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Respiratory metabolism of the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae)

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Abstract. The respiratory metabolism of different polyphenic forms of the pea aphid, including wingless and winged asexual females (virginoparae), sexual females (oviparae) and winged or wingless adult males, was investigated using a micro-respirographic method. The records revealed sub-nanoliter amounts per min of O_2 consumption or CO_2 output. Respiratory metabolism of individuals was monitored for 3 to7 h after removal of the aphid from the food plant. Most of the recordings were for relatively large (3.5 mg), wingless asexual females (virginoparae). These aphids exhibited a continuous and very regular respiratory gas exchange (example: specimen of 3.5 mg body mass consumed 180 nl of O_2 per min; released simultaneously 300 nl CO_2 per min; = standard metabolic rate of 3085 µl O_2/g/h; R.Q. = 1.66). This continuous pattern of respiration occurred only when the aphids were kept at relatively high humidity. By contrast, aphids of various seasonal forms exhibited discontinuous respiratory gas exchange when kept in relatively dry air (atmospheric, room conditions). These patterns can be briefly described as follows: (a) Short and rather small micro-cycles of CO_2 emission, manifested usually by the sudden expiration of 60–120 nl of CO_2 once every 5 min; (b) Larger bursts of 240–480 nl of CO_2 with a periodicity of one hour; (c) Enormously large, discontinuous bursts of 10–14 µl CO_2, duration 10–30 min, repeated with a periodicity of several hours. There was no constant pattern of diffusive CO_2 emission (DGC). The aphids exhibited a pattern of CO_2 release that was appropriate for the external conditions, such as temperature and humidity, and internal physiological conditions such as metabolic activity, availability of reserve substances (carbohydrate, lipid) and water. Certain stages (wingless virginoparae) exhaled volumes of CO_2 greatly in excess of their O_2 consumption (R.Q. over 1.5). Sudden exhalations of CO_2 from the body were a consequence of a bulk production and outflow of CO_2 and not due to the diffusion of CO_2 previously accumulated within the tracheal system. Due to their generally high metabolic activity (1142 to 6780 µl O_2/g/h), aphid tissue and organs produced relatively large amounts of metabolically formed carbonic acid. The resulting respiratory acidaemia was moderated by outbursts of gaseous CO_2, liberated from liquid carbonate buffers by a regulatory mechanism based on enzymatic hydration and neutralization of carbonic acid by discontinuous formation of gaseous CO_2.

INTRODUCTION

A widely accepted 90-year old diffusion theory postulates that insects can easily satisfy their respiratory requirements by the simple diffusion of respiratory gases through spiracles, without the necessity of special ventilatory movements. The theory, generally known as the "diffusion theory of Krogh" (Krogh, 1920), is still used as a working hypothesis for discontinuous cycles in CO_2 release (Lighton, 1996; Chown et al., 2006; Bradley, 2007 for a review). According to this concept, spiracular valves automatically open in response to an increased internal concentration of CO_2, which then diffuses out of the body. This mechanical interpretation of insect respiration, which completely ignores the escape of water vapour, is still frequently used as a theoretical basis for the interpretation of discontinuous cycles in CO_2 release. The cycles, known also as the OCF cycles (for open, closed and "fluttering" spiracles) or discontinuous gas cycles (DGC), were recently described by authors using flow-through, infra-red methods of CO_2 analysis (reviews by Lighton, 1996, 2008; Klok & Chown, 2005; Marrais et al., 2005; Bradley, 2007). The flow-through methods accurately reveal CO_2 leaving the insect body, however, they expose the insect to unnatural, scrubbed, or absolutely dry air. There are many speculations about the function of the tentative openings, closures or "fluttering" of spiracles. In addition, there are complex theories about the presence or absence of DGC in different species of insects (Chown et al., 2006; Gray & Bradley, 2006; Contreras & Bradley, 2009; Lighton, 2008). For example, one of the theories claim that the discontinuous CO_2 emissions (DGC cycles) evolved in insects that live in underground burrows, such as ants or termites (Lighton, 1998; Gibbs & Johnson, 2004). The most complicated hypothesis includes speculations about the general toxicity of aerial oxygen for insects (Hetz & Bradley, 2005; Contreras & Bradley, 2009). The flow-through CO_2 analysis method has also been used to measure respiration in aphids and whiteflies (Salvucci & Craftsman-Brandner, 2000), but only by measuring the respiration of groups composed of several hundred specimens. In addition,

Castañeda et al. (2009, 2010) recently used a combination of a closed respirometric system with flow-through analysis of CO_2 to measure the metabolic rate of the grain aphid, *Sitobion avenae*.

