Role of juvenile hormone in the hypermetabolic production of water revealed by the O₂ consumption and thermovision images of larvae of insects fed a diet of dry food

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Abstract. The young larvae of insects living on dry food produce large amounts of water by the metabolic combustion of dietary lipids. The metabolic production of water needed for larval growth, previously known as hypermetabolic responses to juvenile hormone (JH), is associated with a 10- to 20-fold increase in the rate of O₂ consumption (10,000 µl O₂/g/h in contrast to the usual rate of 500 µl O₂/g/h). Growing and moulting larvae are naturally hypermetabolic due to the endogenous release of JH from the corpora allata. At the last, larval-pupal or larval-adult moult there is no JH and as a consequence the metabolic rate is much lower and the dietary lipid is not metabolized to produce water but stored in the fat body. At this developmental stage, however, a hypermetabolic response can be induced by the exogenous treatment of the last larval instars with a synthetic JH analogue. In *D. vulpinus*, the JH-treated hypermetabolic larvae survive for several weeks without moulting or pupating. In *T. castaneum* and *G. mellonella*, the JH-treated hypermetabolic larvae moult several times but do not pupate. All these larvae consume dry food and the hypermetabolic response to JH is considered to be a secondary feature of a hormone, which is produced by some subordinated endocrine organ. The organ is most probably the controversial prothoracic gland (PG), which is a typical larval endocrine gland that only functions when JH is present. According to our hypothesis, PG activated by JH (not by a hypothetical PTTH) releases an adipokinetic superhormone, which initiates the conversion of dietary lipid into metabolic water. This type of metabolic combustion of dietary lipid produces large quantities of endothermic energy, which is dissipated by the larvae in the form of heat. Thermovision imaging revealed that the body of hypermetabolic larvae of *G. mellonella* can be as hot as 43°C or more. In contrast, the temperature of "cold" normal last instar larvae did not differ significantly from that of their environment. It is highly likely that thermovision will facilitate the elucidation of the currently poorly understood hormonal mechanisms that initiate the production of metabolic water essential for the survival of insects that live in absolutely dry conditions.

INTRODUCTION

Previous studies revealed that insect hormones only affect the metabolism (O₂ consumption) of particular target tissues indirectly, during specific developmental periods and according to a genetically programmed schedule (Sláma et al., 1974, for review). In adult females of the firebug (*Pyrrhocoris apterus*), for example, implantation of active corpora allata as a source of juvenile hormone (JH), greatly increases respiratory metabolism only in females with functional ovaries. In ovariectomised females or males, the hormone had no effect on respiratory metabolism (Sláma, 1964).

In the wax moth (*Galleria mellonella*), implantation of active corpora allata into the last larval instar results in a great increase in respiratory metabolism, which is associated with an increase in feeding and growth of giant supernumerary larval instars (Sehnal & Sláma, 1966). When the synthetic analogues of insect JH became available, they were found to cause a great increase in respiratory metabolism in the last larval instar of different insect species (Sláma et al., 1974). In the carpet beetle (*Dermestes vulpinus*), treatment of the last larval instar with JH analogues caused an incredible, 10- to 20-fold

increase in the rate of O₂ consumption (over 10,000 µl O₂/g/h) and is referred to as the hypermetabolic response to JH (Sláma & Hodková, 1975).

Further studies on hypermetabolic responses to JH revealed a number of important facts. For instance: (1) The treatments were most effective when JH analogues were applied at the very beginning of the last larval instar; (2) Hypermetabolic responses to JH never occurred in larvae that were not allowed to feed; (3) The hypermetabolic response was most pronounced when the food contained a lipid and; (4) The greatly increased rates of O₂ consumption were associated with arrested pupal development and no supernumerary larval moults (Sláma & Kryspin-Sørensen, 1979). The virtual absence of developmental changes in hypermetabolic larvae led several authors to think that JH possibly affected the uncoupling of mitochondrial respiration from oxidative phosphorylation (Sláma & Kryspin-Sørensen, 1979; Sehnal, 1984; Němec, 1985).

According to the principles of insect endocrinology (Pflugfelder, 1958; Wigglesworth, 1965; Novák, 1966, 1975; Sláma et al., 1974), centrally produced hormones induce a complex of affiliated actions indirectly, through

a chain of subordinated peripheral organs or peripheral endocrine glands. The strangest endocrine gland in the larval body is the prothoracic gland (PG), whose functions have been greatly misunderstood and misinterpreted. The original, brain-PG theory of Williams (1952) claimed that the PG is the direct target of the brain hormone and its primary hormonal function is to regulate moults and ecdyses. The theory was considerably elaborated and widely disseminated by Gilbert & Schneiderman (1961) and has persisted as the main hormonal concept in insect developoment (Riddiford, 1996), although it was refuted a long time ago (Williams, 1987) by its creator. We found (Sláma, 1983, 1988, 1998) that the brain-PG model does not correspond with reality. The PG of insects has the specific status of a larval endocrine gland, which is strictly under the control of JH (Sláma & Malá, 1984; Sláma, 1998) and not that of a purely speculative PTTH (prothoracicotropic hormone). Apparently, the PG of insects fulfills some other, hitherto unknown endocrine function (Sláma, 1980, 1983), which is completely different from the regulation of moults and ecdyses (Sláma, 1988, 1998). In this work we investigate the possibility that the PG, although under the control of JH, may be involved in the initiation of hypermetabolic responses.

