RATES AND CYCLES OF OXYGEN CONSUMPTION DURING PUPAL DIAPAUSE IN SARCOPHAGA FLESH FLIES

D. L. DENLINGER, J. H. WILLIS, and G. FRAENKEL

Department of Entomology, University of Illinois, Urbana 61801

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Abstract—The rate of oxygen consumption in non-diapausing flesh flies during the pupal and pharate adult stages follows a U-shaped curve with a minimum of 140 to 150 mm$^3$/g. per hr at 25°C. In diapausing pupae the mean $QO_2$ falls to 10 to 20 mm$^3$/g. per hr. Continuous measurements with single diapausing pupae show that the $QO_2$ is not constant; cycles of high oxygen consumption occur with a periodicity of several days. The cycle periodicity differs with species and temperature. The time between $QO_2$ peaks in Sarcophaga crassipalpis is twice as great at 18°C as it is at 25°C. The cycles of high oxygen consumption are close together in early diapause; in mid-diapause the cycles become farther apart, and as the end of diapause is approached the cycles again become closer together. The time of diapause termination appears unrelated to the phase of the $QO_2$ cycles. Injury produces an increase in the metabolic rate, but an acceleration of diapause termination was not observed.

INTRODUCTION

Survival during the months of diapause necessitates an economical utilization of food reserves. Hence, the rate of metabolism is characteristically low. The low metabolic activity in insect diapause is described as a continuous process involving a fairly constant consumption of oxygen. Although carbon dioxide may be released in bursts occurring at intervals of several hours at 25°C, oxygen uptake in diapausing pupae of Hyalophora cecropia and Agapema galbina occurs at a constant rate (Schneiderman and Williams, 1953, 1955; Buck and Keister, 1955). Such does not appear to be the case in diapausing Sarcophaga pupae. The present investigation shows that the rate of oxygen consumption in flesh flies is not constant. Infradian cycles (cycles occurring less frequently than 1 time/day) of oxygen consumption exist during diapause.

Previous studies with Sarcophaga describe the environmental factors regulating pupal diapause (Fraenkel and Hsiao, 1968; Denlinger, 1971, 1972b). Diapause can be induced by exposure of the adults and larvae to a short daily photophase; low temperature, moisture content of the larval medium, and sex are also influential in diapause induction. Termination of Sarcophaga diapause is temperature dependent. At constant temperatures, the duration of diapause becomes shorter with an increase in temperature. However, even at a constant temperature as high as 28°C, diapause lasts about 2 months.
MATERIALS AND METHODS

Species used in the study include a laboratory strain of *Sarcophaga argyrostoma* (Robineau-Desvoidy), and wild-collected strains of *S. crassipalpis* Macquart from Champaign County, Illinois, and of *S. bullata* Parker from St. Louis County, Missouri. The history of the strains and techniques of rearing are described by Denlinger (1972b). Expressions of pupal weight include the puparium which represents 9 per cent of the total weight.

Oxygen consumption was measured directly with a Scholander respirometer (Mark Co.) using a method described by Scholander *et al.* (1952). The experimental animals were placed in 25 cm³ vessels which were not shaken during the course of the experiments. The carbon dioxide was absorbed on filter paper (Whatman No. 40) moistened with 0.3 ml of 10% potassium hydroxide. When the oxygen concentration in the vessels fell to 15%, a safe level above which respiration is not oxygen-limited in *Phormia regina* pupae (Park and Buck, 1960), the vessels were flushed with fresh air. The pattern of cyclic oxygen consumption described below was not influenced by the time of flushing. Thermobarometers consisting of vessels lacking experimental animals were run during all experiments, and as expected with this respirometer design, the manometer fluid remained at a constant level in the thermobarometers. The vessels were maintained in a constant temperature water-bath at 18 or 25°C within a walk-in temperature cabinet which provided a daily 12 hr photophase.

The vessels containing animals were equilibrated for 1 hr before beginning manometric measurements. When several individuals were placed in the same vessel, measurements were made on an hourly basis for a period of 4 to 6 hr/day. Single individuals were kept in the respirometer continuously (except during flushing) and manometric measurements were made at 24 hr intervals.