An alternative theory of insect respiration, which is also 90-years-old, is based on the well documented presence of ventilatory movements, which are described for a number of taxonomically unrelated insect species (review by Babák, 1921). The ventilatory concept was for a long time over-shadowed by diffusion theory, until advanced electronic devices unequivocally documented that all insects, large or small, actively regulate their breathing by means of previously unknown and delicate ventilatory movements (Sláma, 1988, 1999; Sláma & Coquillaud, 1992). A series of further studies indicate that, during the millions of years of invertebrate evolution, insects acquired the ability to actively control respiration by means of an autonomic neuro-endocrine system (coelopulse), which functions in a way analogous to the parasympathetic nervous system of vertebrates (Sláma, 1991, 1999, 2009). In contrast to theories about the passive diffusion of respiratory gases, insect spiracular valves are programmed to snap-open for millisecond intervals of time in synchrony with bellows-like pumping movements and pulsations in haemocoelic pressure (Sláma, 2010).

The micro-respirographic scanning method can record sub-nanoliter volumes of O_2 consumption or CO_2 release, which makes it useful for measuring the respiration of individuals of relatively small insects (Sláma, 1994, 1999; Sláma & Denlinger, 1992). Previous measurements have revealed that small insects (1 mg body mass) like *Drosophila* (Sláma, 2007) or termites (Sláma et al., 2007), actively ventilate the tracheal system and respire continuously when kept in humid conditions. In dry air, however, they close the spiracular valves and exhibit very special, discontinuous bursts of CO_2 release. In this work we report similar respiratory adaptations in the pea aphid (*Acyrthosiphon pisum*).

MATERIAL AND METHODS

A colony of *Acyrthosiphon pisum* was established from aphids collected in České Budějovice, Czech Republic, 49°N, 14°E) in 1985 and reared on broad bean plants grown in water soaked sawdust in 0.5 l jars. Various developmental stages of this aphid were kept at a constant 22 ± 0.5°C under a 18L : 6D photoperiod. For induction of the sexual morphs, 10 reproductive apterous virginoparae were transferred to plants in a box and kept at a constant 19°C and 12L : 12D photoperiod. Sexual adults were produced by the third generation of aphids reared under these conditions.

For the respirometric recordings aphids were gently transferred from the host plant into disposable, 2 ml capacity, plastic syringes used as respiratory vessels. Syringes with aphids were plugged into the 4 inlet sockets of the thermostatically controlled internal compartment of the respirograph. The aphids were placed on a small piece of either dry or moist filter paper that was moistened with very dilute sulphuric acid, and their locomotory activity reduced by keeping them in darkness. For absorbing the CO_2 we used 0.1 ml of 1.5% KOH. An additional syringe with everything except the aphid was used as a compensatory respirometric vessel. The recordings were started after allowing 15 to 20 min for the temperature to equilibriate.

The methods used for micro-respirographic scanning of O_2 consumption or CO_2 output are those previously described for other insects and ticks (Sláma, 1999, 2009, 2010; Sláma & Coquillaud, 1992; Sláma & Denlinger, 1992; Sláma et al., 2007). This differential manometric system is based on a simple principle, the Boyle-Marriot law of a constant volume. The essential component of the device is a thin beryllium bronze membrane equipped with well matched semiconductor strain-gauge sensors forming the respirographic transducer. An electrically manipulated, miniature inert Hamilton valve, transducer connecting teflon tubing and the respiratory vessels were enclosed within a heavy metallic box thermostatically controlled to 0.05°C. The recording temperatures varied from 23°C to 25°C, unless otherwise stated.