Authors working with larvae of *G. mellonella* noticed a long time ago that jars containing the larvae were noticeably warm (Jindra & Sehnal, 1990). The possibility of an increase in the temperature of hypermetabolic larvae associated with the increase in O_2 consumption (Sláma & Hodková, 1975) prompted us to determine whether they produced heat by using the thermovision imaging that recently became available. This study was made in the hope that the monitoring of the temperature of larvae would be easier than determining their O_2 consumption. The importance of this study was underlined by a possibility that "hot" JH-treated hypermetabolic larvae produce sufficient amounts of metabolic water for their development, which limits larval growth and survival of insects living on dry food. To this end, we reinvestigated the previous data on the O_2 consumption of hypermetabolic larvae (Sehnal & Sláma, 1966; Sláma & Hodková, 1975; Sláma & Kryspin-Sørensen, 1979) and compared the results with new data obtained by thermovision monitoring of larval temperature.

MATERIAL AND METHODS

Larvae of *Dermestes vulpinus* (Coleoptera) and *Galleria mellonella* (Lepidoptera) were obtained from stock cultures maintained at the Research Institute using previously described methods (Sehnal & Sláma, 1966; Sláma & Hodková, 1975). Synthetic analogues of JH were applied topically in a small drop of acetone in dosages of 50 to 100 µg per larva at the beginning of the last larval instar. For inducing hypermetabolic responses in larvae of *G. mellonella* we used methyl-2,7-dimethyl--9-(2-oxolanyl) 2,4 nonadienoate. For larvae of *D. vulpinus* we used topical applications of 50 to 100 µg of ethyl 3,7,11-trimethyl, 11-chloro, 2-dodecenoate (compound No. T-24 in Sláma et al., 1974). These dosages are at least 10-fold greater than the standard ID-50 value. The treated larvae were kept in an incubator in glass jars at 25° to 27°C and supplied with an
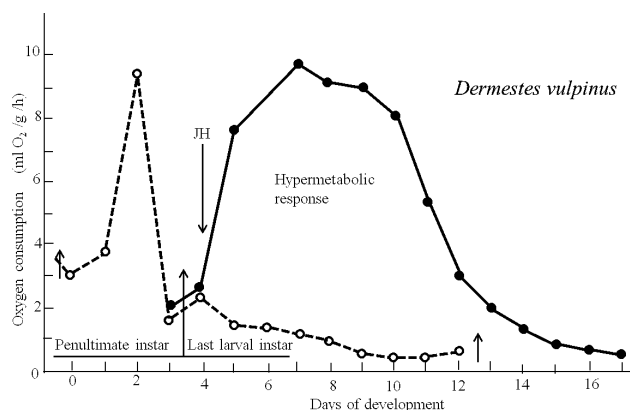
Fig. 1. Hypermetabolic responses in terms of O_2 consumption (full line) induced by topical application of JH analogue to the last larval instar of *Dermestes vulpinus*. Broken line shows the normal course of O_2 consumption. Measurements of larval temperature and water content of the hypermetabolic larvae (see Figs 8 and 9) were made between days 6 and 10 (from Sláma & Hodková, 1975).

abundance of food. The methods used to treat populations of larvae of *G. mellonella* and *T. castaneum* with JH analogues are also described in the legends of Figs 3 and 5.

Methods used for removing PG glands from lavae of *G. mellonella* and measuring the effects of PG extirpation on oxygen consumption are described by Sláma (1983, 1988). Determination of water, lipid, carbohydrate and dry matter content was done using the standard experimental procedures described by Janda et al. (1966) and Němec (1985).

The O_2 consumption of individual hypermetabolic larvae was measured using the scanning microrespirographic method described by Sláma & Denlinger (1992). The temperature changes that occurred in the larvae of *G. mellonella* were monitored using an FLIR infra-red thermovision camera (FLIR B 360 with a 100 µm close-up arrangement, field of view 32 × 24 mm). Thermovision images of larvae in the breeding jars were taken shortly after their removal from the 27°C incubator. The QuickReport software of the FLIR camera produced a detailed analysis of the temperature gradients over the whole thermovision image. The gradients and the calibrated temperature-related colours are provided on the right hand side of each thermovision image in Figs 8 and 9. A large number of additional thermovision images are stored in a database for future evaluation.

RESULTS

Hypermetabolic responses in larvae of *D. vulpinus*

The larvae of *D. vulpinus* were fed on dry calf viscera that contained an abundance of lipid but almost no water. Growth of the young larval instars is very fast, the larval-larval ecdyses occur at less than 3-day intervals. By contrast, duration of the last larval instar without JH, including the whole larval-pupal transformation took much longer, 10 days. Fig. 1 shows the course of O_2 consumption during the penultimate and last larval instars. There is a spontaneous, hypermetabolic rise in O_2 consumption during the juvenile, penultimate larval instar. The rates of O_2 consumption of normal last instar larvae were relatively low. However, the application of a JH analogue results in a great hypermetabolic increase in O_2
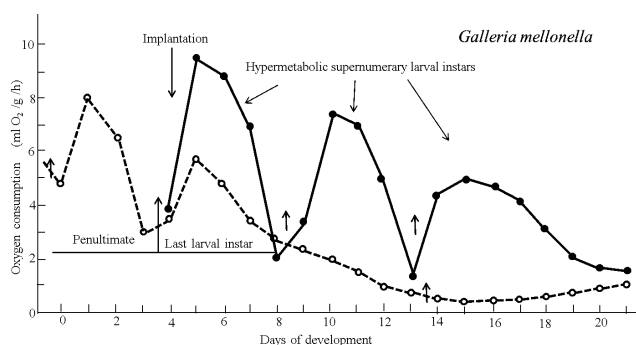
Fig. 2. Hypermetabolic responses in terms of O_2 consumption induced by implantation of corpora allata into the last larval instar of *Galleria mellonella*. The effect of JH is manifested in 3 supernumerary larval, hypermetabolic instars (full line). The normal course of O_2 consumption is indicated by the broken line (arrows indicate ecdyses). Measurements of larval temperature and water content of the supernumerary larval instars (see Figs 8 and 9) were made at intervals of 5 and 7 days (from Sehnal & Sláma, 1966).

consumption. This elevated respiratory metabolism is associated with great increase in food consumption, vigorous ventilatory movements and active locomotory activity. The larval-pupal transformation was inhibited by JH and there were no supernumerary larval instars (Fig. 1).

