Environmental Physiology and Biochemistry of Insects

Edited by
Klaus H. Hoffmann

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With 78 Figures

Springer-Verlag
Berlin Heidelberg New York Tokyo 1985

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Oberer Eselsberg
7900 Ulm, FRG

ISBN 3-540-13762-9 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-13762-9 Springer-Verlag New York Heidelberg Berlin Tokyo

Library of Congress Cataloging in Publication Data. Main entry under title: Environmental physiology and biochemistry of insects. 1. Insects–Physiology. 2. Insects–Ecology. I. Hoffman, Klaus H. (Klaus Hubert), 1946-. QL495.E58 1984 595.7'01 84-14182

Printed in Germany

Offsetprinting: Beltz Offsetdruck, Hemsbach/Bergstraße, Bookbinding: J Schäffer OHG, Grünstadt.

2131/3130-543210

Preface

Of all the zoological classes the insects are the most numerous in species and the most varied in structure. Estimates of the number of species vary from 1 to 10 million, and 10^{18} individuals are estimated to be alive at any given moment. In their evolution, insects are relatively ancient and, therefore, they have proved to be a phenomenally successful biological design which has survived unchanged in its basic winged form during the last 300 m. y. Insects were the first small animals to colonize the land with full success. Their small size opened many more ecological niches to them and permitted a greater diversification than the vertebrates. What is it about this design that has made insects so successful in habitats stretching from arid deserts to the Arctic and Antarctic and from freshwater brooks to hot springs and salines? Is it due to the adaptability of their behavior, physiology, and biochemistry to changing environmental conditions?

Three features of insects are of particular importance in determining their physiological relationship with the environment: their small size, as mentioned above, the impermeability and rigidity of their exoskeleton, and their poikilothermy. Of course, as with any other animals, the insects' success in its environment depends on its ability to maintain its internal state within certain tolerable limits of temperature, osmotic pressure, pH or oxygen concentration (homoeostasis). The mechanisms utilized to control rates of metabolic functions under varying environmental conditions clearly illustrate a key interplay between time and the adaptive strategy. The first strategy is that of the adaptation of life over a period of millions of years (genotypic adaptation). The second concerns the periodic adaptations to the annual cycle or seasons, to the monthly cycle governed by the movement of the moon, and to the 24 h day/night cycle. Such adaptive processes generally require several days or weeks for completion and are customarily referred to as acclimatization if the organism is responding to environmental parameters within its natural ecosystem or as acclimation if the organism is responding to a single environmental factor in a laboratory situation. The third time scale is a very short one and covers the rapid response to acute changes in the immediate environment of the animals.

Knowledge of the physiology and biochemistry of insects has developed extensively over the last 20 years. Reasons for this increased interest in insect physiology and biochemistry are the growing realization that insects can be useful not only as organisms to act as a model system for experimental studies of general principles, but also as an economic model. Since in our view the functions of insects are only really meaningful when studied in the context of the environment with which the organisms are interacting, we shall try to present examples of the major strategies of adaptation of selected physiological and biochemical functions (e.g., growth and development, aerobic and anaerobic energy metabolism, salt and water exchange, respiration, communication and defense) to varying conditions of temperature, light, humidity, salt concentrations, oxygen tension, or food supply. Beyond that, since hormones are involved in the operation of all systems of the insect body, we shall try to give some evidence of our hypotheses that the insect endocrine system is a mediator between environmental factors, such as temperature or light and the (sub)cellular responses, such as protein, fat, and carbohydrate metabolism.

In several chapters previously unpublished data are presented in order to give proof of these new hypotheses.

In general, recent summaries or reviews are cited, but not always the articles on which they are based. Consequently, the present book cannot be a complete coverage of the literature on environmental physiology of insects, but an introduction to it. It should prove useful not only to researches of the Insecta, but also to teachers and graduate students.

