

## ABSENCE OF METABOLIC COLD ADAPTATION AND COMPENSATORY ACCLIMATION IN THE ANTARCTIC FLY, *BELGICA ANTARCTICA*

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**Abstract**—The respiratory metabolism in larvae of the Antarctic fly, *Belgica antarctica* Jacobs (Diptera: Chironomidae) was investigated at Palmer Station, Anvers Island (64°46'S, 64°03'W). Oxygen consumption was linearly related to temperature from 0 to 20°C, respectively, 49 and 338 nl/mg live wt/hr. Maintenance at 0 and 10°C for 8 days had no differential effect on the metabolic rate, suggesting that larvae lack the ability for compensatory acclimation. A comparison of standard metabolism for polar and temperate chironomids revealed no elevation of metabolic rate in polar forms. However, polar species exhibited lower activation energies than temperate forms indicating that the respiratory metabolism of polar chironomids is relatively temperature independent.

**Key Word Index:** Metabolic cold adaptation, compensatory acclimation, Antarctic fly, *Belgica antarctica*, respiration.

### INTRODUCTION

IN RECENT years investigations of the environmental physiology of the Antarctic terrestrial arthropods have revealed a diversity of adaptations for low temperature survival (BAUST and EDWARDS, 1979; BLOCK, 1980; LEE and BAUST, 1981; SOMME, 1978). The wingless chironomid, *Belgica antarctica* Jacobs, is the southernmost free-living holometabolous insect. Larval aggregations are found in a variety of microhabitats associated with the terrestrial alga, *Prasiola crista*, and organic detritus originating from penguin rookeries and elephant seal wallows (STRONG, 1967; PECKMAN, 1971).

Previous investigations of the respiratory physiology of Antarctic terrestrial arthropods have focused on collembola and mites (TILBROOK and BLOCK, 1972; BLOCK, 1977; YOUNG and BLOCK, 1980a). Metabolic cold adaptation refers to an elevation of the basal or standard metabolic rate in polar ectotherms (CLARKE, 1980). Although initially this concept arose from metabolic studies of polar marine ectotherms, it has recently been extended to Antarctic terrestrial mites (BLOCK and YOUNG, 1978; YOUNG, 1979a).

Data on the respiratory metabolism of *B. antarctica* are lacking, therefore, the purpose of this study was to investigate aspects of oxygen consumption in this unique species. The results are analysed with respect to the possible roles that compensatory acclimation of respiration rate and metabolic cold adaptation play in the respiratory physiology of this species.

### MATERIALS AND METHODS

Larvae were collected from Torgersen Island adjacent to Palmer Station, Antarctica (64°46'S, 64°03'W) during February and March, 1980 and January, 1981. Specimens were extracted from substrate composed of the green alga, *Prasiola crista*, and detritus from an

Adelie penguin rookery. Larvae were maintained for 12 hr at the acclimation temperature to insure gut clearance prior to respirometry. This precaution allows for a better measure of the basal or standard metabolism in insects (KEISTER and BUCK, 1974).

Constant-pressure microrespirometers similar to those described by ENGELMANN (1963) and CONRAD-LARSEN (1974) were used to measure oxygen consumption of fourth-instar larvae. Respirometers were made by sealing a 10 or 20  $\mu$ l micropipette into the tip of a 1 ml plastic syringe. Larvae were placed into a 1 cm section of a plastic straw that was half filled with moistened cotton and loaded into the barrel of the syringe. The plunger was weighted, inserted into the syringe barrel and set upright in a water bath ( $\pm 0.02^\circ\text{C}$ ) with the micropipette extending above the surface. A 10% KOH solution was introduced into the micropipette serving as both an absorbant for carbon dioxide and a manometric indicating fluid. For each determination respirometers without larvae served as thermobarometers. One hour was allowed for equilibration with respiration measured for the next two hours. Oxygen uptake was constant during this period.

