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Ecological correlates of protein polymorphisms in a desert and non-desert population of Belonia saturata (Odonata: Anisoptera)

Melissa Jean Hamilton

ECOLOGICAL CORRELATES OF PROTEIN POLYMORPHISMS IN A DESERT AND NON-DESERT POPULATION OF BELONIA SATURATA (ODONATA: ANISOPTERA)

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

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Approved by:

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Dr. David Polcyn, Chair

2-28-96

Date

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Dr. Lisa Shamansky

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Dr. Stuart Sumida

ABSTRACT

Two populations of the Big Red Skimmer Dragonfly, *Belonia saturata* (Odonata: Anisoptera), were studied to determine the thoracic temperatures (T_{th}) maintained during flight, the presence of polymorphic enzyme systems, and to determine the differences in activity of glycerol-3-phosphate dehydrogenase between a desert and non-desert population.

Thoracic temperatures were measured in 57 desert and 26 non-desert individuals. Individuals in the non-desert population maintained a significantly higher T_{th} than the desert population. This may be a result of small sample sizes and the dates that dragonflies were collected from each population, since a previous study on the desert population found a much higher T_{th} over several seasons.

Starch gel electrophoresis was used to determine the presence of polymorphisms in five enzyme systems in the desert population. The level of heterozygosity in desert individuals was 15% for malic enzyme (ME), 8 % for glutamate oxaloacetate transferase (GOT), 8% for superoxidase dismutase (SOD), 31% for glycerol-3- phosphate dehydrogenase (G3PD) and 38% for glucose-6-phosphate dehydrogenase (G6PD). Polymorphisms were present in only three enzyme systems in the non-desert population. The level of heterozygosity in non-desert individuals was 62% for phosphoglucose mutase (PGM), 8% for superoxidase dismutase (SOD) and 38% for glucose-6-phosphate dehydrogenase (G6PD). Phenylalanine proline peptidase (PAP), leucyl glycyl glycine

peptidase (LGG), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) were monomorphic in both populations.

The activity of G3PD was assayed at seven temperatures (30°, 35°, 40°, 45°, 50°, 55° and 60°C) in flight muscle for both populations of *B. saturata*. Enzyme activity was monitored for 30 minutes at 5 minute intervals. The desert population maintained a relatively higher initial activity of G3PD at 40°, 45°, 50°, 55° and 60°C. The activity of G3PD at 40° - 50° is important because that temperature range is well within the range of thoracic temperatures measured for *B. saturata*.

Although body temperatures determined in this study differed between populations, individuals in the desert environment probably experience more extreme temperatures than do conspecifics in the non-desert environment. Polymorphism of the G3PD locus and higher relative activity of G3PD at 40° - 60° in the desert population of *B. saturata* may contribute to increased activity at higher, more extreme ambient air temperatures. These data support the hypothesis that selection favors heterozygosity at the G3PD locus for the population of *B. saturata* in a thermally extreme environment.

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INTRODUCTION

Insects

Terrestrial organisms face a wide range of daily ambient temperatures and intensity of solar radiation. These daily and seasonal fluctuations can be very extreme, posing particular problems for small, (mostly) ectothermic insects. It does not seem to be possible for an organism to have such a wide tolerance of temperature that it can operate equally efficiently over an extremely wide range of environmental temperatures (Heath et al., 1971). To solve this problem, terrestrial organisms select a range of environmental temperatures that permit efficient activity. Presumably, it is at these optimum temperatures that the metabolic machinery works most efficiently (Hochachka, 1976).

Insects represent the most abundant and diverse group of terrestrial organisms. They tend to be seasonal, which reduces the range of environmental temperatures they experience. As adults many species engage in such activities as flight, which is a highly energetic behavior (Heath et al., 1971; Heinrich, 1981; May, 1976; Polcyn, 1994). Metabolic processes ultimately limit activity based on body temperature and oxygen demand during active periods (Somero, 1975). The effects of temperature on an organism are crucial. Temperature establishes distribution limits, the rates of biochemical functions and determines the ultimate survival of an organism. For most insects, especially diurnal insects, temperature control is accomplished by gross movements and body posturing (Heath et al., 1971). Thermal microhabitat selectivity and diurnal and seasonal periodicity are primary adaptations of insects to terrestrial environments (Bartholomew, 1981; Heath et al., 1971).

The first winged insects arose sometime during the Devonian, approximately 350 - 400 million years ago (Wooton, 1976). The evolution of wings capable of sustained, flapping flight was one of the most important evolutionary events in the diversification of insects in the Carboniferous (approximately 300 million years before present). Flapping flight is the most energetically demanding mode of animal locomotion and the flight muscles of insects are the most metabolically active of their tissues (Bartholomew, 1981; Eastbrook and Sacktor, 1958; Kingslover and Koehl, 1985; May, 1979; Sacktor, 1970, 1976). Because the efficiency of muscle contraction is approximately 20%, most of the large energy expenditure during insect flight results in the production of heat. Some of that heat is retained in the thorax, contributing to body temperatures as high as or higher than birds and mammals (Bartholomew, 1981). In some moths, bees, beetles and dragonflies, elevated body temperatures must be attained before flight is even possible (Bartholomew, 1981; May, 1976, 1978, 1987, 1995b; Polcyn, 1994).

Dragonflies

Dragonflies are a well studied group of insects. Like many other organisms, they inhabit a variety of thermal environments and exhibit a range of sizes and thermoregulatory mechanisms (Heinrich and Casey, 1978; Marden, 1995; May, 1976, 1978, 1979, 1987, 1995a, 1995b). Dragonflies inhabit a diverse array of environments including the tropics and temperate zones (Lutz and Pittman, 1970; May 1976, 1995a; Pritchard, 1989; Vogt and Heinrich, 1983), deserts (Polcyn, 1994), as well as oceanic islands (personal observation). *Belonia saturata,* the Big Red Skimmer, is a common

dragonfly found in most habitats from the boreal forest to the equator, including one of the most extreme thermal environments, the desert.

There are three basic requirements for dragonfly survival: 1) water, for larval development, 2) sunlight, as dragonflies are at least partially heliothermic and require a high body temperature for activity (Corbet, 1980: Lutz and Pittman, 1970; May 1976, 1978, 1986, 1987; Polcyn, 1988, 1994; Pritchard, 1989; Vogt and Heinrich, 1983), and 3) other insects as a food source. An important difference in the diverse habitats supporting dragonflies is the ambient thermal environment. The ability of a species to thrive in very different thermal regimes is not unique to odonates; nonetheless, it is a remarkable characteristic that not all taxa possess. Presumably, selection has led to genetically based differences in the metabolic machinery required to provide the energy required by organisms to live in the thermal environments in which they are found (Hochachka, 1976: Low and Somero, 1976; Somero, 1975; Somero and Low, 1976).

Previous work has demonstrated the unique relationship between dragonfly behavior and the ambient temperature of the environment they inhabit (May, 1976, 1978, 1986, 1987; Vogt and Heinrich, 1983; Pritchard, 1989; Polcyn, 1994). Thermoregulation, the maintenance of body temperature relatively independently of ambient air temperature, is achieved by dragonflies through behavioral and physiological mechanisms (May, 1976, 1978, 1995a, 1995b; Polcyn, 1994). During their daily activity dragonflies must be ready to fly instantly in order to catch food, avoid predators or interact with other dragonflies for purposes of territorial defense or mating. Because flight activity varies as a function of body temperature, it is important that the body temperature be maintained as near the

optimum as possible during the active period (May, 1976, 1984; Pezalla, 1979). Dragonflies maintain some of the highest metabolic rates recorded in the animal kingdom (May, 1995b; Polcyn, 1994). Marden (1989) studied total proportion of flight muscle allocation in libellulids and reported that mature libellulid male dragonflies have the highest proportional investment in flight muscle of any animal measured. The thermal window for activity, is defined as the difference between the minimum body temperature at the onset of flight and paralysis due to hyperthermia, or heat torpor (HT). Body temperature is highly regulated within the window by behavioral and/or physiological mechanisms (Heinrich and Casey, 1978; Vogt and Heinrich, 1983). These critical temperatures (optima, heat torpor, etc.) vary with different thermal environments (May, 1978; Polcyn, 1994). The adaptation of dragonflies to the thermal environment is so extreme that temperatures defended by desert populations are lethal to non-desert populations of the same species (Polcyn, 1988).