This book would not have been possible without the helpfulness of many colleagues. Above all I should like to mention my former teacher, Prof. Dr. H. Remmert, for introducing me into insect physiology research. The editor and contributors also wish to thank Dr. D. Czeschlik, Springer-Verlag, for his sustained interest in the presentation of this book. Our students and co-workers deserve special thanks for certain ideas and results discussed in this book. Finally, we are deeply obliged to the Deutsche Forschungsgemeinschaft (DFG) for the establishment of the "Schwerpunktprogramm: Physiologische Mechanismen Ökologischer Anpassungen bei Tieren". Most of the authors are (were) members of this research program and their work was generously supported by the DFG.

Ulm, October 1984 Klaus H. Hoffmann

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Contents

Chapter 8

Chapter 9

Chapter 1

Metabolic and Enzyme Adaptation to Temperature

Klaus H. Hoffmann[1]

Contents

1 Introduction

Temperature determines life of organisms more than many other environmental factors. The cause of this temperature sensitivity lies in the fundamental fact that all organisms are built up by chemical compounds and that all processes of life are made by chemical reactions which follow the laws of thermodynamics. The temperature acts not only upon the rate of all chemical reactions according the Arrhenius equation, but also causes conformational transitions of proteins, phase transitions of lipids, changes in the structure of water, etc. (Alexandrov 1977).

The temperature range for biological activity is relatively small both because of the specific properties of the biomolecules and because of the temperature coeffi-

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Environmental Physiology and Biochemistry
of Insects (ed: by K. H. Hoffmann)

cients of chemical reactions. The relationship between temperature (T) and rate (R) (velocity) of a given reaction (process) is usually defined by its temperature coefficient, Q_{10}. This is the factor by which a rate is speeded up, if temperature increases by 10°C. Generally Q_{10}-values of "normal" chemical reactions and biological processes range between 2 and 4. In other words, without any regulatory mechanisms deviations of only some degrees of centigrade from suitable temperatures would slow down mandatory metabolic, physiological, and behavioral processes to dangerous inactivity or speed up metabolic rates to an extent so that prevailing of food and oxygen would become impossible (Hochachka and Somero 1973). Longer periods below or above a critical temperature cause irreversible destruction of their bioactive structure and function and lead to a desorganization of metabolic processes, if their temperature coefficients differ slightly.

In order to circumvent these difficulties organisms have developed specific adaptations during the course of their evolution to become more or less independent of ambient temperatures. Two particularly prevalent types of adaptations are homeothermy and poikilothermy. True homeothermy is found only in mammals and birds. These organisms use metabolic heat to maintain a relatively high, optimal constant body temperature, whereas the body temperature of poikilotherms corresponds close to that of the external environment. Nevertheless, various species of poikilotherms, including some insects (Sec. 5, this Chap.), are able to maintain the temperature of parts of their body more or less constant while active. Thus, they have been designated as heterotherms. Two other terms, ectothermy and endothermy, refer to the source of heat used to maintain temperatures above or below ambient. Ectothermic organisms gain heat from the environment, while endotherms gain it from their metabolic processes.

Most insects are essentially ectothermic organisms with body temperatures close to ambient. Within their habitats they have to tolerate a considerable temperature range (Bursell 1974a). Two main problems to master are: (1) overcoming longer lasting seasonal adversary temperatures (e.g., low winter temperatures); (2) maintaining an active state during the short-term daily and local fluctuations of ambient temperature (microclimate). The latter is mainly a problem for terrestrial rather than aquatic insects. Both, adaptations to seasonal temperature changes and to daily or local temperature fluctuations make insects survive and function at widely different habitat temperatures. Short-term temperature changes are mastered by altering many aspects of their physiology and biochemistry in a manner that often compensates for changes within a species-specific temperature range. If cooling or overheating surpasses the adaptive capacities of active life, some insects move to a state of concealed life (Chap. 3). They retain, however, the ability to return to normal life upon reestablishment of acceptable temperature conditions.

The adaptive mechanisms can be divided into two types: modificational and genotypic (Precht et al. 1973). Modificational (nongenetic, phenotypic, physiological, capacity) adaptation exists when in an organism the dependence of experimental temperature (ET) is influenced by the previous adaptation temperature (AT). In contrast, genotypic (genetic) adaptations become apparent without any provocative action of a temperature fluctuation.

