SHORT COMMUNICATIONS

Carbon Dioxide Expiration Rate of the Onion Fly, Hylemya antiqua MEIGEN (Diptera: Anthomyiidae) with Reference to Diapause

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Both temperature and photoperiod are involved in the diapause induction of the onion fly, Hylemya antiqua MEIGEN (ISHIKAWA et al., 1987). Discrimination between non-diapausing and diapausing pupae was made by observing the emergence of flies from pupae which were kept under 25°C, 16L-8D (ISHIKAWA et al., 1987). However, a method to discriminate these two at an early pupal stage is needed to study the characteristics of “diapause” physiologically.

Recently, OSAKABE et al. (1982) showed that gas chromatography is useful for estimating the respiratory activity of scarab beetles. We attempted to apply this method to the study of diapause in the onion fly. We first observed the changes of CO₂ expiration rate during the development, and then examined the possibility of discrimination between diapausing and non-diapausing pupae by measuring CO₂ expiration rates.

MATERIALS AND METHODS

Rearing of insects. About 100 newly hatched larvae of the onion fly were reared on an artificial diet (ISHIKAWA et al., 1983) in a petri dish (9 cm in diam.) under 25°C, 16L–8D for non-diapausing growth or under 10°C, 12L–12D to obtain diapausing pupae. For the measurement of CO₂ expiration rate during the development, larvae were selected at random from the non-diapausing culture. All the new pupae were transferred to a moistened filter paper in petri dishes and kept under 25°C, 16L–8D.

Measurement of CO₂ expiration rate. CO₂ expiration rates of 2nd and 3rd instar larvae, pupae and adults were measured individually, while those of eggs (10–50 mg) and 1st instar larvae (10 or 20 larvae) were measured in a group due to the low level of CO₂ expiration from a single individual at these stages. An individual(s) (together with a small piece of diet in the case of larvae) was put in a small vial (2.5 cm in diam. × 5.0 cm), and it was sealed airtight with a sheet of Sealon® film (permeabilities of N₂, O₂ and CO₂ at 20°C are 2.1, 11.6, 26.9 × 10⁻¹¹ ml·cm/cm²·sec·cmHg, respectively; data from Fuji Film Co.) and kept in a chamber of 25±1°C, 16L–8D. Smaller vials (1.0 cm × 5.0 cm) were used for pupae since CO₂ expiration rate of a pupa was fairly low. Vials containing only a piece of diet were used as the control for larvae, while empty vials were used for the others. After 24 hr, 0.5 ml air was sampled from the vial with an airtight syringe and immediately subjected to GC analysis.

Quantification of CO₂. A gas chromatograph (GC-4B, Shimadzu) equipped with a thermal conductivity detector (TCD) and a column (3 mm I.D. × 2 m) packed with Porapak Q (60–80 mesh, Waters Chemicals) was used for quantification of CO₂ under the following conditions. Oven, injection and detection ports: 80, 90 and 90°C. Carrier gas (N₂): 20 ml/min. TCD bridge current and attenuation: 110 mA and 1/8. A calibration curve between quantity of CO₂ and peak area was made before each measurement using high purity CO₂ standard (>99.9%, Japan Oxygen Co.). The amount of CO₂ expired by an individual(s) was obtained by subtracting the amount of CO₂ in the control vial from that in the experimental vial.

RESULTS

Changes in CO₂ expiration rate and body-weight of non-diapausing individuals from egg to adult stages are shown in Fig. 1A. Respiration rates of the egg (ca. 0.04 μl/hr) and the 1st instar larvae (ca. 0.3 μl/hr) remained at a low level. Then, during the 2nd instar expiration increased sharply (up to 37 μl/hr) corresponding to a sharp increase in body-weight. Increase of variation in respiration rates among individuals was also observed. This may reflect a great difference in the growth rates among individuals. High CO₂ expiration rate (25–45
Adults showed a greater CO₂ expiration rate (ca. 2.7 μ/l/mg/hr, Fig. 1B) as compared with pupae, though the body-weight decreased somewhat.

Changes of CO₂ expiration rate during pupal stage were investigated in more detail to examine the differences between diapausing and non-diapausing pupae. As shown in Fig. 2, CO₂ expiration of diapausing pupae showed a monotonous decrease after pupation, and remained at 0.06 μ/l/mg/hr. Meanwhile, expiration of non-diapausing pupae turned to an increase after day 4–5. Though variability of CO₂ expiration rates among individuals also increased as emergence approached, CO₂ expiration rates of diapausing and non-diapausing pupae were significantly different after day 5 (Fig. 2). The minimum expiration rate among non-diapausing pupae and the maximum among diapausing ones did not overlap after day 7 (0.23 and 0.12 μ/l/mg/hr on day 7, respectively). Therefore, under the conditions of this study, it was possible to discriminate between the two kinds of pupae by measuring CO₂ expiration rates after day 7.

DISCUSSION

During the larval stage, CO₂ expiration rate/unit body-weight was highest in the 2nd instar, indicating that metabolism is most active during this period and decreases as the larvae grow. This trend of metabolism is also manifested in the rate of
increase of the body-weight. These changes in CO₂ expiration rate during development are similar to those of a scarab beetle, *Anomala osakana* (Osakabe et al., 1982), though the respiration rate is highest in the 1st instar in this species.

According to the extensive compilation by Keister and Buck (1974), respiration rate (O₂ consumption) of most insects falls within a range of 0.1–1.0 µl/mg/hr. These values were obtained using "resting" insects: deprived of food and restrained in movement. Many factors, both exogenous and endogenous, are known to affect respiration of an insect. Therefore, special care should be taken in evaluating these factors when comparing respiration rates of different species. In the present study, larvae were allowed to feed freely. This may be a cause of their high respiration rate (up to 8.0 µl/mg/hr).

The expiration rate of non-diapausing pupae showed a typical U-shaped curve in the onion fly. This U-shaped change during pupal development seems common in many species (Keister and Buck, 1974). Minimum and maximum of the U-shaped curve differ by species, however, with many showing a minimum of 0.1–0.5 µl/mg/hr and maximum of 0.8–1.7 µl/mg/hr. A similar result was obtained for the onion fly pupa: minimum and maximum of the U-shaped curve were 0.3 and 1.0 µl/mg/hr, respectively. In diapausing pupa, many species showed a respiration rate of less than 0.1 µl/mg/hr; the onion fly was no exception: its expiration rate of diapausing pupa was ca. 0.06 µl/mg/hr.

REFERENCES


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Detection of Alkaline Phosphatase Activity in Developing "Kidney" Embryo of *Bombyx mori* (Lepidoptera: Bombycidae), a Mutant Which Lacks an Endodermal Organ

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Alkaline phosphatases have been utilized as amenable markers for studying the process through which embryos undergo differentiation. The activity level of these enzymes is often shown to increase at the gastrulation stage, i.e. at the formation of intestinal tract (e.g. Whittaker, 1977). Preceding reports have indicated that alkaline phosphatase activity increased in a 2-step mode during embryonic development of *Bombyx mori* (Ito et al., 1954; Mihara et al., 1988). According to the results of histochemical and biochemical studies by Sugai (1957) and Chin (1961), these enzymes of the silkworm eggs seemingly have relevance to the differentiation of endodermal tissues and the completion of the digestive tract prior to hatching. In the present study, we tried to detect the activities of alkaline phosphatase in a mutant called "kidney-shaped egg" (kidney, *ki*), whose embryos differentiate ectodermal organs alone and thus do not produce a digestive tract, the sole endodermal organ of insects (Suzuki and Ichimaru, 1955).

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