Each respirometer contained 12 fourth-instar larvae whose individual weight ranged between 0.8 and 1.2 mg. Larval water content is  $72 \pm 2\%$  of live weight. Each rate ( $\bar{x} \pm \text{SEM}$ ) is based on 8 respirometers per treatment.

### RESULTS

#### *Metabolic rate and temperature*

The standard metabolic rate of field-collected fourth-instar larvae was directly related to the experimental temperature (Fig. 1). Rates varied from 49 to 338 nl/mg live wt/hr at 0 and 20°C. This linear relationship of temperature and metabolic rate has been

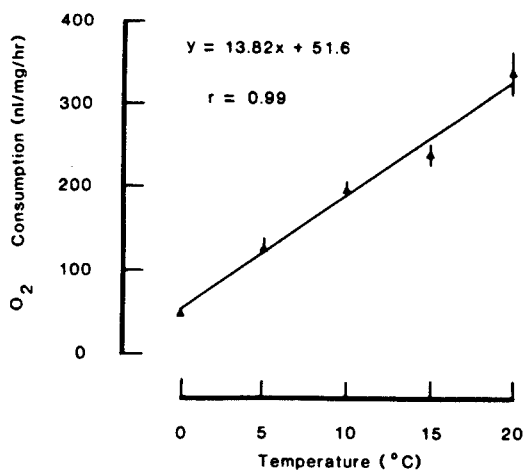


Fig. 1. Effect of temperature on respiratory rate of *Belgica antarctica* larvae. ( $\bar{x} \pm \text{SEM}$ .)

described for a wide variety of insects (KEISTER and BUCK, 1974).

#### Compensatory acclimation of metabolic rate

A commonly used procedure for assessing the capacity of an organism for compensatory acclimation is to maintain animals at differing temperatures for a period of time and then measure and compare metabolic rates (PRECHT *et al.*, 1973). Larvae were maintained for 8 days at 0 and 10°C on their natural substrate. For each laboratory acclimation group respiration rates were similar at all experimental temperatures (Fig. 2). This experiment was repeated in January, 1981 with the same results, no compensatory changes in metabolic rate. This lack of acclimatory response has been classed as Type 4 (no acclimation) by PRECHT (1958).

#### Metabolic cold adaptation

In order to test the hypothesis for metabolic cold adaptation for *B. antarctica*, previous studies of larval respiratory metabolism within the family Chironomidae were examined. Only studies providing data on

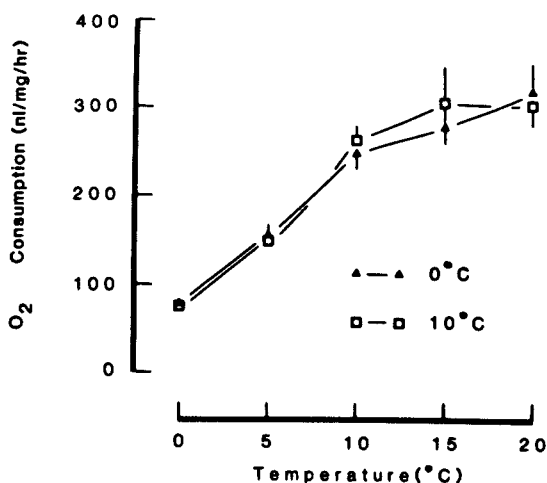


Fig. 2. Effect of temperature on the respiratory rate of *Belgica* larvae lab acclimated to 0 and 10°C. ( $\bar{x} \pm \text{SEM}$ .)