The hypermetabolic conditions outlined in Fig. 1 were confirmed by the results of the thermovision scanning of larval body temperature, which revealed that: (1) The hypermetabolic larvae treated with JH analogue, with the maximum rates of O_2 consumption indicated in Fig. 1, also had the highest body temperature, occasionally exceeding 40°C; (2) The highest rates of O_2 consumption and temperature increase were directly proportional to the dose of the JH-analogue administered and indirectly proportional to the delay in applying the JH after the last larval ecdysis; (3) Temperature of the jar containing the hypermetabolic larvae was up to 10°C higher than that of the incubator; (4) Hypermetabolic larvae treated with JH analogues at the beginning of the last larval instar survived for several weeks without any supernumerary larval moults or pupations and their bodies became bloated due to the increase in the volume of haemolymph; (5) The hypermetabolic response indicated by both the high O_2 consumption and increase in body temperature were strictly dependent on feeding; starved larvae remained "cold" even when treated with large dosages of JH; (6) Finally, larvae treated with JH analogues remained "cold" when fed a lipid free diet. In summary: there were two kinds of last instar larvae of *D. vulpinus* those treated and not treated with JH, which were, respectively, the "hot" hypermetabolic larvae with arrested pupal development and the "cold" normal larvae that pupated after 10 days.

Hypermetabolic responses of larvae of *G. mellonella*

The long dark hairs that cover the larvae of *D. vulpinus* interfere with thermovision recording. For this reason most of the measurements were made on larvae of *G. mellonella*, which have smooth integuments well suited

Fig. 3. Giant, hypermetabolic supernumerary larval instars of *Galleria mellonella*, induced by treatment of the diet with the JH analogue [methyl 2,7dimethyl-9-(2-oxolanyl) 2,4 nonadienoate; 0.1 mg/g of diet].

for thermovision imaging. Fig. 2 shows an overview of the hypermetabolic responses measured in terms of changes in O_2 consumption. These results were obtained over a long period of time following implantation of active corpora allata as the source of JH. The course of the O_2 consumption curves shows that, as in the case of *D. vulpinus*, the reintroduction of JH activity into a last larval instar produces a large hypermetabolic response. This is manifested by renewed, supernumerary, larval-larval cycles of growth and O_2 consumption, which occasionally exceeded 10,000 µl O_2/g/h. Thermovision recordings revealed corresponding cycles in body temperature, oscillating between 43°C and the relatively low temperature of the surrounding diet.

Repeated thermovisual observations were made on a colony of *G. mellonella* larvae which had for a long time been feeding on a diet to which JH analogues had been added. This treatment induced a permanent larval stage with supernumerary larval instars and failure to metamorphose and reproduce. The affected larvae became highly hypertrophic and hypermetabolic. They consumed large amounts of food, produced large quantities of faeces, became bloated with haemolymph and usually moulted two or three times and ended up becoming giant larvae. Due to their intense hypermetabolic activity, the whole colony heated up to more than 44°C. Fig. 3 shows these overheated, hypermetabolic supernumerary larvae leaving their silk tunnels and crawling to the surface to cool off. Fig. 3 further shows that the persistent presence of JH activity completely inhibited the larval-pupal transformation, including the spinning of a cocoon. The 2nd or 3rd
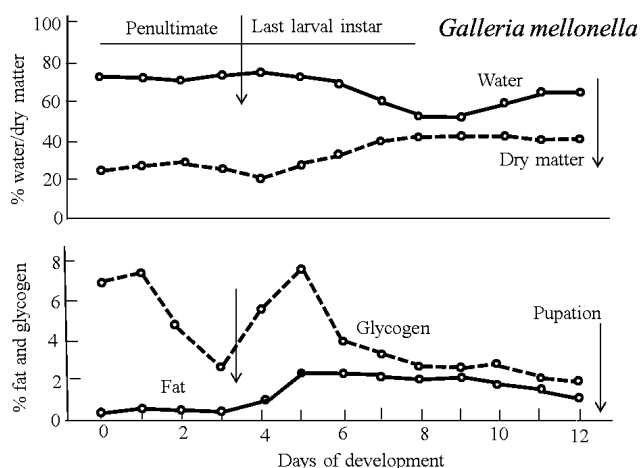
Fig. 4. Changes in the water, dry matter, glycogen and lipid content during the penultimate and last larval instars of *Galleria mellonella* (from Janda et al., 1966).

Fig. 5. The giant hypermetabolic supernumerary larval instars of *Tribolium castaneum* induced by treatment of their diet with the JH analogue (methyl-3,7,11-trimethyl-11chloro-2-dodece-noate; 0.05 mg/g of diet).

supernumerary larval instars ceased feeding and usually survived for a long time without further development.

Thermovisual monitoring revealed that the highest hypermetabolic temperatures of 43°C occurred in the middle of the first or second supernumerary larval instar, which coincides with the maximum consumption of O_2 as shown in Fig. 2. The larval temperatures determined using thermovision are correct, but are relative not absolute values, because the diet was considerably warmed up by the heat dissipated from the larval bodies. For example, in the incubator at 27°C, the temperature of the larval diet was 37°C and that of the hypermetabolic supernumerary larvae was between 37–41°C.