There is a potential for species to display acclimatory responses to short term thermal variations, but some species primarily experience, and are presumably adapted to, a narrow range of ambient temperatures. Further, thermal preference appears to be correlated with habitat (reviewed by May, 1979). The ability of dragonflies to maintain relatively constant body temperatures is determined primarily by the ambient thermal environment, behavior and body size (May, 1976; May, 1984). Dragonflies have been dichotomized into two broad categories, 'fliers' and 'perchers' (Corbet, 1963; Heinrich and Casey, 1978). In these studies, large bodied dragonflies in the Aeshnid family have been classified as "fliers' which can regulate their body temperature over T_a by shunting

blood to their abdomen for enhanced rates of convective cooling (Heinrich and Casey, 1978; May, 1976). Smaller libellulid dragonflies have been classified as 'perchers' (based on thermoregulatory behavior) and apparently cannot shunt blood as a thermoregulatory mechanism. *B. saturata* is one of the largest libellulids and cannot easily be classified as a 'flier' or 'percher', spending roughly equal amounts of time flying and perching (Polcyn, 1994). Although insects are commonly thought of as ectotherms, Heinrich (1993) demonstrated that most large bodied flying insects elevate their thoracic temperature endothermically. For this reason and others, dragonflies are an excellent group of animals to study adaptation to extreme environments.

Glycerol-3-phosphate dehydrogenase

Glycerol-3-phosphate dehydrogenase (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) plays a central role in the intermediate metabolism of insects and is primarily the enzyme that allows insects to maintain the high metabolic rates required to sustain flight (Colgan, 1992; Eastbrook and Sacktor, 1958; May, 1984, 1995b; Polcyn, 1988; Sacktor, 1955, 1970, 1976). Sacktor (1955) showed that the NAD^+-linked cytoplasmic L-glycerol-3-phosphate dehydrogenase is one of the two enzyme activities of the glycerol-phosphate shuttle necessary for the transport of the NADH electrons from the cytosol of a flight muscle cell to the mitochondria for use in oxidative phosphorylation (Figure 1). This shuttle is necessary for oxidation of cytosolic NADH because NADH is impermeable to the inner mitochondrial membrane. In the cytosol, G3PD catalyses the transfer of electrons from NADH to dihydroxyacetone phosphate (DHAP) which is

reduced to glycerol-3-phosphate (G3P). G3P then diffuses across the outer mitochondrial membrane into the mitochondrial intermembrane space. G3P is then reoxidized to DHAP on the outer surface of the inner mitochondrial membrane by the mitochondrial membrane bound G3PD. As a result of this reoxidation, an electron pair is transferred to the FAD prosthetic group of the mitochondrial membrane bound G3PD. The mitochondrial G3PD uses FAD instead of NAD^+ as the electron acceptor. G3PD is a transmembrane protein with a domain on the outer surface of the inner mitochondrial membrane. $FADH_2$ donates the electron pair to ubiquinone (UQ), in the inner mitochondrial membrane, which transfers the electrons to complex III of the electron transport chain. The end result is the formation of two ATP when cytoplasmic NADH, transported by the glycerol-3-phosphate shuttle, is oxidized by the electron transport chain. The DHAP produced by the re-oxidation of the cytoplasmic G3P diffuses back across the outer mitochondrial membrane into the cytosol completing the shuttle and can then be used again in this cyclic process (Eastbrook and Sacktor, 1958; Sacktor, 1955, 1970, 1976; Stryer, 1988). The glycerol-3-phosphate cycle is a mechanism for oxidative utilization of cytoplasmic NADH and it appears to be one of the most important metabolic specializations of insects (Eastbrook and Sacktor, 1958; Sacktor, 1970, 1976). The re-oxidation of NADH by lactate dehydrogenase (LDH) does not occur since insect flight muscles lack lactate dehydrogenase activity almost completely (Eastbrook and Sacktor, 1958; Sacktor, 1955, 1976). The importance of this enzyme in insect flight systems has been extensively studied in several different orders of insects including Diptera (Bewley, 1978; Fernandez-Sousa et al., 1977; Symonds et al., 1995), Hymenoptera (Machado and Contel, 1991a, 1991b;

McGuffin and Collier, 1982), Orthoptera (Colgan, 1987, 1989, 1992), Coleoptera (Jacobson and Hsiao, 1983; Romero-Severson et al., 1986) and Lepidoptera (Pashley, 1983).

There is little variability in the G3PD locus of the few flying insects studied to date (reviewed by Colgan, 1992), although there has been a report of extensive variation in a species of waterstriders in the family Gerridae (Zera, 1981). Zera (1981) attributed the high degree of variation in the G3PD locus in that species to a reduced importance of flight muscles in these hemipterans.

Gilbert (1967) demonstrated that G3P is also a precursor to phosphatidic acid and therefore is a key intermediate in the biosynthesis of phospholipids and triglycerides. In this respect, G3PD may also play a role in lipid metabolism. In *Drosophila* larvae a form of G3PD has been found in the fat body, which is the location of lipid metabolism in some insects (Rechsteiner, 1970).

Larval and adult isozymes of G3PD with different thermal optima have been demonstrated in *Drosophila* (Bewley et al., 1974). The larval isozyme is heat labile while the adult isozyme is heat stable. This is of great importance to the study of dragonflies, which maintain some of the highest body temperatures recorded (Polcyn, 1994). The utilization of an enzyme which functions as an energy shuttle that quickly oxidizes the quantity of fuel needed to sustain flight, and is stable at high temperatures, may be the key to high temperature adaptation in these insects.

Polymorphic enzyme systems have not been studied in dragonflies. If polymorphisms are present in *B. saturata,* what are their functional implications? The

goals of the present study are: 1) to compare the body temperatures of *Belonia saturata* in a desert versus a non-desert population, 2) to determine if polymorphic enzymes are present, and 3) to compare activity levels of glycerol-3-phosphate dehydrogenase between the two populations studied.

