

Insect Adaptations to Changing Environments - Temperature and Humidity

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The most important factors in environment that influence the physiology of insects are temperature and humidity. Insects display a remarkable range of adaptations to changing environments and maintain their internal temperature (thermoregulation) and water content within tolerable limits, despite wide fluctuations in their surroundings. Adaptation is a complex and dynamic state that widely differs in species. Surviving under changing environment in insects depends on dispersal, habitat selection, habitat modification, relationship with ice and water, resistance to cold, diapause and developmental rate, sensitivity to environmental signals and syntheses of variety of cryoprotectant molecules. The mulberry silkworm (*Bombyx mori*) is very delicate and sensitive to environmental fluctuations and unable to survive naturally because of their domestication since ancient times. Thus, the adaptability to environmental conditions in the silkworm is quite different from those of wild insects. Temperature, humidity, air circulation, gases and photoperiod etc. shows a significant interaction in their effect on the physiology of silkworm depending upon the combination of factors and developmental stage affecting growth, development, productivity and quality of silk. An attempt has been made in this article to briefly discuss adaptation in insects with special emphasis on the role of environmental factors and their fluctuations and its significance in the physiology of mulberry silkworm, *B. mori*.

Key words: Adaptation, Temperature, Humidity, *Bombyx mori*

Introduction

Adaptation is characteristic of an organism that makes the adjustment of living matter to environmental conditions either in its lifetime (physiological adaptation) or in a population over many generations (evolutionary adaptation). The ability to adopt is a fundamental property of life and constitutes a basic difference between living and non-living matter. Insects display a remarkable range of adaptations to changing environments. The extensive literature on insect seasonality reveals great diversity and complexity in the adaptations that withstand seasonal adversity and synchronize development with the seasons (Danks, 2007). The basic structure of these adaptations lies in the specific nature of the environment, the components of which in insects are reviewed, especially from the viewpoint of aspects to understand the structure and functions of adaptations. The component responses include dispersal (Bale, 2002; Brower, 1995; Genkai *et al.*, 2005) habitat selection and habitat modification (Powell *et al.*, 2006), resistance to cold (Ramlov, 2000), dryness (Dautel, 1999) and food limitation (Anderson, 1974), diapause (Ando, 1974; Nakamura and Numata, 2000), modification of developmental rate (Honek and Kocourek, 1990), sensitivity to environmental signals (Danks, 2005), life-cycle patterns (Danks, 2000), variation in phenology (Chen *et al.*, 2003) and development (Bennett *et al.*, 2005; Danks, 2007) and all these are the result of environmental pressures. Many types of adversity can prevent the activity or threaten the survival of insects viz. adaptations in response to anoxia (Hoback and Stanley, 2001; Hodgkinson and Bird, 2004), as well as to ice scour, spring spates, floods and other extremes or disturbances (Lytle, 2002; Prowse and Culp, 2003). Insects survive the cold of winter by a wide array of adaptations that have been reviewed many times (Bale, 2002; Danks, 2005; Duman *et al.*, 1991). These studies have led to the discovery of more and more elements that contribute to insect cold-hardi-

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ness. The critical element of adaptations to changing environment is how insect respond to the presence or reliability of the environmental signals, not just the environmental conditions (Danks, 2006). Insects use one or combination of photoperiod, temperature, thermoperiod, moisture, food and other factors to assess the current or future suitability for development or reproduction. Since silkworms are poikilothermic animal, environmental conditions in general and temperature and humidity in particular plays a decisive role in the determination of most of the physiological processes of insects.

In this article an attempt has been made to discuss briefly the advances achieved in relation to adaptation of insects to fluctuating environmental factors in general with special emphasis and significance in mulberry silkworm, *Bombyx mori* (L.).

Temperature

Among various environmental elements, temperature is most critical factor, associated closely to climates and effective through lower and upper limits and through heat accumulation. Lower developmental limits for insects average about 10°C or 11°C (Honek and Kocourek, 1990) and in most temperate species upper limits lie between 20°C and 35°C (Danks, 2007). The impact of temperature is modified by habitat and other physical conditions. Many types of adversity can prevent the activity or even survival of insects. Adaptations are known in response to anoxia (Hoback and Stanley, 2001; Hodgkinson and Bird, 2004) as well as to spring spates and other extremes or disturbances (Lytle, 2002; Prowse and Culp, 2003). However, abundant information is available on survival of insects in cold, dryness and lack of food. The adaptation of insects to cold hardiness has been reviewed by various workers (Bale, 2002; Danks, 2005). These studies have

led to the discovery of more and more elements that contribute to insect cold hardiness and the same are summarized in Table 1.