The recordings were automatically regulated within the preset range of offset limits by electronic systems sensitive to the output voltage of the amplifiers or operated by commands from the PC. In order to explain the basic features of the respirograph the zeroing and offset regulations are described in the legend of Fig. 1. The electrically and temperature compensated respirographic transducer used highly stabilized, low voltage (options 1–4 V), 5 kHz AC feeding current, supplied by a 4-channel electronic tesiometric unit M-1000 (Mikrotechna, Czech Republic). Each module separately amplified and decoded the AC input signals from the transducers, removed all interference and adjusted the DC output signals for the PC, using the DATAQ (Columbus, Ohio) DI-158U hardware and software system.

The respiration of individual aphids was measured during the 1st to 7th h after their removal from a plant. Most of the recordings were made using the relatively large, wingless or winged asexual adult aphids (virginoparae). The recordings of all the other stages were extended for several hours, occasionally overnight. The number (n) of individuals of a particular stage or seasonal form measured is given in the text or in figure legends. For practical reasons, the average values of O_2 consumption were calculated only for wingless or winged virginoparous adults. The appearance of the differential (CO_2–O_2) curves with discontinuous bursts of CO_2 in dry air (~350 ppm CO_2, 30–80% relative humidity) was very variable, which made it difficult to evaluate the results statistically. Therefore, we illustrate and document the data by presenting Figures (1 to 10) of a more or less characteristic set of respirographic records.

RESULTS

In order to decipher the respirographic data, it is essential to know that the pea aphids occur in several seasonal forms that differ in their development, morphology and physiology. The life cycle starts when larvae emerge from overwintering eggs and develop into the spring generation of foundress aphids (fundatrices). These aphids produce summer generations of wingless or winged asexual females (virginoparae). At the end of the year, asexual aphids produce adult males and sexual wingless females (oviparae), which terminate the life cycle by mating and laying eggs. The use of a micro-respirographic method makes it possible for the first time to record the course of O_2 consumption and CO_2 output from individual aphids of various developmental forms.