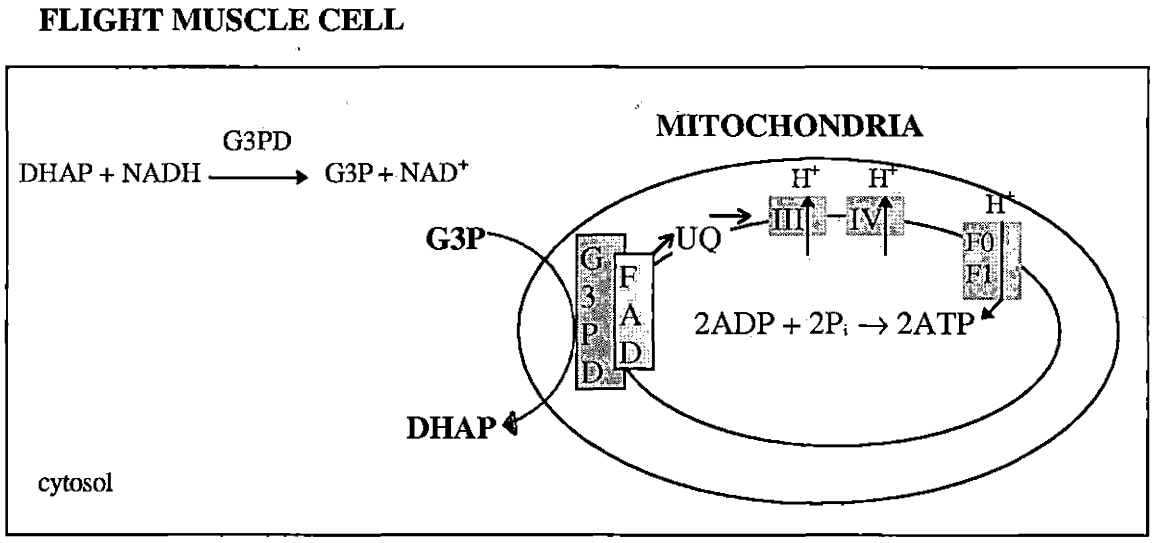

Figure 1: The glycerol-3-phosphate shuttle. In the cytosol, DHAP is reduced to G3P by G3PD in the presence of NADH. G3P crosses the outer mitochondrial membrane and donates a pair of electrons to the FAD prosthetic group of the second form of G3PD which is bound to the inner mitochondrial membrane. On the outer face of the inner mitochondrial membrane G3PD catalyzes the oxidation of G3P to DHAP. DHAP crosses the outer mitochondrial membrane completing the shuttle. FAD donates the electron pair to ubiquinone (UQ) which, in turn donates the electrons to complex III of the electron transport chain.

MATERIALS AND METHODS

Study Sites

The desert population of *Belonia saturata* was collected at the Soda Springs Desert Studies Center at Zzyzx, California, from April 1994 through July 1995. Zzyzx is located in the East Mojave Desert, approximately 80 km southeast of Death Valley, at 290 meters elevation. Weather is typical of the East Mojave Desert, with very hot summers, cold winters and scarce, irregular rainfall (Reid, 1987). A permanent spring-fed pond (Lake Tuende), 0.3 ha in size, supports breeding populations of at least thirteen species of dragonflies. All dragonflies were captured at the ponds edge.

The non-desert population of dragonflies were collected at the Tucker Wildlife Sanctuary (maintained and operated by California State University, Fullerton) from July 1994 through September 1995. Tucker is located in Modjeska Canyon of the Santa Ana Mountains at an elevation of 427 meters. A permanent stream that flows through the canyon supports at least six species of dragonflies. All dragonflies were captured along the streams edge.

Sampling

A total of one hundred and fifty individuals, 73 collected at Zzyzx (desert population) and 77 collected at Tucker (non-desert population), were included in the study. Thoracic temperature (T_{th}) and ambient air temperature (T_a) at the time of capture were measured for 57 desert and 26 non-desert dragonflies. Temperatures were measured

with a 0.001 inch (0.02 mm) diameter copper-constantan thermocouples threaded through and glued into 27 gauge (0.5 mm diameter) hypodermic needles and fixed to plastic 1 ml syringes. Temperatures were read to 0.1°C with a Baily BAT-12 thermocouple thermometer (Bailey Instrument Co., Inc., Saddlebrook, NJ, USA). Immediately after capture with a hand held aerial insect net, the portion of the net containing the insect was placed in the shade. The thermocouple needle was quickly inserted through the net into the thorax of the insect and T_{th} was measured within 10 seconds of capture. Care was taken to avoid touching the insect by hand prior to determining T_{th}. Ambient air temperature was then immediately determined by drying the thermocouple and rapidly waving it through the air at the site of capture. The thermocouple was shaded by hand so that the temperature measured was not influenced by solar radiation. For three weeks in August, 1995, while collecting at Tucker, a Micronta Min-Max Digital Thermometer (Radio Shack) was used to measure ambient air temperature.

The remaining 16 desert and 51 non-desert individuals (of the total number of individuals collected) were used in the G3PD activity assays. Specimens captured for this portion of the study were not handled in any way that would disrupt or degrade the active enzymes. Immediately after capture, the thoracic tissue (mainly flight muscle) was dissected out and sectioned into three samples, placed in 2.0 ml cryovials, labeled and frozen in a liquid nitrogen dewar. The samples were maintained in the dewar until they were transferred to a cryogenic freezer (no more than 48 hours after collection) at -70°C until use in the protein assays as described below.

Electrophoretic Methods

In preparation for electrophoretic analysis, thoracic samples were thawed on ice. A small amount of muscle tissue (approximately 0.1 g) was homogenized in 100 µl of extraction buffer (0.05M Tris-HCl, pH 7.5) using a teflon pestle on a high torque stirrer (Polyscience Corporation). The extract was centrifuged at 18,000 G for five minutes at 5°C and an additional 300 µl of extraction buffer was then added to each tube. The supernatent was used for analysis of leucyl glycyl glycine peptidase (LGG), malic enzyme (ME), phenylalanine proline peptidase (PAP), glutamate-oxaloacetate transaminase (GOT), phosphoglucose isomerase (PGI), superoxidase dismutase (SOD) and phosphoglucomutase (PGM). The remaining extract was diluted by adding 0.6X the original volume of 400 µl and used for analysis of isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PD), malate dehydrogenase (MDH) and glycerol-3-phosphate dehydrogenase (G3PD). Enzyme extract samples, in 10 µl aliquots, were loaded into pre-formed wells (25 µl well size) of 12% horizontal starch gels (Sigma). Three buffer systems were used: a Tris-Borate-EDTA continuous system for ME and GOT [constant voltage of 150 volts]; a Tris-Citrate/LiOH-Borate discontinuous system for PGM, SOD, G3PD, LGG and PAP [constant voltage of 150 volts]; and a Tris-Citrate-EDTA continuous system with 0.36 g EDTA/250 ml gel and 0.36 g EDTA/400 ml electrode buffer for IDH, G6PD and MDH [constant voltage of 100 volts] (Shaw and Prasad, 1970). The gel and electrode buffers (except for Tris-Citrate/LiOH-Borate) were stabilized by adding 5 mg NADP/250 ml buffer. The protein positions were determined

using conventional histochemical stains (Shaw and Prasad 1970). Banding patterns were scored and recorded the same day. Table 1 summarizes the enzyme systems assayed.

Table 1: Enzymes assayed for protein polymorphisms using starch gel electrophoresis in a desert and non-desert population of *Belonia saturata*. Buffers and systems run for each enzyme assayed are also listed.

Enzyme	Abbreviation	Buffer	System
Malic enzyme	ME	Tris-Borate-EDTA	Continuous
Glutamate oxaloacetate transferase	GOT	Tris-Borate-EDTA	Continuous
Phosphoglucose mutase	PGM	Tris-Citrate/LiOH-Borate	Discontinuous
Superoxidase dismutase	SOD	Tris-Citrate/LiOH-Borate	Discontinuous
Glycerol-3- phosphate dehydrogenase	G3PD	Tris-Citrate/LiOH-Borate	Discontinuous
Phenylalanine proline peptidase	PAP	Tris-Citrate/LiOH-Borate	Discontinuous
Leucyl glycyl glycine peptidase	LGG	Tris-Citrate/LiOH-Borate	Discontinuous
Isocitrate dehydrogenase	IDH	Tris-Citrate-EDTA	Continuous
Malate dehydrogenase	MDH	Tris-Citrate-EDTA	Continuous
Glucose-6-phosphate dehydrogenase	G6PD	Tris-Citrate-EDTA	Continuous

Enzyme Activity

Activity assays were run to determine possible differences in activity of glycerol-3-phosphate dehydrogenase (G3PD) between the desert and non-desert populations of *Belonia saturata*. G3PD was selected as the enzyme for activity assays based on its importance in insect flight metabolism and the differences in heterozygosity between the desert and non-desert population.