Insects survive the cold of winter by a wide range of adaptations that have been reviewed many times (Bale, 2002; Danks, 2005) and elements of these responses are compiled, summarized and presented in Table 2. Insects can be injured at low temperatures of 0°C (chilling injury) (Turnock and Fields, 2005). Injury from cold appears to be associated with a breakdown of membrane structure and membrane based ion gradients (Zachariassen *et al.*, 2004); compromised protein structure and enzyme function. Adaptation to temperature below 0°C, include modification in the saturation of membrane fatty acids to maintain function at low temperatures (Bennett *et al.*, 1997; Ohtsu *et al.*, 1998). Shock proteins (Craig *et al.*, 1993) produced in response to chilling are also presumed to protect against low temperatures (Denlinger, 1991; Relina and Gulevsky, 2003). At temperatures below 0°C, most species remain unfrozen because they supercool. Supercooling is enhanced by solutes of low molecular weight (Lee, 1991; Ramlov, 2000). Many species have more than one kind of solute viz., both glycerol and sorbitol. The freezing process depends on the supply of water molecules / 'bound water' (Block, 2002; Block and Zettel, 2003). The cold hardiness of several species in extreme environments includes marked dehydration (Bennett *et al.*, 2005; Ring and Danks, 1994). Information is also available on the pathways of association of other elements in cold hardiness, such as cholesterol (Yi and Lee, 2005), membrane transport of cryoprotectants and water (Izumi *et al.*, 2006). These observations indicate that cold hardiness is not a static condition instituted for the winter, but a series of complex developmental and metabolic patterns. The effects of cold across different temperature ranges and with different times of exposure interact for both chilling and freezing injury, and there is some repair

Table 1. Elements of insect cold hardiness (*c.f.* Danks, 2006)

Elements	Sample features	Main effects on cold hardiness
Region	Climate, weather	Temperature and its variation, freezing rate
Microhabitat	Choice, site features, modification	Insulation, protection against inoculative freezing, freezing rate, effects of ice
Crystallization	(i) Freezing resistance, freezing tolerance.	Absence or presence of ice
	(ii) External nucleators, internal nucleators, ice nucleating proteins	Ice formation
Water relations	Status, availability, dehydration	Ice amount, un-freezable water, water management and solute interactions, supercooling by dehydration
Cryoprotectants	Solutes, antifreeze proteins, shock proteins	Inhibition or modification of ice or injury
Other molecules	Enzymes, antioxidants	Modification of functions or injury

Table 2. Common mechanism of direct resistance to cold in insects (*c.f.* Danks, 2007)

Mechanism	Sample systems or substances
Survive chilling	
a) Protect membranes or membrane functions	Membrane transition temperature lowered by changing composition of fatty acids
b) Protect proteins, enzymes and other functions Prevent freezing	Potential roles of shock proteins and other molecules
a) Lower haemolymph melting point	Manufacture of low molecular weight solutes including glycerol, other polyhydric alcohols, sugars
b) Lower haemolymph freezing point	Antifreeze proteins lower the freezing point (this action is enhanced by glycerol and other molecules)
c) Reduce nucleation sites	Adaptation in structural proteins etc.
d) Eliminate nucleators	Empty gut
e) Mask nucleators	Nucleation sites masked by nucleation inhibitors
f) Reduce water available for freezing process	Associate water with cell constituents, dehydrate passively by readily losing water through cuticle
Survive freezing	
a) Limit supercooling to reduce impact of freezing events	Manufacture of nucleating proteins, cuticular structure readily allowing inoculation
b) Reduce amount and rate of formation of ice	Manufacture low-molecular weight cryoprotectants
c) Protect membrane	Protection provided by association of various cryoprotectants with membranes
d) Protect other functions	Protection provided by association of various cryoprotectants with membranes
e) Rapid cold hardening	Enhanced resistance caused by brief cooling beforehand
f) Prevent recrystallization	Manufacture of antifreeze proteins, manufacture of specific recrystallization inhibitors
g) Modify water status	Un-reactive glassy states mediated especially by carbohydrates such as trehalose.
Dynamic adjustment	
a) Seasonal adjustments of cryoprotectants / Rapid cold hardening	Solutes and antifreeze proteins change through the winter in some species, changing super-cooling point and other features
b) Freezing induced change in super-cooling point	Freezing exposure lower super-cooling points in some species
c) Linkages with diapause	Diapause is prerequisite for some elements of cold hardiness in some species
d) Seasonal adjustment of mitochondria	Mitochondria reduced during winter cold and inactivity
e) Ongoing repairs	Warmer intervals permit injury caused by cold exposures to be repaired in some species
f) Interaction between antifreeze proteins	Enhance action