Puncture wounds for study of injury metabolism were inflicted with a No. 00 insect pin (dia. 0.27 mm) (Adams Co.). A small portion of the dorsal posterior region of the puparium was removed, the puncture was made through the underlying pupal cuticle, and the wound was sealed with paraffin. The intensity of injury was varied by altering depth of pin insertion and number of punctures. A portion of the puparium was also removed in the non-injured controls.

The appearance of various developmental landmarks are used to define stages of adult differentiation. The first visible signs of adult development are the imaginal antennae which appear as white spots on the ventrolateral surface of the head (Fraenkel and Hsiao, 1968). They subsequently move closer together and become elongated. The appearance of red eye coloration and bristle formation serves as points of reference for later periods of development.

RESULTS

**Comparison of diapause and non-diapause metabolism**

Oxygen consumption measurements on single individuals of *S. argyrostoma*, which had been reared under conditions where no diapause is encountered, were initiated when active third instar larvae began contracting to form the white
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The U-curve of metabolic activity for a single individual through the pupal stage and during development of the pharate adult at 25°C is recorded in Fig. 1(A). Other individuals of similar weight showed nearly identical metabolic patterns with the minima in a range of 140 to 150 mm³/g per hr. Each measurement in Fig. 1 is based on the weight of the experimental animal on that day. A rapid weight loss of about 10 per cent during the first 2 days is followed by a gradual weight loss of an additional 10 per cent by the day of adult eclosion.

The first signs of adult development, the antennal spots, can be seen by the fourth day following puparium formation in flies which develop without a diapause. The O₂ consumption continues to fall and reaches the lowest level on the fifth day. As adult development proceeds past the initiation of eye coloration, oxygen consumption increases very rapidly.

In contrast to the situation described above, adult development is not initiated in diapausing pupae. By the fourth day following puparium formation the O₂ consumption of diapause-committed pupae (Fig. 1B) has fallen far below the minimum of the U-curve. Data collected from groups of 10 pupae show a decline in the QO₂ to a mean level of 10 to 20 mm³/g per hr, i.e. 10 per cent of the nadir in non-diapausing individuals.

Fig. 1. Comparison of the oxygen consumption rate in a non-diapause individual from the time of puparium formation to adult eclosion with the rate in a group of 10 diapausing pupae of S. argyrostoma at 25°C.
Infradian cycles of $O_2$ consumption during diapause

If the oxygen consumption is monitored for individual diapausing pupae instead of for groups of pupae as was done in Fig. 1(B), a cyclic pattern of oxygen uptake is observed. $QO_2$ recordings for five different $S. argyrostoma$ pupae are shown in Fig. 2. Each point represents the $QO_2$ for a 24 hr time span. The cyclic peaks occur with a periodicity of 3 to 4 days. On days of high oxygen uptake the rate is 40 to 60 $\text{mm}^3/\text{g per hr}$. In the trough of the cycle an entire day can elapse without any perceptible $O_2$ consumption having occurred. The mean $QO_2$ of a single individual over a several day period is comparable to the rate observed by measuring groups of pupae together in the same vessel.

![Graph showing infradian cycles of oxygen consumption in individual diapausing pupae of $S. argyrostoma$ at 25°C.](image)

**Fig. 2.** Infradian cycles of oxygen consumption in individual diapausing pupae of $S. argyrostoma$ at 25°C.

When individuals of a second species, $S. bullata$, were measured, a similar pattern was evident. Infradian cycles observed on four different pupae are seen in Fig. 3.

A third species, $S. crassipalpis$, also showed regularly occurring peaks of oxygen uptake at 25°C (Fig. 4). Lowering the temperature increased the length of the period. In Fig. 5, the $QO_2$ of 2 $S. crassipalpis$ pupae is recorded throughout the duration of diapause at 18°C.
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Fig. 3. Infradian cycles of oxygen consumption in individual diapusing pupae of S. bullata at 25°C.

Fig. 4. Infradian cycles of oxygen consumption in individual diapausing pupae of S. crassipalpis at 25°C.
Table 1 summarizes the quantitative data collected. Analysis of the data on cycle periodicity with Student’s t-test has shown species and temperature differences to be significant (p < 0.01). In *S. crassipalpis* a 7°C drop in temperature caused the period length to almost double. Correspondingly, the mean $QO_2$ is almost halved by such a temperature decrease. The mean rate of oxygen consumption was calculated by dividing the total oxygen/g consumed by the total number of hours in the experimental period.