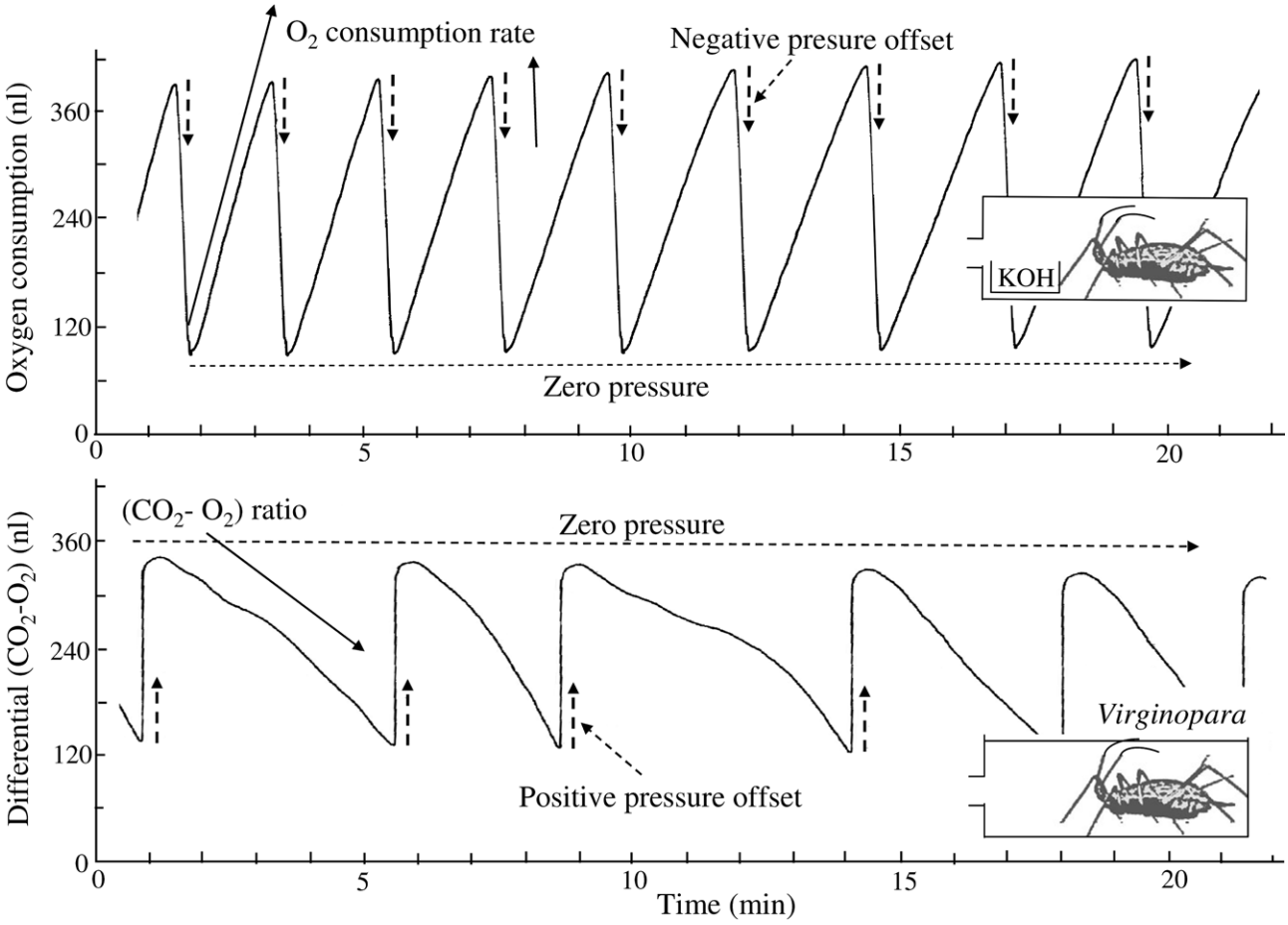

Fig. 1. Scanning respirographic records of a wingless asexual adult aphid (virginopara) of 3.5 mg body mass freshly removed from a plant and kept at 25°C. Upper panel shows cumulative respirographic curve of O_2 consumption (in presence of KOH for absorbing CO_2). There are 9 scanning episodes of negative zero control (preset to –390 nl volume). Lower panel shows differential curve of (CO_2–O_2) ratio without CO_2 absorbent, revealing a predominantly continuous release of CO_2. The offset regulation was set to +130 nl volume.

Continuous O_2 consumption by wingless asexual females (apterous virginoparae) kept in humid conditions

The adult wingless virginoparae of *A. pisum* are the largest individuals, with a body mass of 3–3.5 mg. They feed continuously on a plant and occasionally give birth to small first instar larvae. Fig. 1 (upper part) shows an example of a common, scanning respirographic record of the O_2 consumption (CO_2 absorbed by KOH) of a wingless asexual adult. In principle, the record shows a regular decline in the mechanical pressure within the respiratory vessel, which is calibrated in terms of the corresponding volumes of O_2 consumed by the aphid. It should be noted that the cumulative curve of O_2 consumption was periodically returned to zero pressure by the electronic scanning mechanism whenever the value reached the preset limit. The cumulative O_2 consumption curve is thus divided into 9 scanning episodes, from zero to the offset limit of –380 nl. Evaluation of the whole recording period shows that the aphid exhibited a more or less regular rate of O_2 consumption of 180 nl O_2 per min.

The record in the lower panel of Fig. 1 is for the same aphid as above after removal of the CO_2 absorbent. In this case the respirograph records the differential curve resulting from the combination of two reciprocal variables, i.e. consumption of O_2 and emission of gaseous CO_2. The course of the record in the lower panel of Fig. 1 goes in the opposite direction, which indicates that the release of CO_2 predominates over O_2 consumption. The lower offset limit was set to positive pressure values (+140 nl on the volumetric scale). It is quite common for insects feeding on a carbohydrate rich diet (ants, termites, bees, fruit-flies and aphids) to consume volumes of O_2 similar to that of CO_2 released. In this case the differential respirographic ratio (CO_2 / O_2), known as respiratory quotient (R.Q.) will be close to 1.