of cold injury during winter (Renault *et al.*, 2004; Turnock and Fields, 2005). Diapause is linked to cold hardiness to different degrees. The mechanism of relationship between cold hardiness and diapause is very complex. In several species some aspects of the diapause programme are prerequisite for some aspects of cold hardiness (Hodkova and Hodek, 2004; Danks, 2005; Slachta *et al.*, 2002). Susceptibility to cold shock may also depend on the diapause status (Pitts and Wall, 2006).

Among the various environmental factors that influence

the silkworm cocoon crops, the most important are temperature followed by humidity. Since silkworms are cold-blooded animals, temperature has a direct effect on the growth, development and physiological activity, nutrient absorption, digestion, blood circulation, respiration etc. Temperature plays an important role in egg hatching, larval growth and quality of the cocoons produced, adult fertility etc. With the increase in temperature, the larval growth and development is accelerated resulting reduction in larval duration, cocoons of lower weight and quality,

while at low temperature growth and development is slow leading to prolong larval period, abnormal growth and sensitivity against several diseases. The optimum temperature for healthy growth and higher production of quality cocoon is 26–28°C for 1st instar and 2nd instar, 24–26°C from 3rd to 5th instars and 22–23°C for mounting and 24–25°C for egg incubation. However, for technical reasons, temperature of 25°C is used for all stages. In case of multivoltine strains slightly higher rearing temperature than that required for bivoltine is necessary. The room temperature is generally low during winter and high during summer months and therefore suitable measures are adopted to regulate it to the optimum level. Rearing of 4th and 5th instar larvae at low temperature is better for converting food into silk, since food stays longer in the stomach and the digestion and absorption of nutrient is better. Even though, low temperature has certain advantages, but must be avoided as they decrease the growth rate and increase rearing period affecting the cost of rearing (Upadhyay and Mishra, 1994).

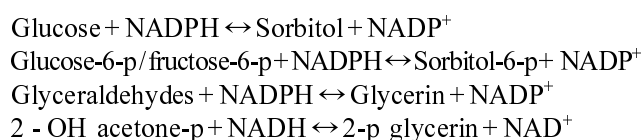
Temperature and embryonic development

The effect of temperature on the development of silkworm has been studied extensively but much attention has not been paid on the effect of temperature on embryonic development. It has been reported that in exothermic organisms, when rate of development is plotted against temperature, a sigmoidal curve is obtained with an almost linear correlation in central temperature range. Attempts have been made to describe the curve more accurately using different mathematical formulas (Bottrell, 1975; Laudien, 1973) but there are also other variables which may affect the rate of embryonic development, such as adaptation phenomenon, genetic variation besides health and age of the organism. Kalthoff (1971) reported that in a given species, different developmental phases may be characterized by different upper and lower limits of temperature and inferred that the correlation between temperature and rate of development may not be the same for all stages of embryonic development. Under extreme environmental conditions, particularly in climates with very hot or cold seasons, a resting stage called 'diapause' (Denlinger, 1985) may ensure the survival of the species. This is important for early embryonic stages, which are often more sensitive to extreme temperatures than any other stages in the life cycle of an insect. However, in some species, sub-zero temperature cannot be avoided and physiological adaptations are required in such cases. While some insects have become freezing tolerant, others have developed mechanism to keep their body below the freezing point, a phenomenon referred as 'supercooling'. The supercooling is common in insect eggs. Eggs of lep-

idopteron *Zeiraphera diniana* may supercool to –51.3°C (Bakke, 1969). Salt (1959) stated that the depression of the freezing point and increased supercooling capacity can be attributed to the accumulation of glycerol in many species. In *Bombyx mori* eggs, at the initiation of diapause, large amounts of sorbitol and glycerol are synthesized from previously accumulated glycogen. After diapause the reaction is reversed and glycogen is synthesized (Yaginuma and Yamashita, 1979).