Approximately 0.1 g of thoracic muscle tissue from four desert and four non-desert dragonflies was homogenized with a teflon pestle in 1.2 ml of extraction buffer (0.05M TRIS-HCl, pH 7.5). The homogenate was then centrifuged at 15,000 g for 10 minutes at 5° C. The supernatant was used in all assays, and was kept on ice until the initiation of the assay.

Glycerol-3-phosphate dehydrogenase (G3PD) was assayed by monitoring the change in absorbance at 340 nm resulting from the oxidation of NADH, by G3PD, in the presence of dihydroxyacetone phosphate (DHAP). The standard assay consisted of 0.2 mM NADH, 0.5 mM DHAP, 0.025 M histidine, 0.025 M Tris, pH 6.6, with a final assay volume of 1 ml. The DHAP substrate was prepared from the dimethyl ketal salt (SIGMA). The reaction was started with the addition of 10 µl of homogenate supernatent (Fernandez-Sousa et al., 1977).

The activity assay was conducted at seven temperatures: 35°C, 40°C, 45°C, 50°C, 55°C and 60°C. Constant temperature water baths were used to maintain the desired assay temperature. Assays were monitored for a total of 30 minutes, with absorbance readings taken every 5 minutes. One milliliter crystal quartz cuvettes were used in the spectrophotometer (Milton Roy Spectronic 3000 Array) to measure absorbance. A control assay (containing the assay mixture without 10 µl of tissue sample) was also conducted at each of the seven temperatures along with the sample assays. At five minute intervals the test tubes containing the assay mixture were removed from the water bath, transferred to a quartz cuvette and the absorbance was then recorded. The assay mixture was immediately transferred back to the test tube and returned to the water bath.

RESULTS

Body Temperature

Ambient air temperature (T_a) and thoracic temperature(T_{th}) were measured in 57 desert individuals and 26 non-desert individuals. Figure 2 shows the relationship between T_{th} and T_a in both the desert and non-desert populations. The range of T_{th} in the desert population was 28.2°C - 43.5°C (mean = 37.6°C ± 2.9°C) over a T_a range of 22.5°C - 38.7°C (mean = 28.5°C ± 4.3°C). The range of T_{th} in the non-desert population was 35.3°C - 42.9°C (mean = 40.0°C ± 2.0°C) over a T_a range of 23.4°C - 36.8°C (mean 29.4°C ± 3.3°C). Table 2 summarizes the temperature data from both populations. For both the desert and non-desert populations, the slope of the relationship between thoracic temperature and air temperature were significantly different from one. The mean T_{th} of the non-desert dragonflies was significantly higher than that of the desert population ($z = 3.70$, $df = 81$, $p < 0.05$). No significant difference was observed between the mean ambient air temperatures experienced by the two populations during the sample periods.

Table 2: Mean ± 1SD, sample size (n) and range of ambient air (T_a) and thoracic (T_{th}) temperatures during diurnal activity for 57 desert and 26 non-desert *Belonia saturata*. Dates of population sampling are also included.

DESERT	NON-DESERT
T_{th} = 37.6°C ± 2.9°C	T_{th} = 40.0°C ± 2.0°C
T_{th} range = 28.2°C - 43.5°C	T_{th} range = 35.3°C - 42.9°C
T_a = 28.5°C ± 4.3°C	T_a = 29.4°C ± 3.3°C
T_a range = 22.5°C - 38.7°C	T_a range = 23.4°C - 36.8°C
n = 57	n = 26
April 1994 - July 1995	July 1994 - September 1995

Protein Polymorphism

The banding patterns on the starch gels revealed polymorphisms in some of the enzyme systems studied. Thirteen individuals from each population were used in this portion of the study. In the desert population, polymorphisms were present in ME, GOT, SOD, G3PD and G6PD. In the non-desert population, polymorphisms were present in PGM, SOD, and G6PD. Neither population was polymorphic for PAP, LGG, IDH or MDH (summarized in Table 3).

Malic enzyme (ME) expressed both a fast and slow allele in the desert population. Eleven of thirteen individuals (85%) expressed only the slow allele and two (15%) expressed the heterozygous condition. All thirteen individuals (100%) in the non-desert population were homozygous for the slow allele.

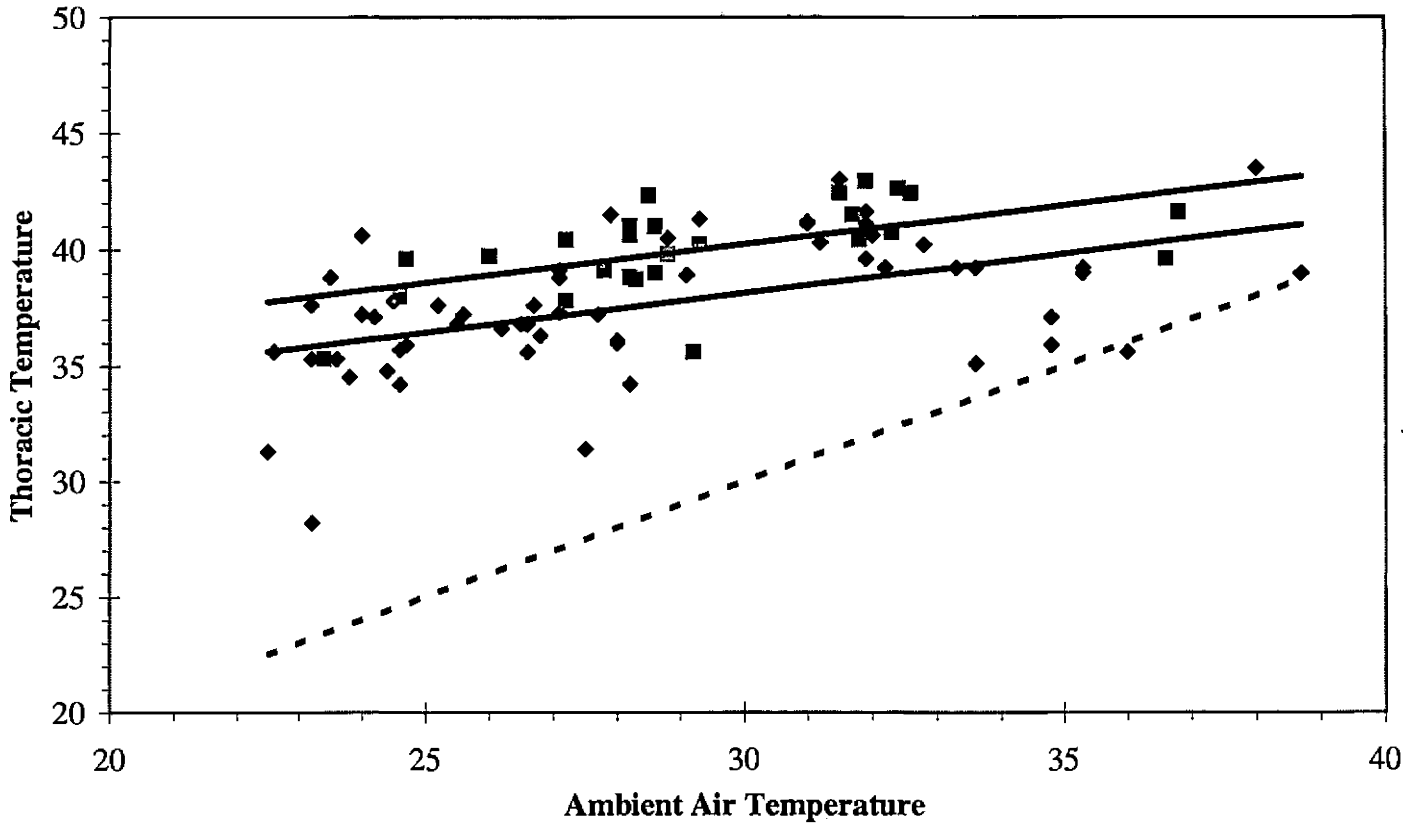

Figure 2: Thoracic temperatures during flight as a function of ambient air temperature for *Belonia saturata* inhabiting desert (◆) and non-desert (■) environments. The regression equation for the desert temperature data plotted is $y = 0.3391x + 27.962$, $r = 0.503$. The regression equation for the non-desert temperature data plotted is $y = 0.3314x + 30.294$, $r = 0.556$. Also plotted is the isothermal line (– –) where thoracic temperature equals air temperature.