The metabolic differences between the relatively "warm", penultimate instar larvae treated with JH and the "cold", last instar larvae of *G. mellonella*, not treated with JH, have been previously investigated. The results in Fig. 4 show that "warm", hypermetabolic larvae in the penultimate instar, indeed contain more water than last instar larvae, but their lipid content is very low. These findings are in agreement with our present conclusion that hypermetabolic larvae produce water by metabolizing lipids. According to our results, 5-day-old hypermetabolic supernumerary instars that received continuous treatment with JH (shown in Fig. 3) contained less than 0.05% of chloroform-methanol soluble lipid but as much as 80% water ($n = 8$). In contrast, 5-day-old, normal last instar larvae contained 2% lipid and only 68% water ($n = 12$). This shows that larvae feeding on dry food use JH and its subordinated targets to metabolically convert dietary lipid into water. The lipids and other nutrients in larvae in the last instar not treated with JH are assimilated and stored in the fat body for later use during metamoprphosis.

Hypermetabolic responses of larvae of *Tribolium castaneum*

The larvae of some insects (Coleoptera, Diptera, Hymenoptera) treated with JH have relatively short larva-larva (L-L) moulting cycles, which when not treated with JH are usually 3-times longer. These different develop-

mental programmes can never run synchronously in all cells (all or none effect of JH). The final results are either perfect larvae or perfect pupae without intermediate forms. Coleopteran species that consume dry food (*Tribolium castaneum*) have a large and variable number of JH-dependent L-L instars, until they obtain enough water for larval growth and metamorphosis. Treatment of their diet with a JH analogue causes a similar dramatic change in the condition of the colony as in the case of *G. mellonella*. In *T. castaneum* it results in the development of relatively large larvae that are unable to pupate. Fig. 5 shows these large larvae, which are bloated with haemolymph. They survive for a long time in a morphologically perfect larval form unable to pupate or develop into larval-pupal intermediates.

In comparison to normal, fully grown, or "cold" last instar larvae, the hypermetabolic, JH-treated or "warm" supernumerary larvae of *T. castaneum* were 2.5- to 4.2-fold heavier. A group of fully grown normal larvae contained 31.4% dry matter and 68.6% water ($n = 20$), whereas the giant larvae contained only 18.7% dry matter and as much as 81.3% water ($n = 14$). In terms of respiratory metabolism, the O_2 consumption of normal larvae was 500–700 μl O_2/g/h and that of the JH-treated large larvae was on average 9,535 μl O_2/g/h ($n = 8$), with two peak values of 12,000 and 13,400 μl O_2/g/h. Based on the differences in O_2 consumption, the hypermetabolic larvae potentially produced 17 times more metabolic water per unit time than normal last instar larvae.

Prothoracic glands a possible subordinated target of JH

The principal endocrinological role of insect JH is the inhibition of morphogenesis. During larval somatic growth, JH integrates a number of associated physiological functions via subordinated organs or endocrine glands. In the regulation of hypermetabolism the most important target of JH is the prothoracic gland (PG), whose endocrine functions have been seriously misinter-
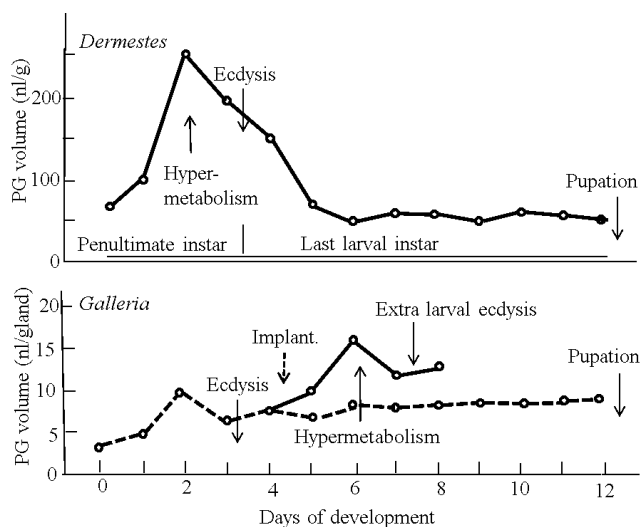
Fig. 6. Changes in the volume of the prothoracic glands during the penultimate and last larval instars of *Dermestes vulpinus* (from Sláma, 1980) and *Galleria mellonella* (from Sláma & Malá, 1984).

Fig. 7. Microrespirographic record of infradian cycles in O_2 consumption of diapausing pupae of *Sarcophaga crassipalpis* (from Sláma & Denlinger, 1992, adapted).

preted (for references see discussion). The data in Fig. 6 provide clear experimental evidence that the PG of *D. vulpinus* and *G. mellonella* larvae are true targets of JH, because these glands can only grow and function in the presence of JH. During the last larval instar, when JH is absent, the PG cells survive until they disintegrate in the prepupal period. In *G. mellonella*, a supernumerary cycle of growth and endocrine activity of the PG was induced in the last larval instar by implanting corpora allata (Fig. 6). And, as a matter of fact, this treatment also results in a hypermetabolic cycle (Fig. 2). Based on these data, we redefine the endocrine status of the PG, as a typical larval endocrine gland whose functions are regulated by JH, not PTTH.

Existence of hypermetabolic responses independent of feeding

The metabolic conversion of dietary lipid into water raises a lot of new questions. A few of them can be formulated as follows: (1) Can a hypermetabolic response occur in phytophagous insects whose food is rich in water? (2) Is the PG and hypermetabolic responses to JH needed in the adult stages of insects that also consume dry food (cockroaches, grasshoppers)? (3) Is only dietary lipid used as a substrate for the hypermetabolic production of water? and (4) Can an insect in a nonfeeding stage with a low metabolic rate use lipid stored in their fat body for the hypermetabolic production of water?