With the initiation of diapause, the above reaction is right oriented while during termination; it is left oriented (Yaginuma and Yamashita, 1977, 1978). The experiment with ¹⁴C glycine showed that sorbitol is totally derived from glycogen, while glycerin is produced only when glycogen content reached the lowest level. About the mechanism of reversible reaction, following steps exists -



Diapausing silkworm eggs chilled at 5°C for at least three months when transferred to 25°C, the eggs resumed embryogenesis and hatches within two weeks (Yamashita *et al.*, 1988). Soaking the pre-chilled diapausing eggs into hot solution can also bring about hatching (Yamashita, 1984). In these eggs, sorbitol content began to decrease after continuous chilling for at least two months or by treatment with HCl on one month chilled eggs. Greater fall in sorbitol content took place in eggs chilled for longer periods. Conversion of sorbitol to glycogen does not take place if eggs are kept continuously at 25°C (Yamashita *et al.*, 1988) even for more than six months. After diapause, all sorbitol in the eggs is converted into glycogen. During embryogenesis, the main carbohydrate consumed is glycogen. The glycogen-sorbitol-glycogen metabolic process is roughly the same as the process of diapause onset, maintenance and termination. In other words, the change of carbohydrate metabolism of the silkworm eggs is a close relative to the phenomenon of diapause.

Temperature and physiological effects: Temperature is a parameter in developmental cycle which can be manipulated experimentally but their effect is very complex for interpretation. The physiological explanation for embryonic death after exposure to lethal temperature is likely to be highly complex and probably species specific. Kittlans (1961) stated that temperature above 33°C leads to abnor-

mal development in one species of *Leptinotarsa* and two species in *Epilachna* and suggested that at these temperatures yolk cannot be degraded with high rate of development and as a consequence, undegraded yolk comes to lie in abnormal positions and thereby disturbs normal morphogenesis. On the other hand, when these species were kept at 10°C (just above the lower lethal temperature) the yolk reserves become depleted prior to hatching and embryos died. On the cuticular level, extreme temperatures may induce drastic physiological changes with the result that development becomes abnormal. Silkworm eggs can be induced to undergo thermal androgenesis when exposed to 40°C during the meiotic division. The heat-shock phenomenon has attracted much attention as a model system for the induction of specific genes by exogenous treatment. When *Drosophila* cells are exposed to 37°C, a small number of so called heat-shock genes become activated almost immediately. Cold treatment of embryos may also cause embryonic death or abnormal development. Cold treatment of *Bombyx mori* silkworm eggs leads to formation of tetraploid individuals which lay large eggs (Kawamura and Nakada, 1981).

Temperature and food consumption and utilization: Variations in the fluctuations of temperature prevent insects from attaining their physiological potential performance and they achieve it only if placed in an ideal and favorable environment. As a consequence of natural selection imposed by less ideal environmental conditions, insects have evolved certain abilities to evaluate their environment and to make decisions involving physiological, behavioral and genetic responses. These responses frequently involve changes in the consumption and utilization of food, rate and time of feeding behavior, metabolism, enzyme synthesis, nutrient storages, flight behavior and other physiological and behavioral process. Natural environments exhibit large amount of variation in abiotic components (temperature, humidity etc.) which play an important role on the consumption and utilization of food. Variation in environmental factors away from the conditions that allows insects to achieve their ideal performance may reduce their performance unless compensated for by changes in their physiology and behavior. Variable temperature regimes may influence performance differently compared to constant temperature; growth performance is often stimulated in fluctuating temperature regime (Scriber and Slansky, 1981). Insects have also evolved various enzymatic and metabolic adaptations that allow them to survive and develop in a broad range of temperatures. Temperature acclimation, physiological and behavioral thermoregulation allows individual insects to compensate to various degrees for changes in ambient temperature (Heinrich, 1981).