Both, modificational and genotypic adaptations involve all levels of biological organization ranging from molecular processes (this Chap.) to population dynamics (Chap. 2).

2 Temperature Dependence of Metabolism and Activity

Oxygen consumption is often taken as a measure of the overall metabolic rat of an animal. Its measurement has been employed more than any other experimental parameter to monitor changes in insects' metabolism associated with temperature (for a summary of data see Keister and Buck 1974). Figure 1.1 depicts the usual relationship between resting metabolic rate (per unit weight) and body temperature in insects. Of course, a relationship like that is found only in those situations where body temperature approximates the ambient temperature. The relationship between metabolic rate and body (ambient) temperature has Q_{10}'s of 2-3 and is usually only approximately exponential. It does not truely follow the exponential Arrhenius function with temperature coefficient, Q_{10}, being constant over all temperature ranges. When, however, metabolic range is not a strictly exponential function of temperature, then Q_{10} varies with the particular range of temperature considered. When Q_{10} changes with temperature it typically does so continuously, that means there is a steady drop in Q_{10} with increasing ambient temperature:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)} \qquad \text{van't Hoff equation,}$$

where R_2 is the rate at any temperature T_2 (in °C) and R_1 is the rate at any lower temperature T_1. The formula indicates that the Q_{10} value per se is temperature dependent.

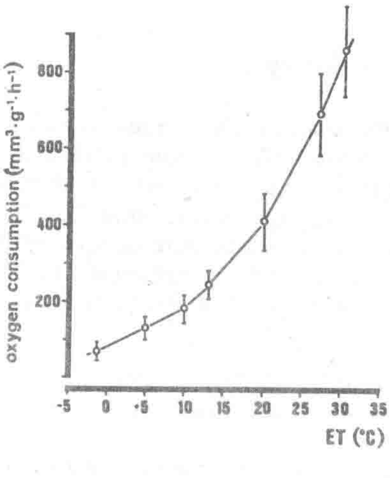

Fig. 1.1. Oxygen consumption of male adult crickets, *Gryllus bimaculatus*, at several experimental temperatures. *Vertical bars* represent SD. (After Hoffmann 1973)

In last instar larvae of the house cricket, *Acheta domesticus*, Q_{10} for oxygen consumption is about 2 in the normal temperature range 20°–40°C (Roe et al. 1980). Standard metabolic rate of male blowflies *Calliphora erythrocephala*, as it was measured by oxygen consumption of the whole animal and of isolated flight muscle tissue, is temperature dependent over the range 10°–30°C with Q_{10} values of 1.34–2.90 (Tribe and Bowler 1968). In the cockroach *Periplaneta americana*, the level of metabolic rate depends strongly on the acute experimental temperature (Welbers 1976) only during the activity phase. In the resting metabolism of *Periplaneta* an influence of the temperature pretreatment has been shown (see p. 12, this Chap.). Curves similar to that in Fig. 1.1 have also been obtained for single enzymatic reactions. It would, however, be unjustified to refer the overall respiration-temperature curve directly to enzymic activity. In a system in which overall gas exchange almost certainly involves a complex of sequential and parallel reactions, it is illusory to apply an analysis based on assumed single rate-limiting or "master" reactions (Keister and Buck 1974).

Many other physiological processes in insects (e.g., motoric activity, heart rate, or electric activities) also generally show a nearly exponential thermal dependency. In mosquitoes *Aedes sollicitans*, fed on a single meal of sugar, the rate of triglyceride synthesis exactly followed the Arrhenius equation over the entire range from 10°–30°C (van Handel 1966). In starving mosquitoes, the logarithm of the time in which 50% of the calories were used, again followed the Arrhenius relation with Q_{10}'s of 2.1–2.3 (van Handel 1973). Even processes such as development and reproduction (Chap. 2) or behavioral activities, which are based on the interaction of many single reactions like those described above, show similar temperature coefficients.