**Table 1—Mean rate of oxygen consumption and cycle periodicity during pupal diapause in species of *Sarcophaga***

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp. (°C)</th>
<th>No. of pupae</th>
<th>No. of cycles observed</th>
<th>Cycle periodicity mean days (range)</th>
<th>Oxygen consumption (mean mm³/g per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. argyrostoma</em></td>
<td>25</td>
<td>9</td>
<td>26</td>
<td>3.7 (3-5)</td>
<td>18.7</td>
</tr>
<tr>
<td><em>S. bullata</em></td>
<td>25</td>
<td>9</td>
<td>26</td>
<td>7.5 (5-10)</td>
<td>16.1</td>
</tr>
<tr>
<td><em>S. crassipalpis</em></td>
<td>25</td>
<td>9</td>
<td>40</td>
<td>5.1 (4-6)</td>
<td>18.4</td>
</tr>
<tr>
<td><em>S. crassipalpis</em></td>
<td>18</td>
<td>7</td>
<td>70</td>
<td>9.8 (6-14)</td>
<td>8.5</td>
</tr>
</tbody>
</table>

**Changes in cycle periodicity during diapause**

Measurement of oxygen consumption throughout the course of diapause in *S. crassipalpis* at 18°C (Fig. 5) revealed that the periodicity of the $QO_2$ cycles is not constant throughout diapause. At the beginning of diapause, the peaks are close together. They gradually become farther apart, and as the breaking of diapause is approached, the cycles again become shorter. The same general pattern of changing cycle duration occurred in all seven of the pupae examined at 18°C. The change in period length throughout diapause is plotted in Fig. 6 for four representative individuals.

**Termination of diapause**

The $QO_2$ at the termination of diapause was observed for numerous animals including the individuals recorded in Figs. 3(A) and (B), Figs. 4(D), and Figs. 5(A) and (B). The increase of $QO_2$ associated with the break of diapause is not closely correlated with a particular phase of the metabolic cycle. In some cases the increase in $QO_2$ proceeds directly from the top of a metabolic peak (Fig. 3A; Figs. 5A, B), and in other examples the change in $QO_2$ characteristic of diapause termination does not occur until after a metabolic peak is past (Fig. 3A; Fig. 4D).

The $QO_2$ increases rapidly to a level corresponding to the minimum of the U-curve in non-diapause animals. Adult antennal disks, the first morphological signs of diapause termination, are apparent 3 to 4 days after the first detectable increase in oxygen consumption at 25°C; at 18°C, the antennal spots can be seen 4 to 5 days after the change in $QO_2$. As the antennal disks migrate toward each other and elongate the $QO_2$ remains at a fairly constant level. As development
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S. crassipalpis in diapause – 18°C

TIME (days)

O₂ CONSUMPTION (mm³/g/hr)

Fig. 5. Infradian cycles of oxygen consumption throughout diapause for two individual pupae of S. crassipalpis at 18°C.

Fig. 6. Change in periodicity of oxygen consumption cycles throughout the course of diapause in four individuals of S. crassipalpis at 18°C.
progresses beyond this stage, the $QO_2$ again increases rapidly to the same values observed for comparable stages in uninterrupted imaginal development (Fig. 1A).

Injury metabolism

Diapausing *S. argyrostoma* pupae were injured 10 days after the formation of the puparium at 25°C. Three intensities of injury were performed: A, one puncture to a depth of 2 mm; B, one 4 mm puncture; C, two 4 mm punctures. Groups of 8 to 10 pupae receiving the same injury were placed in the same vessel and measurement of the oxygen consumption was made daily over a period of 4 hr. Group measurements partially masked individual cycles. Mean oxygen uptake for the groups of pupae over a period of 40 days is recorded in Table 2. Within the first 10 days after injury an increase in $QO_2$ is correlated well with intensity of injury. This difference is later obliterated and the mean daily $QO_2$ over all 40 days of observation is similar for all injured pupae. The mean $QO_2$ of injured pupae is > 10 mm$^3$/g per hr higher than the $QO_2$ of non-injured pupae.

Pupae injured with more than two punctures in the posterior abdominal region did not survive. Four of the 10 pupae in group C died before termination of diapause, but all other individuals eventually initiated adult development and all but one emerged successfully. No differences were observed in the time required to break diapause in the non-injured and injured pupae.