Glutamate oxaloacetate transferase (GOT) was expressed as a slow and fast allele in the desert population. Twelve of thirteen individuals (92%) expressed only the slow allele and one individual (8%) expressed the heterozygous condition. All thirteen individuals (100%) in the non-desert population expressed the slow allele.

Phosphoglucose mutase (PGM) was 100% homozygous for the fast allele in the desert population. In the non-desert population, five of thirteen individuals (38%) expressed only the fast allele and eight individuals (62%) expressed the heterozygous condition.

Superoxidase dismutase (SOD) was expressed as a slow and fast allele in the desert population. Twelve of thirteen individuals (92%) expressed only the fast allele, while only one individual (8%) expressed only the slow allele. There were no heterozygotes in the desert population. In the non-desert population, twelve of thirteen (92%) expressed the fast allele with one individual (8%) expressing the heterozygous condition.

Glycerol-3-phosphate dehydrogenase (G3PD) was the most diverse enzyme system in the desert population. One of thirteen individuals (8%) expressed only the fast allele, four individuals (31%) expressed the heterozygous condition and eight individuals (61%) expressed only the slow allele. All thirteen individuals (100%) in the non-desert population were homozygous for the slow allele.

Glucose-6-phosphate dehydrogenase (G6PD) was expressed as a fast and slow allele in both the desert and non-desert populations. In the desert population, eight of thirteen individuals (62%) expressed only the slow allele and five individuals (38%)

expressed the heterozygous condition. In the non-desert population, twelve of thirteen individuals (92%) expressed only the slow allele while only one individual (8%) expressed the heterozygous condition.

Leucyl glycyl glycine peptidase (LGG), phenylalanine proline peptidase (PAP), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) were monomorphic in both the desert and non-desert populations.

Table 3: Results of starch gel electrophoresis assays. Assays were run to determine the presence of protein polymorphisms in a desert and non-desert population of *Belonia saturata*. Abbreviations of enzymes are defined in the text.

	ME	GOT	PGM	SOD	G3PD	G6PD	PAP	LGG	IDH	MDH
Desert										
n = 13										
slow	11	12	0	1	8	8	0	0	0	0
fast	0	0	13	12	1	0	13	13	13	13
heterozygous	2	1	0	0	4	5	0	0	0	0
%	15%	8%	0%	8%	31%	38%	0%	0%	0%	0%
Non-Desert										
n = 13										
slow	13	13	0	0	13	12	0	0	0	0
fast	0	0	5	12	0	0	13	13	13	13
heterozygous	0	0	8	1	0	1	0	0	0	0
%	0%	0%	62%	8%	0%	8%	0%	0%	0%	0%

G3PD Activity

The activity of glycerol-3-phosphate dehydrogenase (G3PD) was assayed at seven temperatures to determine if there were differences in the temperature sensitivity of this enzyme between the desert and non-desert population. The reaction rate during the first 5 minutes was determined to be 100% activity (Table 4). The activity during subsequent 5 minute intervals was then determined relative to the initial activity from 0 - 5 minutes.

Student t-tests were performed on the activity during each 5 minute interval. No significant difference between the mean activity was observed at any temperature from 10 - 30 minutes. To determine differential enzyme activity between the desert and non-desert population, differences in initial activity of G3PD from 0 - 5 minutes were evaluated with analyses of variance (General Linear Models Procedures, SAS, 1985). Absorbance at 340 nm was the dependent variable and time was the covariate. The slope of the covariate-dependent variable regression was homogeneous, the residuals were normally distributed and the variances of the residuals were homogeneous as required to satisfy the assumptions of the model. There was no significant difference in G3PD activity from 10 - 30 minutes between the desert and non-desert population at any of the seven temperatures assayed (Figures 3 - 9). There was no significant difference in initial G3PD activity (activity from 0 - 5 minutes) at 30°C or at 35°C. The desert population had a significantly higher initial activity at 40°C ($F = 6.90$, $df = 1$, $p < 0.05$), at 45°C ($F = 95.20$, $df = 1$, $p < 0.05$), at 50° ($F = 181.16$, $df = 1$, $p < 0.05$), at 55°C ($F = 133.07$, $df = 1$, $p < 0.05$) and at 60°C ($F = 97.55$, $df = 1$, $p < 0.05$). Figure 10 represents the temperature dependence of the initial activity of G3PD in the desert and non-desert populations of *B. saturata*.

Table 4: Activity of glycerol-3-phosphate dehydrogenase from *Belonia saturata* collected in desert and non-desert habitats. Data is reported as percent activity and slope (slope reported is change in absorbance over time from 0 -5 min only). * Denotes a significant difference between the desert and non-desert population in the slope of the regression line plotted as absorbance at 340 nm vs time.

	Activity 0 -5 min	Activity 5 - 10 min	Activity 10 - 15 min	Activity 15 - 20 min	Activity 20 - 25 min	Activity 25 - 30 min	Slope 0-5 min
30°C							
Desert	100	44.23	5.89	0.75	0.52	0.32	-0.154
Non-Desert	100	38.14	15.87	7.84	4.65	1.84	-0.125
35°C							
Desert	100	24.52	0.84	0.41	2.01	0.62	-0.185
Non-Desert	100	30.17	10.92	2.86	0.81	0.18	-0.141
40°C							
Desert	100	11.01	0.49	0.47	1.04	0.89	-0.205*
Non-Desert	100	11.33	0.50	0.78	0.63	0.60	-0.172
45°C							
Desert	100	1.98	0.94	0.59	0.70	0.30	-0.226*
Non-Desert	100	5.95	0.56	1.17	0.86	1.40	-0.160
50°C							
Desert	100	0.87	0.59	0.73	0.61	0.26	-0.230*
Non-Desert	100	0.91	0.53	0.41	0.28	0.25	-0.178
55°C							
Desert	100	0.90	0.75	0.52	0.55	0.09	-0.225*
Non-Desert	100	1.23	0.61	0.52	0.42	0.53	-0.181
60°C							
Desert	100	0.49	1.19	0.23	0.55	0.36	-0.234*
Non-Desert	100	4.14	0.37	0.27	0.41	1.09	-0.182