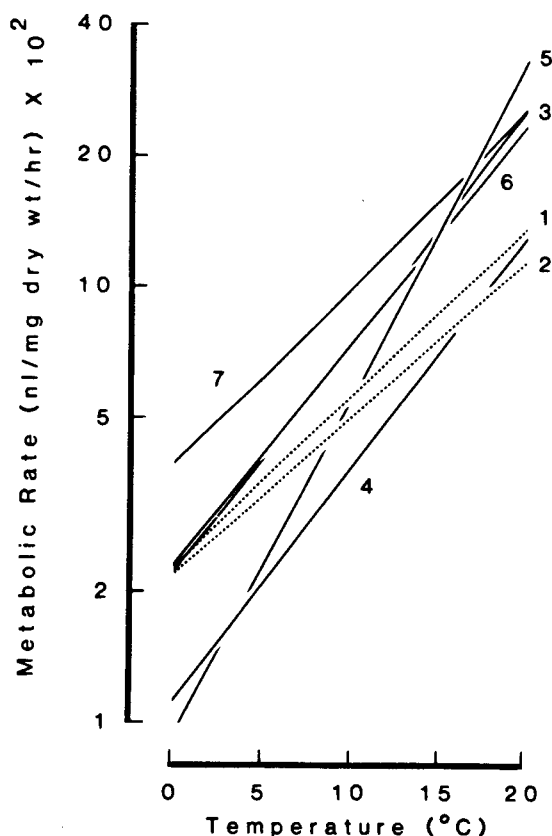


Fig. 3. Linear regression of  $\log_{10}$  respiratory rate on temperature for two polar species, *Belgica antarctica* and *Chironomus* (---), and 5 species of temperate chironomids (—). Regression lines for each species are numbered as in Table 1.

standard metabolic rates for larval chironomids tested at naturally experienced environmental temperatures were included. When possible, rates for larvae of a comparable size to *B. antarctica* were selected. In order to facilitate interspecific comparisons all metabolic rates are expressed in nl/mg dry wt/hr.

The respiratory metabolism of 2 polar and 5 temperate species is summarized in Fig. 3. An examination of this figure reveals that the respiratory metabolism of the two polar species, *B. antarctica* and *Chironomus* spp. (SCHOLANDER *et al.*, 1953) occupy an intermediate position relative to those of temperate forms. Since the metabolic rates of the temperate species numbered 3, 6 and 7 lie above the lines for the Arctic and Antarctic species, it suggests that metabolic cold adaptation is lacking in these polar species.

For comparative studies of metabolic rate the Arrhenius activation energy ( $E_a$ ) provides a convenient measure of the temperature dependence of metabolic processes which is independent of the particular temperature range investigated. The Arrhenius equation may be presented as follows (CALOW, 1977):

$$\log_{10} C_2 - \log_{10} C_1 = \frac{-E_a}{2.3 R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right).$$

In this equation  $C_1$  and  $C_2$  are rates obtained at temperatures  $T_1$  and  $T_2$  in degrees Kelvin,  $E_a$  equals the activation energy Kcal/mole, the factor 2.3 converts in

Table 1. Linear regression equations and activation energies ( $E_a$ ) for the polar species, *Belgica antarctica* and *Chironomus*, and 5 species of temperate chironomids

ID No.	Taxon	Linear regression equation*	Activation energy (kcal/mole)	Reference
Polar:				
1	<i>Belgica antarctica</i>	$y = -3.11x + 13.76$	14.3	Present study
2	<i>Chironomus</i>	$y = -2.97x + 13.21$	13.7	SCHOLANDER <i>et al.</i> (1953)†
Temperate:				
3	<i>Glyptotendipes</i>	$y = -4.25x + 17.90$	19.6	McFARLANE and McLUSKY (1972)‡
4	<i>Polypedilum</i>	$y = -3.84x + 16.15$	17.7	McFARLANE and McLUSKY (1972)‡
5	<i>Stictochironomus</i>	$y = -6.25x + 24.82$	28.8	McFARLANE and McLUSKY (1972)‡
6	<i>Chironomus</i>	$y = -3.92x + 16.71$	18.0	McFARLANE and McLUSKY (1972)‡
7	<i>Chironomus riparius</i>	$y = -3.39x + 14.96$	15.6	EDWARDS (1958)§

\*  $y = \log_{10}$  metabolic rate (nl/mg dry weight/hr),  $x = 1/T(^{\circ}\text{K}) \times 10^3$ .