Summarizing, the temperature coefficient, Q_{10}, can be used to describe the thermal sensitivity of many quantitiable physiological rate functions in insects. One could assume that this conflicts with the ideas of thermal adaptation. However, thermal adaptation of insects does not seem to be a rare phenomenon, as will be evidenced in the following sections.

3 The Relationship of Time and Adaptive Strategy

The relative independence of live processes from changes in temperature is achieved through a variety of adaptive mechanisms that have evolved in many poikilotherms during the course of evolution. To fully appreciate these phenomena it is necessary to clearly discriminate between the effects of acute (experimental temperature, ET) and chronic (adaptation temperature, AT) exposure to temperature changes. As has been discussed by Hochachka and Somero (1973) and Hazel and Prosser (1974), such compensations to temperature may occur over at least three distinct time-course periods:

1. instantaneous temperature compensation (acute temperature change);
2. thermal compensation associated with a period of adaptation (chronic temperature change, nongenetic adaptation);
3. evolutionary rate compensation (chronic temperature change, genetic adaptation).

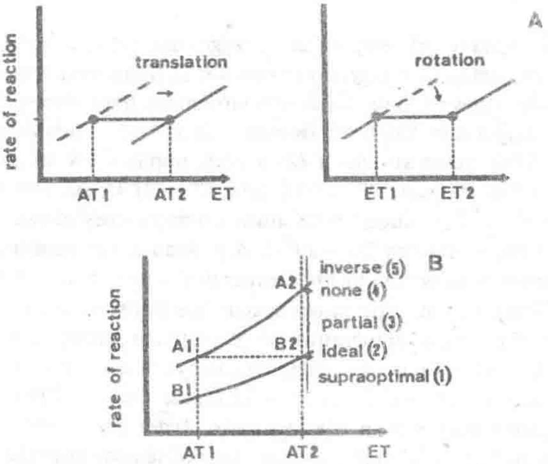

Fig. 1.2 A. The two main types of response to changes of temperature usually termed "adaptive" in ectotherms. Prosser (1958) refers in the first case to a translation of the rate in reaction/temperature curve, and in the second to a rotation. (After Wieser 1973). **B** Types of capacity adaptation (1–5) according to Precht (1949). Adaptation temperature for curve A1-A2: AT1, for curve B1-B2: AT2. (After Precht et al. 1973)

The first generalizations regarding a temperature independence of physiological processes in poikilotherms are according to Precht (1949) and Prosser (1958). After Prosser the reaction rate (R) responds to two distinct ways (Fig. 1.2A): The one shows a plateau of temperature independence when animals are acclimatized (abscissa: AT = ET), whereas the plateau of the other occurs in the course of acute temperature changes (abscissa: ET). Prosser refers in the first case to a translation of the R-T curve (up or to left with cold, down or to right with cold) and in the latter to a rotation (clockwise – Q_{10} reduced in cold, counterclockwise – Q_{10} increased in cold). It is irrelevant, however, whether plateaus of this kind occur in the acclimatized or in nonacclimatized animals, since such cases only differ with respect to the responsible mechanisms and not in principle (Wieser 1973). Typically the R-T curve is steeply in the lower temperature range (Q_{10} ~ 2) and then flattens out (Q_{10} in the middle range ~ 1). Therefore, it is perhaps generally significant that high Q_{10} values are so often found at the lower end of biological temperature ranges. Here many ectothermic organisms are obliged to speed up their metabolism as much as possible. It seems that temperature-insensitive phases frequently occur around the mean temperature expected in the usual environment of the animals. Figure 1.2B presents the type classification of temperature adaptations by Precht (1949) (supraoptimal, ideal, partial, none, and inverse compensation).

Most fundamental research on temperature adaptation has been done with ectothermic vertebrates and intertidal mollusks. Insects, as behavioral thermoregulators (see p. 29, this Chap.) were presumed for a long time to posses little capacity for metabolic compensation against thermal fluctuations. However, since insects have radiated successfully into a wide variety of habitats with different microclimates, important temperature adaptations at the physiological and biochemical level should also have been evolved during evolution in these animals.