The results presented in Fig. 1 indicate that the 3.5 mg aphid consumed 180 nl of O_2/min (= 3085 µl O_2 / g / h, expressed in standard metabolic rate or SMR). Moreover, the 120 nl/min rate of the (CO_2–O_2) ratio in opposite direction shows that gaseous CO_2 was released at a velocity of 300 nl/min (with 180 nl/min correction added for the reciprocal O_2 consumption). The R.Q. value for this aphid is 1.66, which is far greater than that commonly recorded for carbohydrate metabolism. Evidently,

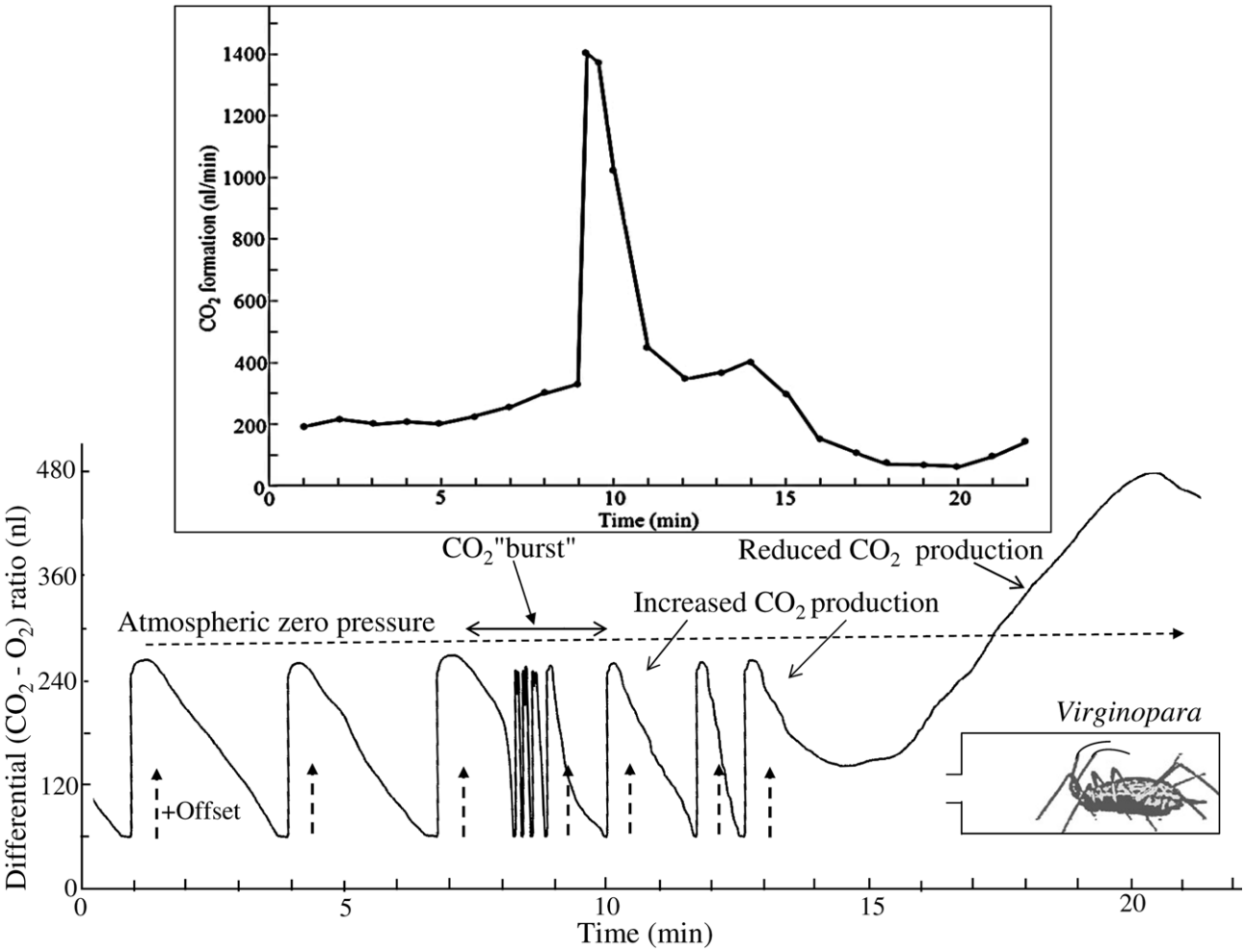

Fig. 2. Scanning respirographic record of the differential (CO_2–O_2) ratio of an asexual wingless aphid (virginopara) of 3.5 mg body mass recorded at 25°C. O_2 consumption rate of this aphid was determined one hour earlier (150 nl O_2/min, = 2570 µl O_2/g/h). Note that the predominant release of CO_2 was suddenly enhanced by a sudden outburst of gaseous CO_2 (between min 8 and 9). Inset shows conversion of the cumulative data into nl/min.

the aphid uses some other, incompletely known metabolic pathway.

Discontinuous CO_2 release by other wingless asexual females (apterous virginoparae) kept in dry conditions

The micro-respirographic records described in Fig. 1 are characteristic of continuous gas exchange. In our experiments, continuous respiration was always associated with relatively high humidity (highly diluted, 1.5% KOH solution or a filter paper acidified with water containing traces of sulphuric acid). It is obvious that under humid conditions the aphids freely exhaled CO_2 by ventilation, diffusion or by a combination of both, without substantial risk of sudden water loss. In dry air and without the KOH