† Calculations based on Fig. 11 assuming dry weight of larvae is 25% of live weight.

‡ Calculations based on Table 3.

§ Calculations based on Fig. 2 for larvae of 1 mg dry weight at 10 and 20 C.

to  $\log_{10}$  and  $R$  equals the gas constant (1.96 cal/mole). From the slope of the linear regression lines for an Arrhenius plot the activation energy may be calculated ( $E_a = -4.6 \times \text{slope}$ ).

Linear regression analysis of an Arrhenius plot of the data in Fig. 1 yielded a correlation coefficient of  $r = -0.96$ . Linear regression equations and corresponding activation energies for the species of Fig. 3 are summarized in Table 1. The lowest activation energies were found in the two polar species. Activation energies of temperate species ranged from 1.1 to 2.1 times higher than polar forms.

## DISCUSSION

### Compensatory acclimation of metabolic rate

Compensatory acclimation specifically refers to metabolic rate changes which occur after long-term exposure, usually of the order of days or weeks, to altered environmental conditions (PROSSER, 1975). For example, for an organism which was transferred from a lower temperature ( $T_1$ ) allowed to acclimate to a second higher temperature ( $T_2$ ), complete compensatory acclimation would occur if the initial metabolic rate at  $T_1$  were equal to the rate at  $T_2$  after acclimation. Seasonal patterns of compensatory acclimation have been reported for a wide variety of ectotherms (see reviews of BULLOCK, 1955; HAZEL and PROSSER, 1974).

To date, all data indicate that the capacity for compensatory acclimation of respiration rate is lacking in Antarctic terrestrial arthropods. YOUNG (1979b) found no evidence for compensatory acclimation of respiration rate in the cryptostigmatid mite, *Alaskozetes antarcticus*. For males, females, and engorged nymphs of the Antarctic tick, *Ixodes uriae* laboratory acclimation to 0 and 10°C revealed no compensatory changes in oxygen consumption (LEE and BAUST, 1982). Likewise, similar results were obtained in this study for *B. antarctica* (Fig. 2).

From studies of compensatory acclimation in a variety of metabolic processes a general picture is emerging. Animals from constant environments generally lack compensatory capacity while those from variable habitats may have considerable acclimatory potential

(SOMERO *et al.*, 1968; HAZEL and PROSSER, 1974; PROSSER, 1975 and FEDER, 1978). The microhabitat of *B. antarctica* is surprisingly thermal constant as temperatures remain between 0 and -2 C for over 300 days of the year (BAUST, 1980; BAUST and LEE, 1981). Microhabitat temperatures range from +11.2 C in February to 0.8 C by the end of March (LEE and BAUST, 1981). This period, not only represents a transition from austral mid-summer to autumn, but closely approximates the maximal yearly amplitude of temperature variation within the larval microhabitat. Thus, the observed lack of compensatory acclimation in *B. antarctica* is consistent with the thermal constancy of its microhabitat and suggests a lack of seasonal change in respiratory metabolism. The evolutionary history of this species may also play a role. With the exception of a single report (EDWARDS, 1958), larval chironomids from temperate regions lack seasonal acclimation of respiratory metabolism (McFARLANE and McLUSKY, 1972).

### Metabolic cold adaptation

Historically the concept of metabolic cold adaptation has been a controversial issue among comparative physiologists. An early report by SCHOLANDER *et al.* (1953) provides evidence in support of metabolic cold adaptation in aquatic ectotherms including fish, molluscs and crustaceans from the Arctic. This hypothesis has received additional support from a number of studies cited in BLOCK and YOUNG (1978). However, HOLETON (1974) has suggested that the previous reports of elevated metabolism in polar fish are an artifact of the experimental technique, and are not an accurate measure of basal or standard metabolism. Recent reviews of metabolic cold adaptation in fish (EVERSON, 1977) and marine invertebrates (CLARKE, 1980) have concluded that polar ectotherms are characterized by slow growth rates, reduced annual reproductive output, a low standard metabolism and a lack of metabolic cold adaptation.