The last question can be answered positively by the so far little understood infradian cycles in O_2 consumption that were recorded in diapausing pupae of the flesh-fly (*Sarcophaga crassipalpis*) by Denlinger and his co-workers. Fig. 7 shows that these infradian cycles in O_2 consumption can be characterized by relatively large peaks in respiration, which are repeated every 4 days. These peak values are more than 10 times greater than the basic respiratory rate, which is a common symptom of a
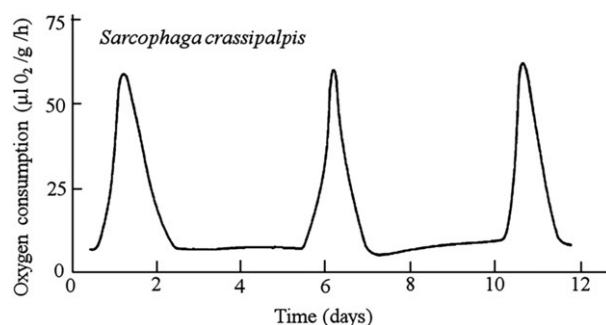
hypermetabolic response. Evidently, during the long period spent in diapause, the pupae used a homeostatically controlled mechanism to compensate for respiratory water loss by the hypermetabolic production of water every 4 days. The R.Q. of 0.7 confirmed that the substrate used for the metabolic production of water in this case was also a lipid.

Detection of hypermetabolic responses by thermovision

The discovery of the hypermetabolic production of water came from measurements of O_2 consumption (Figs 1 and 2). We anticipated, but never confirmed it by direct experimental evidence, that a massive burning of fat should produce endothermic energy that ought to be dissipated as heat. Theoretically, most insects use aerobic respiratory metabolism, whose end products are carbonic acid and water. Under usual metabolic conditions (O_2 consumption 500–1000 µl /g/h), the water obtained from oxidative metabolism may be used to compensate for respiratory water loss, but is insufficient for larval growth. Under these conditions, endothermic energy is conserved and stored in the form of macroergic phosphate bonds by oxidative phosphorylation.

In contrast to the usual metabolic conditions cited above, the hypermetabolic larvae consume 10- to 20-times more O_2. This results in amounts of endothermic energy that cannot be fully converted into macroergic phosphate bonds and need to be dissipated from the mitochondria of the metabolising cells as heat, which can be easily recorded using a thermovision camera. The current thermovision cameras can easily distinguish the "warmer", hypermetabolic larvae from the relatively "cold", normal larvae. In this study we used thermovision to record the temperatures of a number of insect larvae. The final results will be published elsewhere. For practical reasons and limited space we show in this preliminary report only selected thermovision images of larvae of *G. mellonella*.

The first image (A) in Fig. 8 is a good general description of the thermovision method used in this study. It shows the increased body temperature (38°C) of a relatively small, naturally hypermetabolic penultimate larval instar of *G. mellonella*. This larva is at the same stage of O_2 consumption as that indicated by day 1 in Fig. 2. The
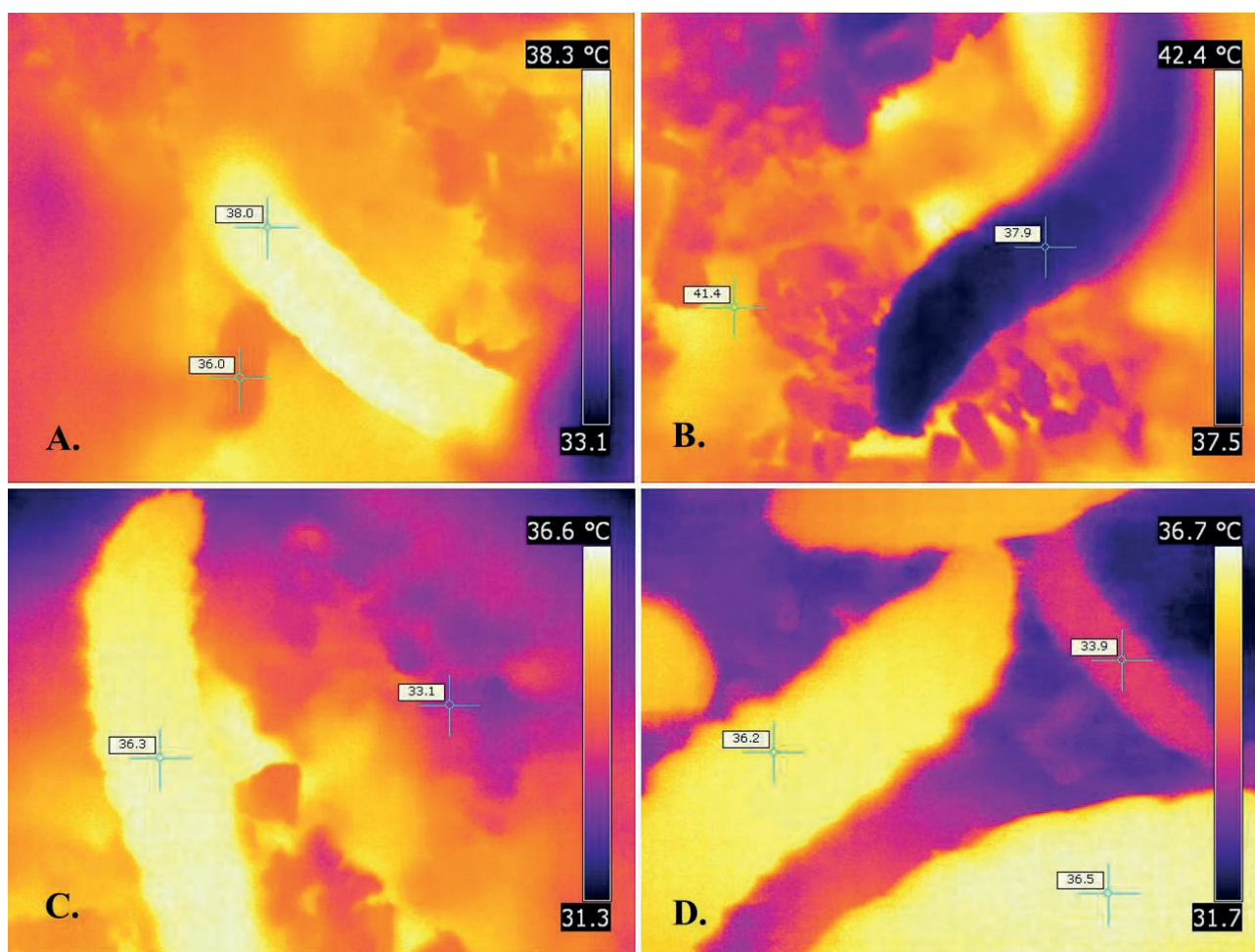
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Fig. 8. Selected thermovision images showing hypermetabolic larvae of *G. mellonella* feeding on a diet that were taken using a FLIR thermovision camera. The gradient in the colour scales on the right hand side of the images shows the selected range of temperature/colour conversion. Images (A) and (B) record the elevated body temperature of the hypermetabolic penultimate instar larvae. Images (C) and (D) show increased body temperature of the giant, hypermetabolic supernumerary larvae produced by adding a JH analogue to their diet (see Fig. 2). Temperature of the room in which the larvae were kept was 27°C.

