSS statistical package (NCSS, 1995).

RESULTS

Among the three developmental stages examined, the larval stage required the longest time and the egg stage the shortest (Table 1). All three stages required less time at higher temperatures in both sexes. However, the developmental time (D) of the egg stage at 30°C was longer than suggested by a linear relationship between developmental rate (1/D) and temperature. Thus, data for 30°C were omitted when regression was carried out to obtain the parameters K and T₀. There was no difference in 1/D from egg to adult between the sexes at 15, 20, or 25°C (ANCOVA with temperature being the covariate, p>0.05; data at 30°C omitted).

T₀ of both sexes was about 12°C at the larval stage (Table 2), and above 13°C at the other two stages and for the total developmental period from egg to adult. K from egg to adult, obtained by summing the value of three stages, was ca. 293 and 291 degree-days (DD) for male and female, respectively.

The sex ratios of the adults were not significantly different from 0.5 at each temperature (binomial test, p>0.05). The mortality rate from egg to adult was lowest at 25°C and highest at 30°C. The among-temperature variation in mortality was attributed mostly to that during the pupal stage (Fig. 1). Temperature significantly affected mortality at the pupal stage (log-linear model, p=0.0195 for the interaction), but not at the larval stage (p>0.05).

Head width was largest at 18°C, and decreased significantly with increasing temperature in both sexes (Table 3; GLM, two-way ANOVA, p<
DISCUSSION

The developmental period of each of the three stages was always longer at lower temperatures than at higher temperatures, the same as in most other ectotherms. However, at 30°C, the length of the egg period was longer than expected from a linear regression of the other three temperatures. Furthermore, at this temperature, mortality during the pupal stage was high. These results suggest detrimental effects of high temperature on development. It is likely that more serious detrimental effects would occur above 30°C, since other Carpophilus species show that below 30°C the developmental rates increase as temperature increase, but 32.5°C or over, the rates are mostly the same (James and Vogele, 2000). Considering that C. marginellus is distributed widely in warm regions of the world (Gillogly, 1982; Kirejtshuk, 1998), tolerance to relatively high temperatures would be expected. In addition, although the free-living stages of this species would be directly exposed to air temperature, the pupal stage in the soil would be somewhat insulated (Skinner et al., 2004), and larvae would choose an appropriate depth for pupation according to the temperature (Dimou et al., 2003). Thus, the detrimental effect of high temperature on pupation under natural conditions might be smaller than that determined in this study.

The sex ratio of the emerged adults suggests that although temperatures affect the mortality rate, this effect is the same on both sexes. This result differs from that of H. ocularis, in which mortality of males tended to be high at low (15°C) and high (30°C) temperature (Tsukada et al., 2005a). The species-specific characteristics that make such difference between the two species are not known. Other than the temperature itself among the rearing conditions, difference in humidity in the vials also might have affected the result. During the pupal stage we did not control the water content of the tissue paper not to disturb the insect. Therefore, evaporation rate of water via the cotton plug would be different among the temperature conditions, i.e., it is likely that at low temperature condition the water content of pupation site was higher and at high temperature it was lower than the optimal.

It is well known that in the favorable temperature range, insects that develop at lower temperatures attain a larger size at eclosion (Atkinson, 1994). In this study, the adults were consistently larger at lower temperatures. This also suggests that the temperatures we used are in the favorable range, and C. marginellus follows the general rule on the relationship of body size and temperature. Although we did not examine the effect of body size on fecundity and longevity, it is frequently shown that larger individuals lay more eggs and live longer (Sopow and Quiring, 1998; Rodriguez et al., 1999). Therefore, rearing this insect at lower temperatures has a likely merit of larger fecundity. Besides temperature, the density of the larvae and type of food also affect development. It is worthwhile to carry out additional experiments to confirm the best conditions for mass rearing of this beetle.

The developmental biology of Carpophilus spp. has been studied by James and Vogele (2000). The thermal constant from egg to adult for three Carpophilus species ranged from 260.4 to 320.0 DD, and $T_o$ ranged from 14.6 to 15.4°C under constant temperature (James and Vogele, 2000). The thermal constant and threshold for C. marginellus obtained here were similar to those of the congeneric species. On the other hand, H. ocularis has a smaller body and requires a lower cumulative temperature (Tsukada et al., 2005a). Another nitidulid species, Librodor japonicus, with larger body size, requires ca. 800 DD until eclosion (Okada and Miyatake, 2007). Thus, larger species tend to require more day-degrees in this family.

This study revealed developmental characteristics of C. marginellus that are indispensable for mass rearing, but prerequisites for mass release must yet be investigated. Considering that this species visits Annona flowers, we assume that mass-released insects will visit the flowers readily.

<table>
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<tr>
<th>Temperature</th>
<th>$\delta$</th>
<th>$\varphi$</th>
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<tbody>
<tr>
<td>18°C</td>
<td>0.759±0.008 (44)</td>
<td>0.760±0.008 (42)</td>
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<tr>
<td>20°C</td>
<td>0.755±0.009 (35)</td>
<td>0.754±0.008 (36)</td>
</tr>
<tr>
<td>25°C</td>
<td>0.736±0.005 (46)</td>
<td>0.729±0.005 (40)</td>
</tr>
<tr>
<td>30°C</td>
<td>0.714±0.008 (27)</td>
<td>0.718±0.007 (33)</td>
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0.0024). Sex and interaction of sex and temperature did not show significant effects ($p > 0.05$).

Table 3. Head width (mm) of adult C. marginellus reared at four temperatures. Mean±SD ($n$)
Nevertheless, their behavior on the flowers should be studied to clarify their pollination ability, because not all flower visitors are effective pollinators (Waser et al., 1996). Furthermore, mass rearing can change the characteristics of insects genetically (Miyatake and Yamagishi, 1999). Therefore, important traits such as propensity to visit flowers should be monitored over a long period. Finally, harmful effects on dried foods and orchard fruits should be considered. A possible way to minimize harm to other crops and foods would be to use fine mesh on the openings of greenhouses. Detailed studies are required to evaluate the effect of such efforts before mass release is tried.

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REFERENCES