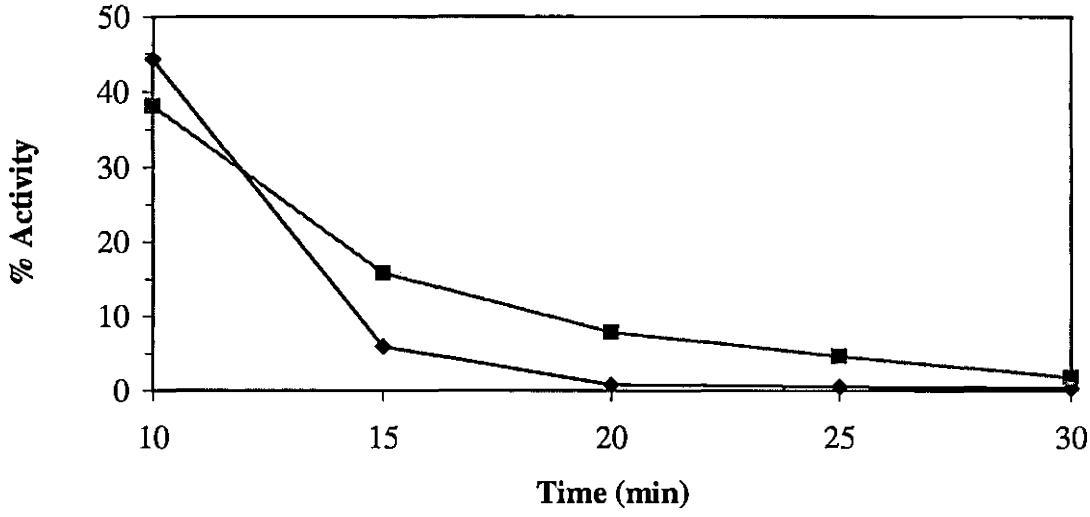

Figure 3: Percent G3PD activity from 10 - 30 minutes at 30°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

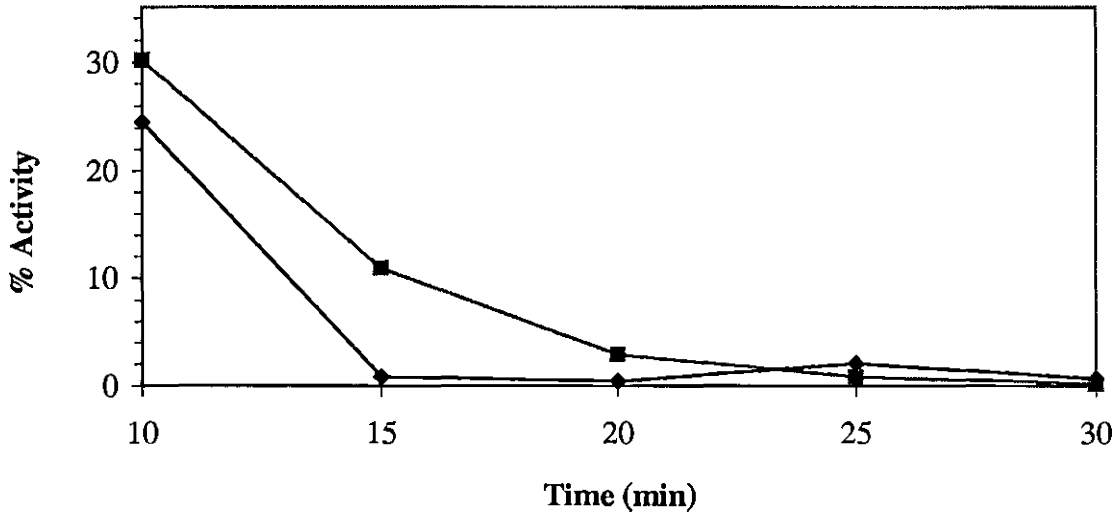

Figure 4: Percent G3PD activity from 10 - 30 minutes at 35°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

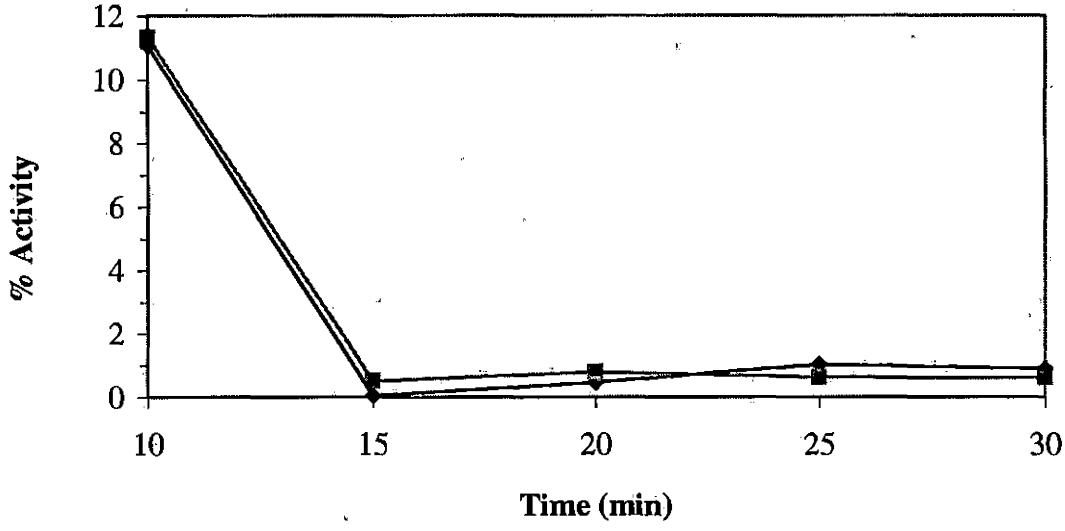

Figure 5: Percent G3PD activity from 10 - 30 minutes at 40°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

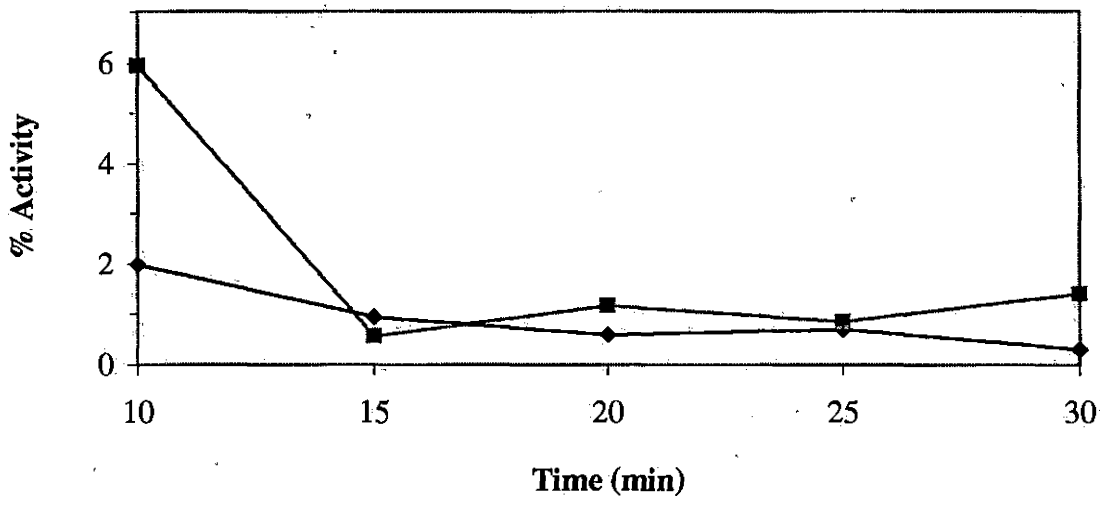

Figure 6: Percent G3PD activity from 10 - 30 minutes at 45°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