scale of the temperature gradients on the right hand side of the figure (calibrated from 33.1°C to 38.3°C) can be identified by the different colours. Note that the background temperature of the diet was also elevated due to the heat dissipating from the hypermetabolic larvae. There is a smaller and somewhat colder larva (36°C), which may not be feeding or has recently moulted, underneath the hypermetabolic larva. The temperature of the colony was much higher than that of the incubator in which the colony was kept (27°C).

Image (B) in Fig. 8 shows a "hot" hypermetabolic penultimate larval instar (37.9°C), which has crawled on to the surface of the diet (41.1°C) in order to cool down. The scale of temperatures on the right hand side of the figure (from 37.5°C to 42.4°C) illustrates the versatility of the method for distinguishing the slightly cooler larva (recorded in blue) from the relatively warmer backround. Image (C) in Fig. 8 shows the elevated body temperature of a hypermetabolic larva (36.3°C) of *G. mellonella* produced by treatment with a JH analogue. This larva is at the same stage of O_2 consumption as that recorded on day 6 in Fig. 2. The relatively high background temperature

of the diet (33.1°C) was due to the heat dissipating from the hypermetabolic larvae. Image (D) in Fig. 8 shows the elevated body temperature of two supernumerary hypermetabolic larvae of *G. mellonella* (36.2 and 36.5°C), at the same stage of O_2 consumption as that recorded on day 11 in Fig. 2. The smaller and relatively colder (33.9°C) larva was presumably at a stage of development just prior to ecdysis.

The thermovision image (E) in Fig. 9 indicates that the body temperature (35.1°C) of the giant hypermetabolic larva of *G. mellonella* was elevated and that it is bloated with haemolymph due to its increased water content (it corresponds to the stage of O_2 consumption in Fig. 2 indicated by the full line on day 10). In contrast, image (F) in Fig. 9 shows the body temperature of "cold", normal last instar larvae (corresponding to the stage of O_2 consumption in Fig. 2 indicated by the broken line on day 10). Unlike the "hot", hypermetabolic penultimate instar larvae, the temperature of normal last larval instars is close to ambient, which makes them poorly discernible by thermovision. Image (G) in Fig. 9 provides an example of a "cold" larva of *G. mellonella*, whose O_2 consumption is at
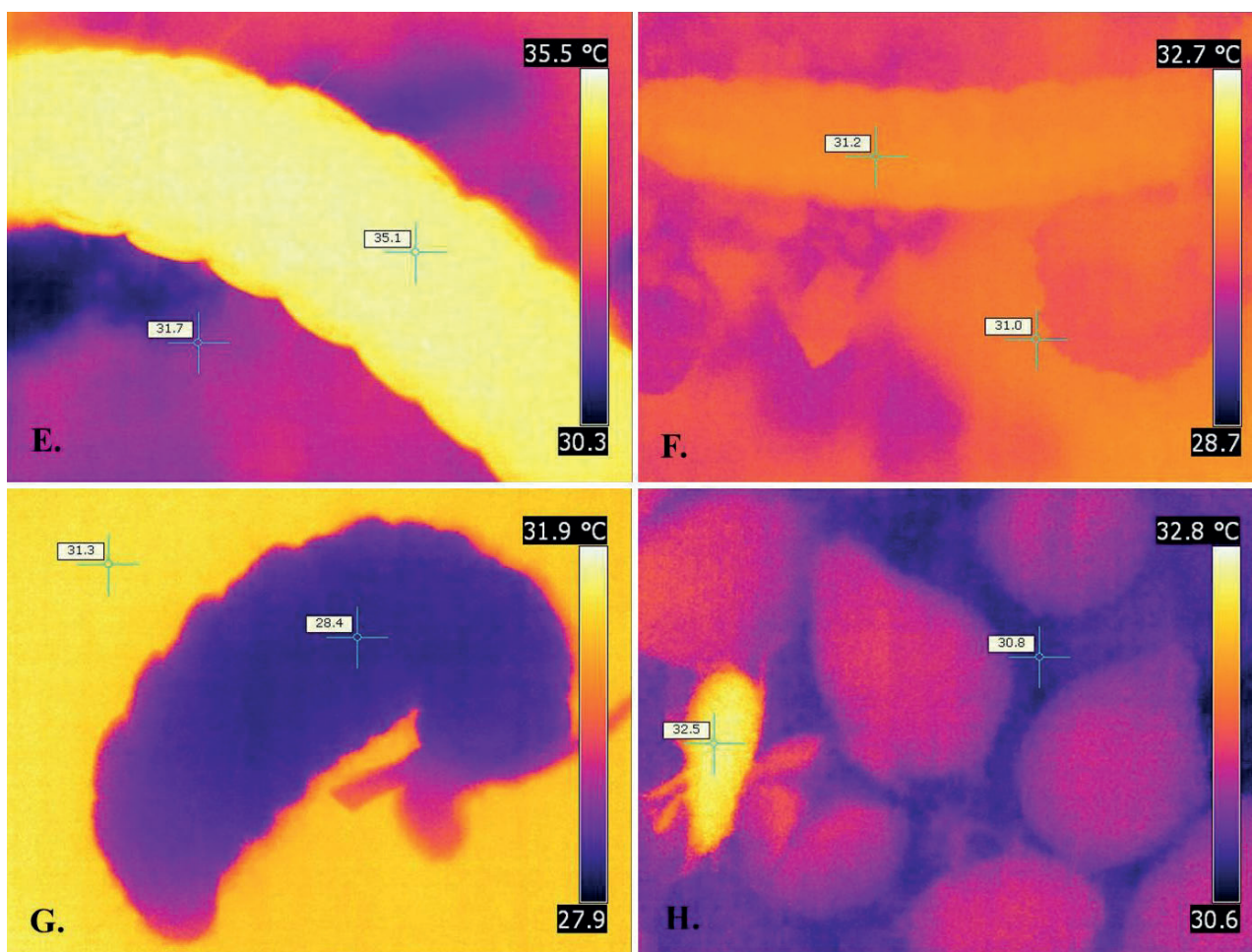
Fig. 9. Selected thermovision images of the body temperature of *G. mellonella* (E to G) and *Pyrrhocoris apterus* (H). E – record of the temperature of a giant hypermetabolic larva, 6 days after treatment of the last larval instar with a JH analogue. F – that of a similar aged "cold" untreated last larval instar. G – that of a ligatured, "cold" last instar larva transferred to filter paper for better visibility; H – that of the elevated body temperature of a naturally hypermetabolic, 4th instar larva of *P. apterus*, fed on dry linden seeds and not provided with water.