3.1 Instantaneous Temperature Compensation

Metabolic adaptations to acute (short-term) temperature changes are well-illustrated for intertidal animals, but not restricted to this group of organisms. Some insect species subject to less drastic fluctuations in their thermal environment have also succeeded in compensating acute temperature fluctuations over at least a small temperature range (Keister and Buck 1974). Some strains of *Drosophila* pupae show an approximately independent respiration rate at 18°–22°C and 27°–30°C, on either side of the rearing temperature of 25°C. A dung beetle shows no respiratory increase between 12°–24°C and an ant none between 20°–34°C. A plateau of temperature independence, or even a minimum, also occurs in the temperature-respiration curve of some Carabidae (Schmidt 1956). In the same insect species the transpiration rate remained constant within a certain narrow temperature range. In the antarctic beetle *Hydromedion sparsutum* activities of the digestive enzymes α-amylase and proteases are temperature compensated between 5°–15°C (Q_{10} ~1.3); (Haderspeck and Hoffmann 1983). The rate of glycogen synthesis in the mosquito *Aedes sollicitans* increases with temperatures from 10°–22.5°C, but is independent of temperatures between 22.5°–30°C (van Handel 1966).

In a second group of insects, plateaus in the R-T plot are found at temperatures considerably lower than the rearing temperature and at which locomotion may be stopped or restricted (e.g., respiration rate in larvae of *Phormia* between 10° and 15°C (Keister and Buck 1974) or in *Drosophila* pupae at 15°C). Such regions of constancy in respiration-temperature plots are difficult to explain with respect to an unitary respiring system, but can be visualized as the resultant of a temporary progressive slowing of the metabolic rate of one reaction system superimposed on continuing increase in rate of a second process (Fig. 1.3). That temperature independence of whole-animal metabolism may result from properties of mitochondria is postulated by the low Q_{10} values reported to succinate and pyruvate oxidation (measured at low substrate concentrations; standard metabolic rates) in mitochondrial preparations from the desert locust, *Schistocerca gregaria* (Newell 1967). In addition, α-glycerophosphate- and ADP-stimulated respiration of blowfly sarcosomes, *Calliphora erythrocephala*, have a temperature independent plateau at intermedial temperatures (Davison 1971). In contrast, in blowfly mitochondria Tribe and Bowler (1968) and Danks and Tribe (1979) found no strong evidence for a plateau of temperature independence in respiration. Fleming and Miquel (1983) have observed different effects of temperature on the metabolic rate of young and old *Drosophila*. The Q_{10} over the entire temperature range was almost 1.5 times as great for the adult flies (Q_{10} = 2.8) as it was for the young flies (Q_{10} = 1.8), but there was no difference in respiration rates at the temperature in which the flies were raised.

Meyer (1978) has pointed out that short high and low temperature pulses immediately increase the anaerobic metabolism of nonfeeding *Callitroga macellaria* larvae, resulting in a drastic increase in formation of polyols or polyolphosphates. Starvation appears to be an important factor that affects both the level and the relation to temperature of metabolism (see Chap. 4). Immediate changes in temperature also often have disturbing effects on animals. For example, a rise in temperature frequently causes a temporary, but drastic increase in motoric activity. Such overshoots are also

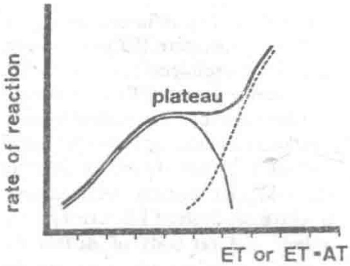

Fig. 1.3. Rate in reaction/temperature curve, where a plateau may be caused by the overlap of two separate R/T curves. (After Wieser 1973)

found in the speed of movements of insects (Precht et al. 1973). Additionally, rapid changes in temperature can have transitory effects on endogenous rhythm of insects in the form of acceleration or retardation.