Temperature and biochemistry of digestion

In a series of investigations carried out at different constant temperatures on *Tenebrio molitor* and *Morimus funereus* beetles, established that long-term adaptation is mediated via the retrocerebral complex (affecting cytological state of cerebral neurosecretory cells) and is a programming of the steady state level of mid-gut proteolytic and amylolytic activities. These two beetle species differ in their temperature requirements viz., *T. molitor* develops well at 23°C and *M. funereus* at 13°C. The effect of temperature along with starvation / ligation, on the digestive physiology of these beetles indicated that retrocerebral trophic factor (stimulating mid-gut protease) is involved in the process, while mid-gut amylase synthesis is apparently independent of this factor. Protease decreases in *Tenebrio* larvae when kept at high temperature and extracts of retrocerebral complex elevate mid-gut protease in ligated or temperature inactivated larvae. These results demonstrate a positive control of mid-gut protease activity, resulting from either reversible inactivation of the cerebral neurosecretory mechanism by temperature or physiological separation from the retrocerebral source as by ligation.

Temperature and fecundity

The rate of egg production varies with temperature; accelerated up to a point and then falls-off rapidly (Mathur and Lal, 1994). But the temperature limits between which reproduction can occur are often much narrower than the range of temperature over which the other activities of the same species remain normal. Females of *Locusta migratoria* fail to mature their eggs when the day and night temperature alternate between 30°C and 20°C, *Pediculus* will not lay eggs below 25°C, *Anopheles quadrimaculatus* will not lay below 12°C. The male seems to be more sensitive than females to abnormal temperatures. When *Euchalcidia caryobori* exposed to 16°C for 10 days, the female still laid the total number of eggs, but 70% of males were sterile. In *Drosophila* kept at 32°C, 50% of the females and 96% of males were sterile. These males were able to copulate but no ejaculation of sperm occurred as the sperm in the male organs lost their motility and subsequently degenerated. In the silkworm, *Bombyx mori*, maximum ovulation and fecundity with minimum retention was observed at temperature $25.36 \pm 0.17^\circ\text{C}$ (optimum) and any fluctuation from optimum level decreased ovulation, oviposition, fecundity and increased retention of eggs (Mathur *et al.*, 1988).

Temperature and embryonic diapause

A critical element of adaptations is how insects respond to the presence and reliability of environmental signals, not just the environmental conditions themselves. Insects use

Table 3. Features of environmental signals used by insects

Element	Description	Sample factors and properties
Reliable	Correlated with seasonal position and possibility of seasonal change	Photoperiod, thermo-period reliable, food often reliable, other factors mostly unreliable
Frequent	Available for regular monitoring	Temperature, moisture, photoperiod, thermo-period frequent, other factors mostly intermittent or variable
Recognizable	Sensors available and seasonal rate of change high	Sensors available for most factors but most factors with low rates of change

Table 4. Effects of temperature and photoperiod on egg diapause in bivoltine breeds of *Bombyx mori* (*c.f.* Singh and Samson, 1999)

Temperature and photoperiod during incubation		Temperature °C		Resulting moths laying diapause and non-diapause eggs
		Developmental stage		
		I – II	IV - pupal	
25°C	Light	25 or 20	25	Diapause
	Dark	25 or 20	25	Diapause
20°C	Light	25 or 20	25 or 20	Diapause
		20	25	Diapause << Non-diapause
	Dark	25	25	Diapause < Non-diapause
		25	20	Diapause >> Non-diapause
15°C	Light	20	25	Diapause < Non-diapause
		25	25	Diapause > Non-diapause
		25	20	Diapause >> Non-diapause
	Dark	25 or 20	25 or 20	Non-diapause

Note: Light -16 hrs light and 8 hrs dark; Dark -16 hrs dark and 8 hrs light

one or a combination of photoperiod, temperature, thermo-period, moisture, food and other factors to assess the current or future suitability of habitats for development or reproduction. The best cues are reliable, frequent and easily recognized (Table 3).

The determination of diapause is almost maternal in mulberry silkworm (*Bombyx mori*), i.e. temperature and photoperiod are most efficient at the embryonic stage of previous generation (Table 4, 5) and only supplemental in the post-embryonic stages. The sensitive embryonic stages begin just after blastokinesis. Incubation of bivoltine eggs at high temperature results in induction of diapause in the next generation and low temperature incubation results in the production of non-diapause eggs (*c.f.*, Yokoyama, 1973). Incubation as low as 15°C causes production of non-diapause eggs in the next generation, whereas, diapause eggs are induced by incubation at 25°C. When eggs are incubated at intermediate temperature of 20°C, the developmental fate remains undetermined in the embryos. High temperature at younger larval stages and low temperature at late larval stages acts to induce diapause eggs (Tazima, 1978). Egg diapause is regulated by photoperiod as well as temperature during embryonic stage of the female and is completely independent of photoperiod during post-embryonic development. Thus, pho-