Recently, the hypothesis of metabolic cold adaptation has been extended to include Antarctic terrestrial mites (BLOCK and YOUNG, 1978). The respiratory metabolism of Antarctic cryptostigmatid and mesostigmatid mites is elevated 2-4 times over that of temperate species. In contrast, SCHOLANDER *et al.* (1953)

did not find elevated metabolic rates in several terrestrial invertebrates in the Arctic. Nor, was the respiratory metabolism of the Antarctic tick, *Ixodes uriae*, (LEE and BAUST, 1982) or of *B. antarctica* in the present study conspicuously elevated when compared with temperate species (Fig. 3).

Obviously, polar ectotherms must have evolved mechanisms which allow normal physiological processes to occur at low temperature. At the biochemical level this likely requires a reduction in the free energy of activation ( $\Delta G^{2+}$ ) which serves as an 'energy barrier' controlling the rate of enzymatic reactions (HOCHACHKA and SOMERO, 1973; HAZEL and PROSSER, 1974). The magnitude of  $\Delta G^{2+}$  is a function of the relative contribution of the enthalpy ( $\Delta H$ ) and the entropy ( $\Delta S$ ). One measure of  $\Delta H$  is the Arrhenius activation energy ( $E_a$ ). For a number of enzymes the  $E_a$  is directly correlated with the environmental temperature to which an organism is adapted (LOW *et al.*, 1973; JOHNSTON and GOLDSPIK, 1975; JOHNSTON and WALESBY, 1977).

The Arrhenius activation energy ( $E_a$ ) may also be calculated for processes at the whole animal level (i.e. respiratory metabolism). At this level the  $E_a$  is a dimensionless number derived from the slope of the Arrhenius equation which reflects the relative temperature dependence of the process.  $E_a$  is particularly useful because it is independent of the temperature range over which it is initially measured and, thus, is useful for interspecific comparisons. However, the extension of  $E_a$  to multiple chain enzyme systems has been criticized because it reflects only the enthalpy factor of  $\Delta G^{2+}$  (LOW *et al.*, 1973; KEISTER and BUCK, 1974).

Recently, YOUNG (1979a) has suggested that the drawbacks of the application of  $E_a$  to whole animal systems may have been overemphasized.  $E_a$ 's derived from the respiration rates of 4 polar mites fell in the lower end of the range when compared with 23 temperate species (YOUNG, 1979a).

An examination of Table 1 reveals that the two polar species had the lowest  $E_a$ 's when compared with temperate forms. The low  $E_a$ 's of cold-adapted chironomids indicate relative temperature independence of respiratory metabolism which in itself may be thought of as a form of metabolic adaptation to low temperature. The data of MCFARLANE and MCLUSKY from Loch Leven (1972) tentatively suggest that  $E_a$ 's may be correlated with natural habitat temperatures for temperate species. They describe *Chironomus* as a deep water species which had an  $E_a$  of 18.0, while the shallow water *Stictochironomus* had the highest  $E_a$  (28.8). Additional comparative studies which seasonally monitor habitat temperatures and respiration rates are needed in order to test the hypothesis that  $E_a$ 's reflect an ectotherm's thermal environment.

Antarctic terrestrial arthropods display species specific patterns of respiratory response. Some exhibit an elevated basal metabolism, others do not. This interspecific diversity is also reflected in the parameters associated with cold-hardiness (YOUNG and BLOCK, 1980b; LEE and BAUST, 1981). A given species may, or may not, be freezing tolerant, produce cryoprotectants or supercool extensively. Further, these factors may vary seasonally. Research is needed on a species by species basis to integrate the observed pat-

terns of respiratory response with the overall physiology, particularly as it relates to low temperature adaptation.

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