**DISCUSSION**

Flies reared under conditions simulating the long days and warm weather of early summer metamorphose from a larva to an adult through a pupal stage which is uninterrupted by diapause. Metabolic activity during this period follows a U-shaped curve characteristic of the endopterygotes. A minimum of 140 to 150 mm$^3$ $O_2$/g per hr in the U-curve for *S. argyrostoma* is quite comparable to that observed by TAYLOR (1927) in *S. sarracenioides* at 25°C.

 Interruption of development with an overwintering diapause is correlated with a fall in metabolic activity far below the minimum of the U-curve to a level
of 10 to 20 mm³/g per hr at 25°C. The oxygen consumption rate during diapause is only one-tenth of the rate observed in the comparable morphological stage of non-diapause pupae. The low cost of maintenance during diapause makes possible the low metabolic rate. Even less of the available energy stored is used at lower temperatures. At 25°C, diapausing pupae of *S. crassipalpis* are maintained at a rate of 184 mm³/g per hr, whereas at 18°C, the cost of maintenance falls to 8.5 mm³/g per hr. Continuation of this trend implies extremely low metabolic activity in pupae overwintering out-of-doors.

The low rate of oxygen consumption in *Sarcophaga* at 25°C is nearly identical to the rate in diapausing Lepidoptera pupae at the same temperature. A rate of 16 mm³/g per hr has been recorded in *Hyalophora cecropia* (SCHNEIDERMAN and WILLIAMS, 1953), 14 mm³/g per hr in *Antheraea pernyii* (WAKU, 1957), and 20 mm³/g per hr in *Agapema galbina* (Buck and Keister, 1955). The similarity of metabolic activity during pupal diapause in *Sarcophaga* and various Lepidoptera is of special interest considering the fact that a Cecropia pupa weighs 40 to 60 times as much as a *Sarcophaga* pupa. The general trend of an increase in metabolic rate with a decrease in body size found in the homeotherms has also been observed with insects and other poikilotherms (ZETHEN, 1953, 1955; PROSSER, 1962). A comparison of the metabolic rates during pharate adult development in the two insect groups [e.g. 300 mm³/g per hr in *H. cecropia* (SCHNEIDERMAN and WILLIAMS, 1953) and >800 mm³/g per hr in *S. argyrostroma* at adult eclosion] does show the trend of increased metabolism with decreased body size. However, such a relationship apparently does not exist between diapausing pupae of *Sarcophaga* and Lepidoptera.

Although mean rates of diapause oxygen consumption are similar for the two taxa, the analogy breaks down upon examination of the oxygen consumption patterns of individual pupae. The constant rate of oxygen consumption observed in diapausing Lepidoptera pupae (SCHNEIDERMAN and WILLIAMS, 1953, 1955; BUCK and KEISTER, 1955) is not observed in *Sarcophaga*. Instead, infradian cycles of oxygen consumption with a periodicity of several days occur throughout diapause. Different species have different cycle periodicities, and examination of *S. crassipalpis* has shown the mean time between peaks of oxygen consumption to be twice as great at 18°C as it is at 25°C. At 18°C, the cycles are farther apart in mid-diapause than they are during early or late diapause.

The cycles of oxygen consumption appear to be unrelated to the carbon dioxide bursts observed in the diapausing Lepidoptera. At 25°C, the carbon dioxide bursts occur with a mean frequency of 7.4 hr in *H. cecropia* (SCHNEIDERMAN and WILLIAMS, 1955) and 3 to 5 hr in *A. galbina* (BUCK and KEISTER, 1955). The duration of carbon dioxide bursts is short, 10 to 14 min in *A. galbina*. By contrast the peaks of oxygen consumption observed in *Sarcophaga* are several days apart and the duration of the peak may extend over several days. Preliminary observations on the evolution of carbon dioxide recorded in vessels devoid of potassium hydroxide has shown an increase in carbon dioxide production in phase with the expected periods of high oxygen consumption, thus indicating a
general respiratory increase during the peaks. Since readings were made only once daily, carbon dioxide bursts of the nature observed in the Lepidoptera could not have been observed. That the peaks of oxygen consumption in *Sarcophaga* could represent storage of oxygen within the insect in a fashion analogous to carbon dioxide storage is deemed unlikely; such a method of oxygen storage is unknown.