What are the mechanisms for such temperature independence? A nearly instantaneous increase in enzymes' abilities to bind substrate as temperature is lowered might represent the most prevalent mechanism (Hochachka and Somero 1973). Although studies in this direction have not been accomplished, there are some lines of evidence that are consistent with the hypothesis that positive thermal modulation of substrate binding (that means an increase in apparent Michaelis constant, K_m, for substrate with increasing experimental temperature in a temperature range above the temperature of minimal K_m; see also p. 18, this Chap.) is important in effecting immediate metabolic compensation. For insects, however, only a few data are available that demonstrate the influence of experimental temperature on substrate binding, and these few data do not evaluate this concept. Within the habitat temperature range of three cricket species, *Gryllus campestris, G. bimaculatus,* and *Acheta domesticus,* K_m values of the glycolytic enzymes pyruvate kinase for phosphoenolpyruvate are hardly influenced by experimental temperature (Fig. 1.4A). A positive temperature modulation of enzyme-substrate affinity is avoided within their physiological temperature range (Hoffmann 1976a; Hoffmann and Marstatt 1977). Selection rather may have led to the development of enzyme species ("eurytolerant" enzymes) that are able to maintain their ligand-binding abilities at relative stable levels over the full range of habitat temperatures. An increase in app. K_m values at experimental temperatures beyond the habitat temperature, as can be observed from Fig. 1.4A, may render their metabolic rate less sensitive to extreme situations.

Temperature also influences the rate of enzymatically catalyzed reactions by determining the proportion of molecules in a given population that possess sufficient energy (energy of activation, E_a; enthalpy of activation, $\triangle H^{\neq}$) to react and form an activated complex. A temperature dependence of the barrier of free energy of activation, therefore, is proposed as another possible molecular basis for spontaneous temperature compensation. A biphasic nature of Arrhenius plots (ln rate vs 1/T), and that means different E_a values at different experimental temperatures, are a common phenomenon in membrane-bound enzymes and perhaps reflect changes in the membrane lipid component (for details see Sec. 3.2.6, this Chap.). Recent results of Wood and Nordin (1980) on mitochondrial respiration of the blowfly *Protophormia terranovae* and the housefly, *Musca domestica,* show that for both insect species, ADP re-