toperiod becomes effective in regulation to development only when eggs are incubated at an intermediate temperature. In these eggs, long photoperiod causes induction of diapause and short photoperiod non-arrested state of development. Chilling is generally effective in terminating diapause. Chilling is reported to reduce water absorption by diapause eggs of *Chorthippus brunneus*. In *Leptopterna dolabrata* (mired bug), chilling elevates oxygen uptake in diapause eggs, thereby indicating a gradual transition from diapause to post-diapause (Braune, 1976), and chilling also elevates glycerol content in diapause eggs of melon beetle, *Atrachya menetriesi*.

Humidity

Water forms a large proportion of insect tissues and survival depends on the ability to maintain and to balance water in the body. There is no limiting range of humidity and most insect can develop at any humidity provided they are able to control their water balance. The water content in insects ranges from less than 50% to more than 90% of the total body weight and there may be much variation within the same species even when reared at identical conditions (Mathur and Lal, 1994). In *Calandra*

granaria, the water content is only 46–47%, while in *Talea polyphemus* caterpillar, it amounts to 90–92%. The water content in mulberry silkworm larvae ranges from 77–79% by weight, but adults have only 64–69% water. In *Popillia japonica*, the larvae contain 78–81% of water, the pupae 74% and the adult 67%.

Humidity and rearing: Abiotic factor that has significant impact on the performance of insects in terrestrial environments is humidity. Humidity interacts with the availability of free water and with the water content of the food. Demands in humidity vary depending on the biological circle. Humidity mostly shows indirect effect on growth and development. In silkworm, humidity influences physiology through withering of leaves and sanitation of rearing beds. Under too dry conditions, the leaves wither very fast and become unsuitable for feed, resulting in retarded growth of larvae which makes them weak and easily susceptible to diseases and other adverse conditions.

Ordinarily high relative humidity during rearing of young age silkworm larvae results in lesser loss of silkworm larvae than low relative humidity. During late age silkworm rearing, low humidity reduces their loss. However, during moult and throughout the moulting phase, the humidity should invariably be maintained slightly lower than in the rearing period. Humidity during rearing phase of 1st, 2nd, 3rd, 4th and 5th instar should be maintained at 85%, 85%, 80%, 75% and 70% respectively for healthy growth of larvae and good quality cocoon. During mounting the room should be well ventilated and optimum humidity is to be maintained at 70–75%. During egg incubation, it is important that humidity should be maintained at 80% on an average for normal growth of embryo. If humidity falls below 70% during incubation, the hatching invariably is low. For regulating humidity in the rearing bed, paraffin paper and wet foam pads or paper bands are used. If humidity is too low, sprinkling of water on the floor may be found useful to increase it.

Humidity and embryonic diapause

Ando (1974) classified diapause into three groups based on the requirement of water for embryonic development viz., sufficient water is stored in the egg at the time of oviposition, so that embryogenesis is completed till hatching as in *Bombyx mori*; water is absorbed into egg before diapause establishment and utilized for post-diapause development as in crickets; and water is absorbed mainly after diapause termination for the completion of embryogenesis as in grasshoppers. The mechanism of water absorption takes place through egg shells. In silkworm, *Bombyx mori*, hydrocarbons comprise the major lipid of the egg shell and are believed to take part in water evaporation. Humidity may affect metabolism and hence the rate of

development of insects. *Ptinus* eggs take 15 days to develop at 20°C and a relative humidity of 30%, but at 90% humidity, the incubation period is reduced to 10 days. In *Locusta*, larval development is faster at 70% relative humidity, being slower at lower and higher humidity. Humidity affects diapause nature of eggs and voltinism in silkworm only if the incubation temperature is between 15°C–25°C (Table 5).