absorbent, however, the respiratory situation is more complex. The differential curves (CO_2–O_2) of the respirographic records show large variations, from predominately O_2 consumption to a mainly predominating reciprocal course of CO_2 release. The respirographic record in Fig. 2 provides an example of this. There is a predominant release of CO_2 with a sudden burst of gaseous CO_2 (see min. 8–9 of the recording). After conversion of the cumulative (CO_2–O_2) curve into nl of gas exchange per min, the sudden burst of CO_2 is more apparent (see inset in Fig. 2).

Based on the rate of O_2 consumption (150 nl/min) and average (CO_2–O_2) ratio of 80 nl/min, the aphid in Fig. 2 released CO_2 at an average rate of 230 nl/min, with a R.Q. of 1.53. Moreover, during the relatively short CO_2 burst, the aphid exhaled 11.6 µl of CO_2, i.e. a volume equivalent to 3 times that of its body.

In order to obtain more data on the homeostatic mechanism, which is essential for aphid survival, we recorded differential (CO_2–O_2) exchange of several dozen wingless virginoparous females of *A. pisum*. Evaluation of individual records (n = 36) did not reveal any common pattern of discontinuous CO_2 release. Instead of the stereotypic gas cycles (DGC), well known from the literature, the aphids kept and measured in dry conditions exhibited all kinds of special respiratory adaptations, ranging from continuous CO_2 release, through occasional micro-cycles of CO_2 output, to relatively enormous bursts of CO_2, occurring sometimes with a periodicity of several hours. The records in Fig. 3 show an example of the rather common CO_2 release pattern characterized mainly by a