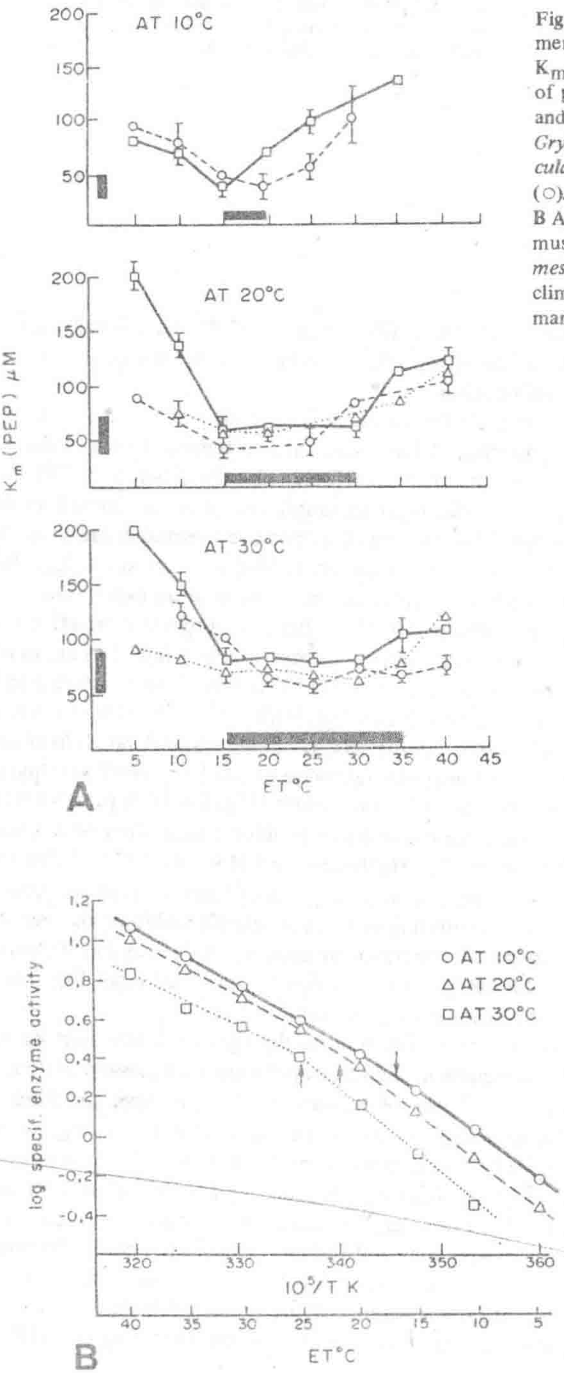

Fig. 1.4. A The influence of experimental temperature (*ET*) on the app. K_m of phosphoenolpyruvate (*PEP*) of pyruvate kinase (*PK*) from muscle and fat body of three cricket species: *Gryllus campestris* (□), *Gryllus bimaculatus* (△), and *Acheta domesticus* (○). *AT* acclimation temperatures. B Arrhenius plots of PK activity from muscle and fat body of *Acheta domesticus* after periods at various acclimation temperatures. (After Hoffmann and Marstatt 1977)

quiring step(s) in oxidative phosphorylation become rate limiting below an ET of 11.5°C. The observed increase in E_a below 11.5°C may slow depletion of energy reserves when their mobility is impaired due to a temperature decrease. Breaks in Arrhenius plots have also been described for cytoplasmic enzymes, e.g., for pyruvate kinase of Trichoptera and Plecoptera larvae, of the blue alder leaf beetle, *Agelastica alni*, of the flour beetle, *Tenebrio molitor* (Hoffmann 1976a), and of various cricket species (Fig. 1.4B). These findings indicate that cytoplasmic enzymes can also exist in at least two temperature dependent (per se induced) conformational states. An Arrhenius plot of phosphofructokinase from the cold-hardy gall fly larva, *Eurosta solidaginis*, (see also Sec. 4, this Chap.) shows an activation energy of 19,800 cal/mole (82.8 kJ/mole) and a very high Q_{10} of 3.64 beetween 10° and 0°C (Storey 1982). In addition, kinetic properties of this enzyme are strongly negatively modulated by low temperature.

A third mechanism for maintenance of temperature independent catalytic function is referable to the effect of experimental temperature on the interactions between enzymes and various modulating metabolites (Hazel and Prosser 1974). In the house cricket, *Acheta domesticus*, pyruvate kinase activity is stabilized by fructose-1, 6-bisphosphate against inactivation by elevated temperatures (Hoffmann 1975). In gall fly larvae, *Eurosta solidaginis*, the effects of physiological levels of AMP on the activity of phosphofructokinase (PFK) are drastically lessened at low temperatures. In addition, two compounds, which are normally not effectors of PFK, gylcerol-3-phosphate and sorbitol, both decrease enzyme affinity for fructose-6-phosphate at low temperature. PFK in cold-hardy *Eurosta solidaginis* is an excellent example of the exploitative strategy used by these larvae with respect to temperature changes. Rather than producing compensatory effects to allow continued, normal glycolytic flux at all temperatures, temperature-modulator effects on the enzyme enhance the direct flux of carbohydrates into sorbitol (a cryoprotectant in this animal species; see p. 26 at low temperatures.

A temperature dependent conformation change resulting in an interconversion between two forms of an enzyme, each exhibiting distinctly different kinetic properties, has been reported as an additional mechanism of immediate compensation, also for insect enzymes. The activity of pyruvate kinase from *Protophormia terranovae* displays cooperativity with respect to phosphoenolpyruvate at 20°C (Hill coefficient, $n_H = 1.5$), but cooperativity appears negligible at 0°C ($n_H = 1.0$) (Fig.1.5). Thus, while the maximum velocity decreases by a factor of 16 at saturating substrate concentrations, at physiological levels of PEP the decrease is only about sevenfold (Wood et al. 1977).

From above results it appears that the picture on immediate temperature compensation is rather complex and that the temperature relationship of any single enzymic reaction can probably not be used as an argument for or against the occurrence of temperature compensation of whole animal metabolism.