a minimum, which was obtained by ligaturing off the head before spinning. These "permanent" larvae survive 3 months without development. In this case the treatment with high doses of JH analogues does not result in a hypermetabolic rise in O_2 consumption.

The thermovision image (H) in Fig. 9 provides an example of the existence of hypermetabolic responses in exopterygote insects. The warmer (32.5°C), hypermetabolic penultimate 4th instar larva of a fire bug (*Pyrrhocoris apterus*) was reared on dry linden seeds (30.8°C) and not supplied with water. The seeds contain an abundance of plant oil, which can be combusted and converted into metabolic water. Preliminary measurements revealed that the body temperature of specimens provided with water is never so high. We conclude that thermovision imaging may provide an easier way to identify water producing, hypermetabolic insect larvae than measuring the rates of O_2 consumption and CO_2 release. Based on the above data, we think there is an endocrine center, whose secretion of an adipokinetic superhormone is controlled by JH and that it is this hormone that is responsible for the hypermetabolic conversion of dietary lipid into water.

DISCUSSION

Insects are poikilothermic invertebrate animals whose body temperature is similar to that of their environment. It is known, however, that ectothermic insects can occasionally cool down by evaporating water or warm up by intensive muscular activity (Wigglesworth, 1965; Heinrich, 1993). These examples of insects regulating their temperture have been the subject of numerous publications and review articles (reviews by May, 1979; Heinrich, 2007; Jones & Oldroyd, 2007). Perhaps the best example of heat production by insects are overwintering honey bees, which under freezing conditions can heat up bee hives to 30°C or more. They do this by metabolizing carbohydrates (Lindauer, 1954; review by Jones & Oldroyd, 2007). Investigations using thermovision revealed that the thoraces of resting bees are warmer than their abdomens (Kovac et al., 2007). Another example of heat production in insects can be found in sphingid moths, which warm up their flight muscles before take-off (review by Heinrich, 1993, 2007). These examples of heat production are based on well founded physio-

logical reasoning. In contrast to this, our findings of marked heat production by hypermetabolic larvae of *D. vulpinus*, *T. castaneum* or *G. mellonella* would appear to be the unwanted side effect of a more important physiological process, i. e. supply of water for larval growth.

There are several ways in which a growing insect larva can obtain sufficient water for its growth: (1) Dietary intake of water with food; (2) Absorption of water from the environment; (3) Metabolic water obtained by regular respiratory metabolism and, as we have now demonstrated; (4) Hypermetabolic production of large amounts of water by oxidative combustion of dietary lipid or carbohydrate. Regular levels of respiratory metabolism also result in the production of small amounts of water, but this is mainly used to compensation for that lost during respiration (Kuznetzoff, 1953). Our results indicate, however, that a hypermetabolic larva by using some 12- to 40-times more oxygen per unit of body mass produces 12- to 40-times more water. It is known (Sláma & Hodková, 1975; Sláma & Kryspin-Sørensen, 1979) that the combustion of 1 mg of a trilinoleate lipid by *D. vulpinus* larvae requires 1.97 ml of O_2, and results in the exhalation of 1.46 ml of CO_2 and production of 0.95 mg of metabolic water. In other words, one gram of hypermetabolic *D. vulpinus* larvae utilize 10 ml of oxygen per hour, oxidize 5 mg of triglyceride and what is more important produces 4.75 mg of metabolic water per hour (Sláma & Kryspin-Sørensen, 1979).