Insects which are not in diapause commonly show circadian rhythms of oxygen consumption which are correlated with the insect's rhythm of activity. Such phenomena have been observed in the grasshopper *Romalea microptera* (FINGERMAN et al., 1958) and in the cockroaches *Blattella germanica* (BECK, 1964) and *Periplaneta americana* (RICHARDS and HALBERG, 1964). An ultradian rhythm with two daily peaks of oxygen consumption in the fiddler crab *Uca* has been correlated with the rhythm of the lunar tide (BROWN, 1959). A photoperiodically entrained metabolic rhythm with a periodicity of 8 hr occurs in the European corn borer, *Ostrinia nubilalis* (BECK, 1964). However, in pupal diapause metabolic rhythms are not associated with locomotor activity, and the increase of period length with a decrease in temperature and the change in cycle length during diapause in *Sarcophaga* denies the existence of a relationship correlated with a regularly occurring environmental 'zeitgeber'.

The metabolic rhythms in *Sarcophaga* may reflect the pattern of a particular method of energy utilization during pupal diapause. Metabolic end products such as lactic acid or pyruvate may not be oxidized continuously, but instead they may accumulate during the trough of the cycle until a critical amount has been stored. The peaks of oxygen uptake could also be associated with the periodic production of high energy compounds, the supply of which may be utilized as the pupa progresses toward the next peak. An analysis of the concentrations of certain key metabolites in relation to the phase of the cycle should resolve the validity of the proposed theory.

The regular sequential change in cycle periodicity throughout diapause exemplifies the dynamics of the diapause state. The absence of morphological development does not imply physiological homogeneity, a fact long recognized in the literature by ANDREWARTHA's (1952) term 'diapause development'. The meaning behind the increase in cycle periodicity in mid-diapause is still obscure. The pattern is somewhat reminiscent of the arousal cycles observed in hibernating mammals like the ground squirrel *Citellus lateralis* (PENGELLEY and FISHER, 1961; TWENTE and TWENTE, 1967). Mammalian hibernation is not a prolonged period of constant torpor. Rather, periods of quiescence alternate with periods of arousal. The periods of arousal are more frequent in early and late hibernation than during mid-hibernation. As in *Sarcophaga* diapause, the duration of uninterrupted torpor in mammalian hibernation is longer at low temperatures (PENGELLEY and FISHER, 1961; HOFFMAN, 1964). The trigger which starts the mammalian arousals remains unknown (PENGELLEY and FISHER, 1961; STRUMWASSER et al., 1967).

If the normal course of *Sarcophaga* diapause is interrupted by an injury, the organism will respond with an increase in the metabolic rate. The capacity to
increase metabolism above the diapause level presumably for purposes of wound healing increases the cost of maintenance, but the observed successful emergence of adult flies from puparia in which the pupae had been wounded during diapause implies that there are sufficient food reserves to maintain the pupae at twice the normal metabolic rate and still to complete development.

Following injury in diapause Saturniidae pupae, metabolism increases immediately to a peak and then gradually returns to the diapause level (Harvey and Williams, 1961). In Sarcophaga the \( QO_2 \) makes a less marked initial rise, but within the 40 days of the experiment there is no return to the metabolic rate of the non-injured pupae. The mean \( QO_2 \) for groups of injured pupae does not approach the \( QO_2 \) observed for non-diapause pupae in the same morphological stage. An acceleration in the breaking of diapause was not apparent. Even the most severe injury in \( H. cecropia \) does not terminate diapause (Schneiderman and Williams, 1953; Harvey and Williams, 1961); however, pupal diapause in Antheraea polyphemus (McDaniel and Berry, 1967) has been terminated by injury. The effect of injury at various stages during diapause and on \( QO_2 \) cycles remains to be investigated.

The injury metabolism further demonstrates that diapause is not a static state. The organism can respond to an emergency situation requiring wound healing. Despite an excess metabolic expenditure following injury, the pupae can be maintained at a sufficiently economic level to permit survival. The margin of potentially useable energy which can be tapped for purposes of body repair under laboratory conditions may play an important rôle under natural conditions.

In field experiments conducted in Illinois, 20 per cent of \( S. bullata \) pupae entered diapause during August (Denlinger, 1972a). High temperatures during this time of the year dictate a high diapause \( QO_2 \) and thus a greater total energy expenditure throughout diapause than would be required of pupae entering diapause in September or October.

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