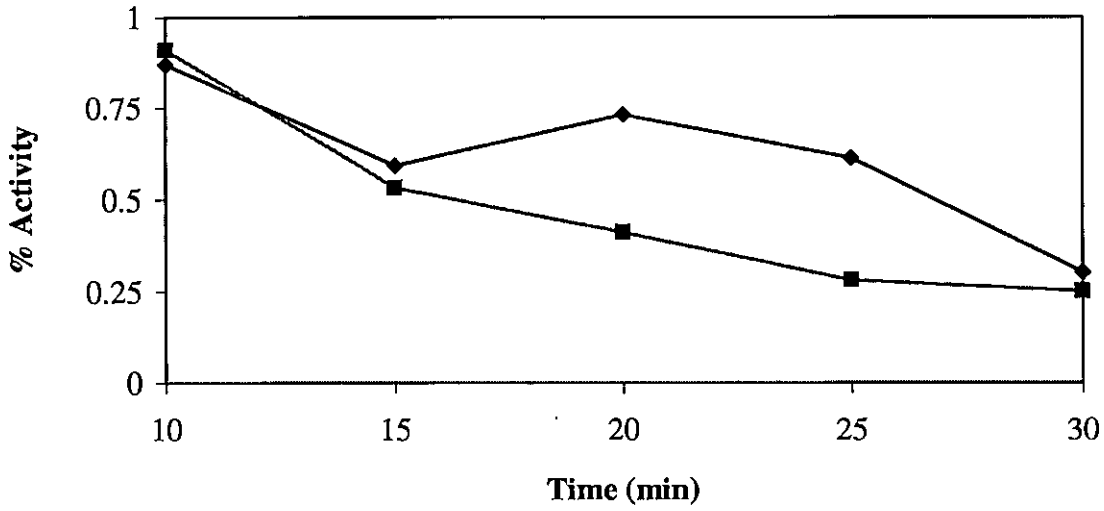

Figure 7: Percent G3PD activity from 10 - 30 minutes at 50°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

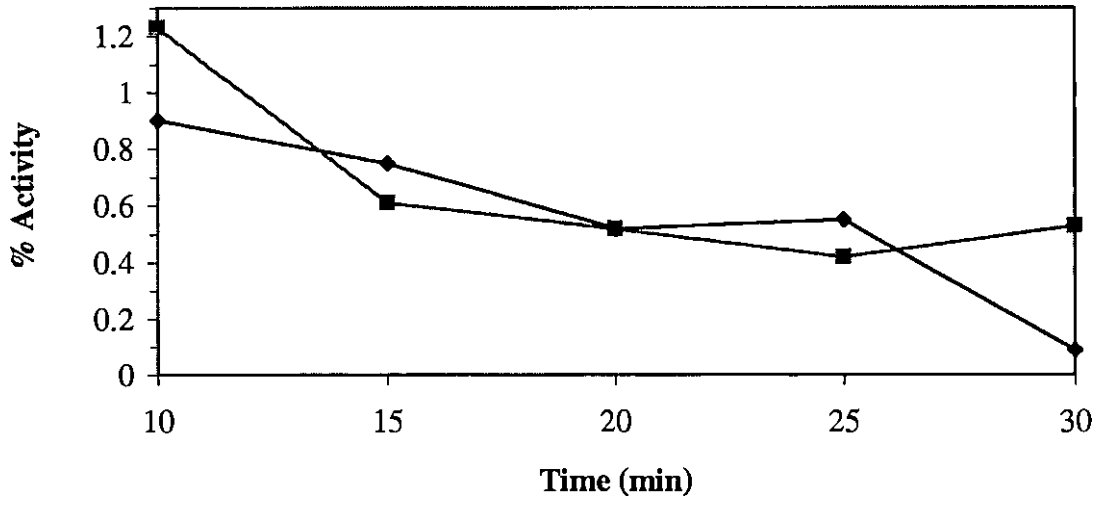

Figure 8: Percent G3PD activity from 10 - 30 minutes at 55°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

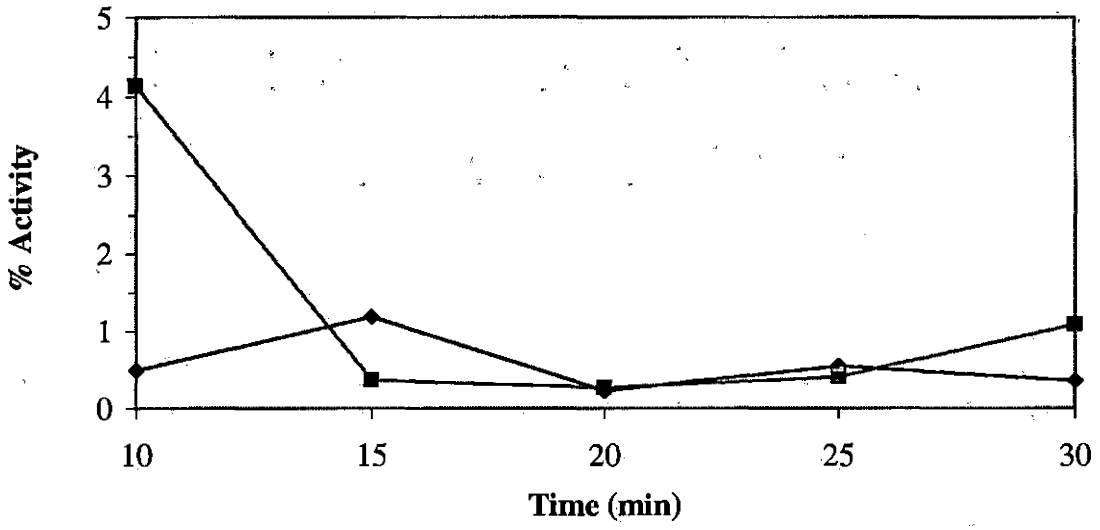

Figure 9: Percent G3PD activity from 10 - 30 minutes at 60°C of both desert (♦) and non-desert (■) populations of *Belonia saturata.*

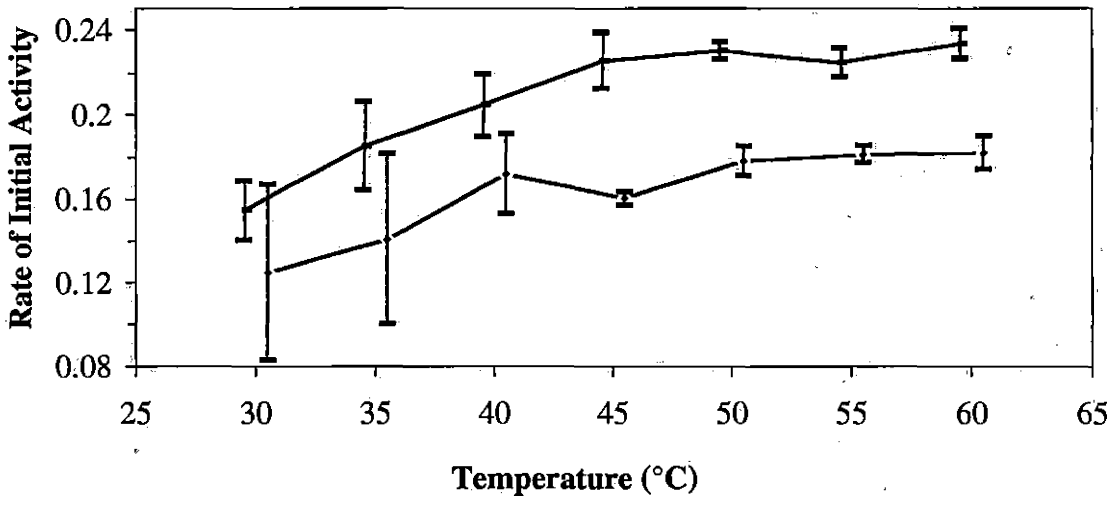

Figure 10: Percent G3PD activity from 0 - 5 minutes at 30° - 60°C of both desert (-) and non-desert (♦) populations of *Belonia saturata.*

DISCUSSION

Body Temperature

The data reported here suggests that the desert population of *B. saturata* maintain lower body temperatures than their conspecifics in the non-desert population. However, this is misleading for several reasons. Body temperature is reported as a mean body temperature over all individuals measured. An individual dragonfly does not experience a mean body temperature. There are only two critical temperatures that an active adult may experience, the minimum temperature needed to sustain flight and heat torpor. Somewhere between those two critical temperatures (the "thermal window") there may be an optimum temperature for activity. Presumably, this is the body temperature at which the dragonfly is the most metabolically efficient. Polcyn (1994) reported a minimum flight temperature of 22.6°C (± 2.5) and a heat torpor of 53.3°C (± 0.9) for a desert population of *B. saturata*. Polcyn (1994) also reported a mean T_{th} of 42.1°C (± 2.2, range = 29.8 - 47.7, n = 81). The mean T_{th} of *B. saturata* reported in the current study is 37.6°C (± 2.9, range = 28.2 - 43.5, n = 57), which is 4.5°C lower than that reported by Polcyn (1994), although the data reported are for the same desert population. The simplest explanation for this discrepancy in body temperature data is that the sample size of the current study was too small. Another possibility is that individuals were not collected from the desert site on the hottest days of the season (May - September, 1995). Based on data from Polcyn (1994), it appears that *B. saturata* may experience more extreme ambient air temperatures in the desert environment than the non-desert population of *B. saturata*. Since ambient air

temperature affects body temperature, and sampling did occur during periods of maximum ambient air temperature in the non-desert site, the body temperature measured in the non-desert population may represent the upper extremes of body temperature in that population.