The increase in water content of JH-treated hypermetabolic larvae of *D. vulpinus* was experimentally demonstrated by Němec (1985). Caterpillars of the last instar of a phytophagous noctuid species (*Spodoptera*), which receive sufficient water from the plant food, do not respond to JH by hypermetabolic responses in O_2 consumption (Kryspin-Sørensen et al., 1977). They showed no hypermetabolic responses in spite of other typical symptoms of JH action, such as supernumerary larval instars, increased growth of the last instar larvae and inhibited pupation. This shows that larvae that obtain enough water from their food do not have to burn lipids stored in their fat body.

According to Němec (1985), the endothermic energy obtained from hypermetabolism exceeds the limits of oxidative phosphorylation, because the large accumulation of ATP becomes a rate-limiting factor of cellular intermediary metabolism. Similar conclusions about the uncoupling of oxidation from oxidative phosphorylation were drawn by Chefurka (1978), who considered JH analogues as novel uncouplers of oxidative phosphorylation with protonophoric activity. According to Sehnal (1984), hypermetabolic responses induced in *D. vulpinus* by JH analogues (Sláma & Hodková, 1975; Sláma & Kryspin-Sørensen, 1979) could be considered as typical uncoupling symptoms. It is argued by Karlson (quoted in Sehnal, 1984), however, that the uncoupling of mitochondrial oxidation from oxidative phosphorylation has never been identified as part of some general physiological process. Relationships between the production of metabolic water and heat dissipation in larvae of *G. mellonella*

were extensively investigated by Jindra & Sehnal (1990). They found that the last instar larvae respond to a decrease in dietary water by reducing their energetic net growth efficiency from 42 to 36%. This was mainly associated with an increase in the production of metabolic water and the uncoupling of mitochondrial respiration from oxidative phosphorylation. Unfortunately, these studies were made only on the "cold" last instar larvae that lacked JH, stored the dietary lipid in their fat body and did not use it for the hypermetabolic production of water.

Proving that the PG is a secondary target of JH and is involved in hypermetabolic responses presents an experimental challenge. The story of the PG hormone starts with Williams (1952), who proposed a theory for the regulation of insect development, known as the brain-prothoracic gland theory. The theory was championed by Gilbert & Schneiderman (1961) who elaborated a model in which PG regulates moults and ecdyses after being activated by a special prothoracicotropic hormone (PTTH). Karlson (1966) suggested that the hormonal function of PG could depend on the secretion of ecdysone. These widely accepted hormonal schemes were challenged when Delbecque & Sláma (1980) described an autonomic, brain-independent regulation of moults and demonstrated that the production of the PG hormone is independent of the level of ecdysone in an insect's body. Moreover, Sláma (1983) successfully removed the PG from a large number of penultimate instar larvae of *G. mellonella* and this had no effect on the timing of larval or pupal moults. The effect of PG extirpation on O_2 consumption has been carefully investigated in the last larval instar of *G. mellonella*, where its removal had no effect on the rate of O_2 consumption (Sláma, 1983), which is fully consistent with our recent conclusions that PG is virtually inactive during the last larval instar. Finally, Sláma & Malá (1984) have demonstrated the functional subordination of PG to JH (not to some hypothetic PTTH as is still generally believed).

In 1987, Williams reported a massive production of ecdysteroids in the pupal midgut of *M. sexta*, which did not involve the PG. This led him to refute the validity of his original, brain-PG theory, which was never accepted by his students (Riddiford, 1996; Willis, 1996; Goodman & Granger, 2009). The new endocrine status of PG was defined as an endocrine gland subordinated to JH, which in insects is part of the autonomic neuroendocrine system analogous to the adrenal glands of vertebrates (Sláma, 1998).

The above listed, controversial functions of insect PG are still incomplete as they do not include the many speculations about the secretion of ecdysteroids. As far as the hypermetabolic responses to JH is concerned, however, ecdysone and ecdysteroids are not relevant, because they drastically inhibit the rate of O_2 consumption in larvae that are still feeding (Sláma & Kryspin-Sørensen, 1979). The low content of lipid and high content of water recorded in hypermetabolic larvae (Sláma & Hodková, 1975; Sláma & Kryspin-Sørensen, 1979) are fully consis-

tent with the experimental results of Janda et al. (1966) and Němec (1985). Although only lipids appear to be the exclusive substrate for the hypermetabolic production of water in *D. vulpinus* (Sláma & Kryspin-Sørensen, 1979), this fact may not be true for all insects. During winter honey-bees heat the colony by metbolizing honey, i.e. carbohydrate. The diet of *G. mellonella* larvae also contains abundant carbohydrate and wax, which are potential candidates for the hypermetabolic production of water in this species. Diapausing pupae of the fleshfly, *S. crassipalpis*, which exhibit a 4-day infradian hypermetabolic cycle in O_2 consumption (Denlinger et al., 1972; Sláma & Denlinger, 1992), also use JH for metabolic stimulation (Denlinger et al., 1984) and a lipid stored in the fat body is used as the fuel (Sláma & Denlinger, 1992).

The new method of thermovision imaging (Figs 8 and 9) provides a new way of studying thermoregulation in insect physiology. The easy distinction and segregation of "hot" hypemetabolic penultimate larvae from "cold" larvae will help identify specimens that have ceased producing JH and are ready to pupate. In this study we show that thermovision techniques can be used to study survival of insects that feed on dry food. The finding of "cold" last instar larvae apparently lacking JH and "hot", hypermetabolic larvae produced by JH, supports the basic endocrinological conceptions of Novák (1966, 1975), Sláma (1975, 1980) and Sláma et al. (1974), which are based on the virtual absence of JH in the last larval instars of all Exopterygote and Endopterygote insects.

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