Humidity and respiration: The effect of humidity on respiration is complicated by a number of factors, most important of which is the water content of the insect. Humidity effects are very closely associated with temperature effects i.e. water loss by desiccation, spiracular diffusion, retention of ingested water and production of metabolic water. In alternating wet and dry conditions, *Drosophila* have a higher respiration in saturated air than in dry air (Mathur and Lal, 1994). Mealworm subjected to various humidities at temperature from 8°C to 37°C show no variation in CO₂ output. It has been reported that water content of the insects may not be a limiting factor as starved *Papilla* larvae at different relative humidity does not show any effect. Utilization of tissue water is rapid in those insects where lowered respiration and death follows starvation. From these, it can be inferred that changes in humidity have no direct effect on respiration, but indirectly may modify metabolism through temperature, nutrition and metabolic water changes.

Ventilation

Silkworms like any other animal require fresh air for their various physiological activities. The freshness of air can be determined by its CO₂ contents. Due to respiration, carbonic acid gas is released in the rearing bed and rearing room. Although atmospheric CO₂ content is generally 0.03–0.04% in the rearing room but it can increase due to firewood, coal, smoke of fuel, carbon dioxide produced due to fermentation of leaves on rearing beds, respiration of mulberry leaves, carbon mono-oxide, ammonia and sulphur dioxide etc. and these gases are injurious to growth, development and health of silkworm larvae. When built up of these poisonous gases reaches beyond the tolerance limit of silkworm larvae, they start to show the symptoms of sluggishness and even stop to feed. Young silkworm larvae are more susceptible to the poisonous gases and hence artificial circulation of air is extremely useful in bringing down the temperature and humidity besides removing the poisonous gases from the rearing room. Carbon dioxide content exceeding 1% in rearing room is reported injurious for silkworm larval health and the relation between concentration of CO₂ in a rearing room and mortality of silkworm is lin-

Table 5. Relationship between the conditions of incubation, rearing, moulting and diapausing character of resultant egg in bivoltine silkworm breeds (*c.f.*, Tazima, 1978; Singh and Saratchandra, 2004)

Developmental stage	Environmental conditions	Effect	Remarks
During incubation	Temperature		
	High	Diapausing	Whole eggs at 25°C or above
	Low	Non-diapausing	Whole eggs at 15°C or above
	Humidity		
	Humid	Diapausing	Humidity affects voltinism only when incubated at 15~25°C.
	Dry	Non-diapausing	
Early larva	Photoperiod		
	Light	Diapausing	Light (18 hrs or more a day). Photoperiod affects only when incubated at 15~25°C
	Dark	Non-diapausing	In case of 12 hrs darkness a day
	Temperature		
Late larva	High	Diapausing	Only when incubated at 15~25°C
	Low	Non-diapausing	
	Temperature		
Mounting and later	High	Diapausing	Only when incubated at 15~25°C
	Low	Non-diapausing	
	Temperature		

ear (Singh and Saratchandra, 2004). Rajan and Himantharaj (2005) stated that air current of 1.0 m/sec during 5th age rearing reduce the larval mortality and improves ingestion, digestibility, larval weight, cocoon weight and pupation rate compared to those recorded under no ventilation condition. However, 1% CO₂, 1% formaldehyde gas, 0.02% SO₂ and 0.1% NH₃ in the air in rearing house is safer limit.

Photoperiod

Silkworm is an insect of small day with positive phototaxis. It constantly influences the physiology of silkworm. Silkworms are photosensitive and generally have a tendency to crawl towards the dim light. Larvae of silkworm do not prefer either strong light or complete darkness but usually light phase in contrast to the dark phase activates the larvae which prefer light within the range of 15~30 lux. Patil and Gowda (1986) reported that if silkworm larvae are fed in complete darkness their duration is longer and cocoon quality is poor. Rearing in either complete darkness or in bright light leads to irregularity in growth and moulting. Light phase usually makes larval duration longer than the dark phase. Photo phase for rearing of silkworms should be 16 hours light per day fol-

lowed by a Scot phase of 8 hours. It is advisable to rear silkworms in dim light during the daytime and in the dark during at night for healthy growth of larvae. Rearing of silkworms in continuous light delayed growth considerably leading to appearance of pentamoulters and reduced larval and cocoon weights besides cocoon quality.

Future research strategies

Seasonal responses of insects to environmental signals and adaptations to survive under changing situations require detailed simultaneous analyses of natural environments that drive the patterns of response.

Study on long term analysis of habitat variability for a species is required to understand how seasonal adaptations such as prolonged diapause are maintained and governed in a given species.

Different species have different divergent strategies to survive changing environments in the same place. Therefore, multiple simultaneous experimental approaches are needed to identify the components of such strategies.

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