Furthermore, it has recently been shown that there are marked changes in physiology and thermal sensitivity in flight muscles during adult maturation in a libellulid dragonfly (Marden, 1995). Many dragonflies emerge as adults at approximately one-half their eventual adult body mass (Anholt et al., 1991; Marden, 1989). Muscle maturity is achieved by hypertrophy of the flight musculature (increase in cell size and increase in number of mitochondria). There are also changes in behavior as these dragonflies mature (Marden, 1995). Marden (1995) has shown that teneral (newly emerged) *Libellula pulchella* do not experience body temperatures above 40°C, whereas fully mature adults routinely experience body temperatures in excess of 45°C. Relative maturity was not determined for the individuals included in the present study, and since *B. saturata* were collected early in the season, the probability of an increased number of teneral and/or immature dragonflies active at this time was likely quite high. Thus, differences in body temperatures due to relative muscle maturity may also have contributed to the lower mean body temperature reported for the desert population. It also follows that the adult *B. saturata* included in this study from the non-desert population were collected towards the end of the season when fewer teneral *B. saturata* are active. An increase in the presence of fully mature individuals should be expected which should give a more accurate representation of the body temperatures maintained by adults of this species.

Protein Polymorphism

The presence of protein polymorphisms have been interpreted primarily in terms of selective pressures. If there is a high degree of polymorphism at a given locus it is generally interpreted as resulting from weak selective pressure at that locus (Colgan, 1992; Gibson et al., 1991; Jacobson and Hsiao, 1983; Zera, 1981). Conversely, a low degree or absence of polymorphism is interpreted as the result of strong selection at that locus. However, heterozygote advantage has also been shown in several organisms, so until the functional significance is determined for the product of any given locus, any interpretation of the selective advantage of polymorphisms present at a given locus can only be speculative, at best. As Symonds et al. (1995) point out, "the study of adaptive potential of allozyme variants in natural populations requires a demonstration that the electrophoretic variation reflects a functional difference via which natural selection can discriminate between alternative alleles."

There have been no studies on the genetic variability of enzyme systems in dragonflies. The data reported in this study show the desert population to have more polymorphic enzyme systems (five: ME, GOT, SOD, G3PD, G6PD) than the non-desert population (three: PGM, SOD, G6PD). There were four loci that were monomorphic in both populations (PAP, LGG, IDH, MDH). Heterozygosity of more loci in the desert population may be interpreted as contributing to greater reproductive success. However, until the adaptive significance of these allozymes, under these environmental settings, can be documented, it remains unclear what forces have acted to shape the gene pools.

Glycerol-3-phosphate dehydrogenase

Glycerol-3-phosphate-dehydrogenase, an important enzyme mediating rates of aerobic metabolism, has been widely studied and characterized in several orders of insects. The only study which has been conducted on G3PD in odonates demonstrated a lack of a thoracic tissue specific isozyme of G3PD in libellulids (Colgan, 1992). In other insects, *Drosophila*, for example, there are tissue specific forms of G3PD that have been isolated, and each form has its own functional characteristics (reviewed by Colgan, 1992). Unfortunately, this sheds little light on the functional significance of G3PD in dragonflies, including *B. saturata.*

G3PD is completely functional in flight metabolism whether the fuel being metabolized is carbohydrates or lipids (Eastbrook and Sacktor, 1958; Sacktor, 1970, 1976). The primary fuel source utilized by dragonflies, in general, and *B. saturata*, specifically, is not known. The current dogma is that small-bodied insects with asynchronous-type flight muscles use carbohydrates as their primary fuel source, and large-bodied, flying insects with synchronous-type muscles use lipids as their primary fuel source (Eastbrook and Sacktor, 1958; Sacktor, 1970, 1976). This is a generalization, at best. The "typical" small-bodied insect studied tends to be *Drosophila*, a common laboratory insect, but is perhaps not the best representative of small insects in general. The typical large-bodied insects studied are the Sphinx Moth, *Manduca sexta*, and several other Lepidopterans and Orthopterans. Dragonflies are atypical insects physiologically and behaviorally, so it is unclear where dragonflies fit in this highly dichotomized spectrum.

The data from the current study (Figure 10) suggest that the desert population maintain a higher relative activity of G3PD at 40°, 45°, 50°, 55° and 60°C than their non-desert counterparts. Polcyn (1994) reported heat torpor in *B. saturata* as 53.3° (± 0.9), so in a natural population of *B. saturata* enzyme activity at 55° and 60°C should not have any functional significance. *Belonia saturata* most likely experience more extreme body temperatures in the desert environment than the population of *B. saturata* in the non-desert environment and the thermosensitivity of G3PD suggests that the desert population may maintain higher enzyme activity levels at greater body temperatures than the non-desert population. Although the desert population was polymorphic for G3PD, the genotypes of the individuals included in the activity assays of this study are unknown. Thus, further research will be required to determine if the increased activity of G3PD in the desert population was due to heterozygosity at this locus or some other contributing factor(s).

There was no significant difference in activity of G3PD between the desert and non-desert populations at either 30° or 35°C. This may also be attributed to the differences in thermal sensitivities of teneral and immature dragonflies. Marden (1995) showed that unlike adults, teneral libellulid dragonflies did not experience T_{th} above 40°C. As seen in larval versus adult *D. melanogaster* there may be a relationship between teneral vs adult thermosensitivity and expression of G3PD (Bewley et al., 1974). Body temperatures between 30° and 35° were measured in flying individuals from both populations in the current study, so they do represent realistic conditions. Although these

temperatures are on the low end of the reported body temperatures for *B. saturata,* they are above the minimum temperature required for flight in *B. saturata* (22.6° ±2.5) as reported by Polcyn (1994) yet, as discussed above, may reflect thermal preferences of tenerals.

There are still gaps in our understanding of the biology and mechanisms used by dragonflies to enable them to maintain activity in such a wide range of habitats, especially thermally extreme habitats such as the Mojave desert. Individuals in the desert population may be able to be more active at higher ambient air temperatures due to higher activity of G3PD at elevated body temperatures. This may be attributed to the polymorphic G3PD locus in the desert population, with heterozygosity at the G3PD locus permitting individuals to be more active at higher ambient air temperatures. Conversely, the lack of polymorphism at the G3PD locus in the non-desert population may contribute to lower levels of activity of G3PD at high temperatures resulting in lower activity of *B. saturata* individuals at higher ambient air temperatures overall, or the maintenance of a lower body temperature in non-desert populations. Further work on the differences in G3PD activity between homozygotes and heterozygotes should shed light on the adaptive significance of this enzyme system in this group of widely dispersed animals.

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