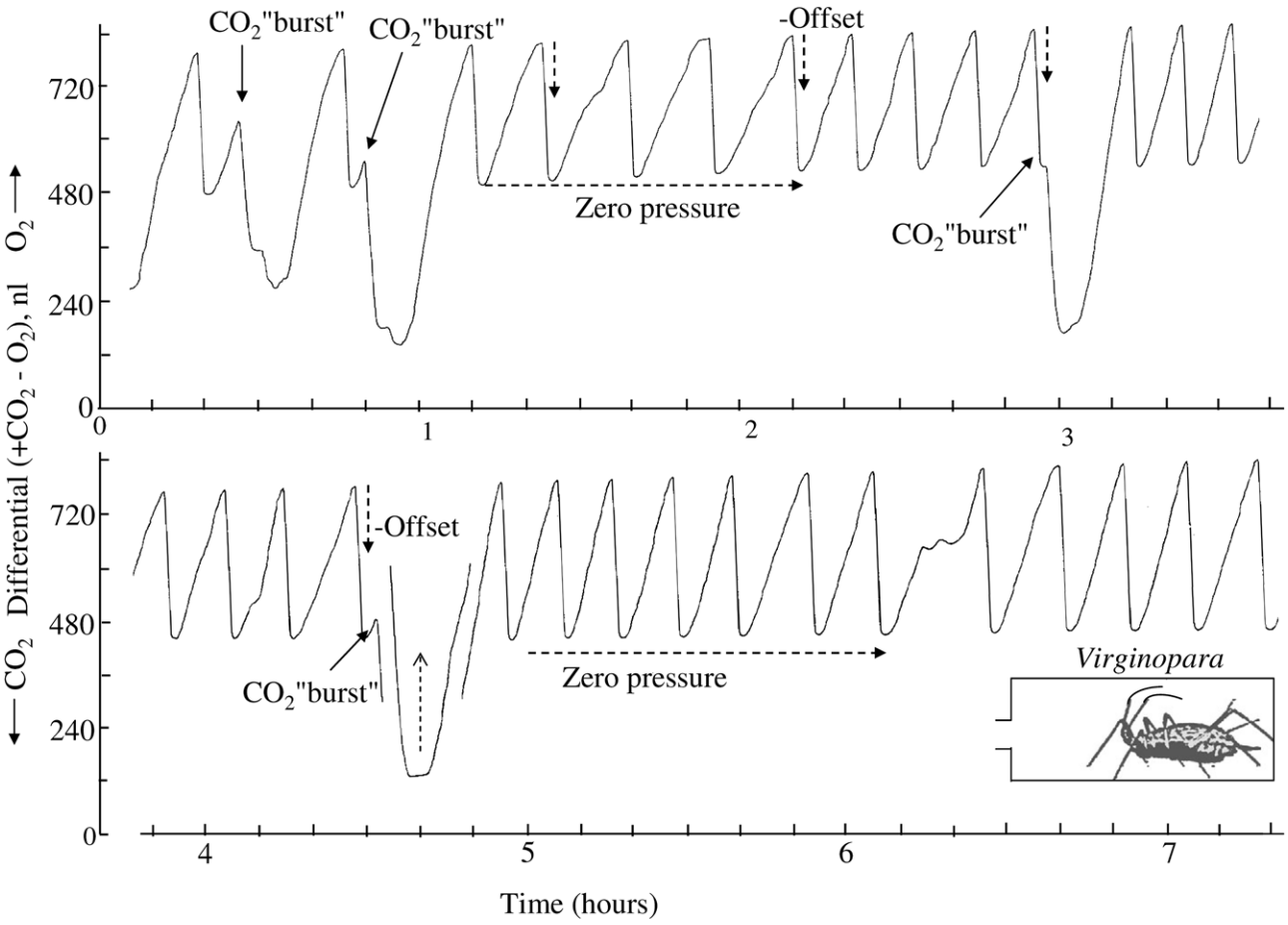

Fig. 3. Scanning respirographic record of the differential (CO_2–O_2) ratio of a wingless asexual adult aphid (virginopara) of 3 mg body mass recorded at 23°C, several hours after its removal from a plant. The record shows a predominant consumption of O_2 over CO_2 production, intercalated by occasional outbursts of 300- to 500-nl volumes of CO_2, with a periodicity of close to 2 h.

predominant consumption of O_2 with occasional corrections of acidaemia by smaller and shorter releases of CO_2.

Respiratory metabolism of winged asexual adults (alate virginoparae)

The anatomical and morphological structure of winged virginoparae differs from that of their wingless counterparts. This is mainly due to the presence of wings and large indirect flight muscles. These relatively lightweight, flying aphids are usually smaller (1.0 to 1.5 mg). They are able to survive for some time away from their food plant. Their ability to survive, breathe, retain water and fly is really miraculous. The records in Fig. 4 are representative examples of the respiratory metabolism of a winged asexual aphid. The upper record recorded in humid conditions (highly diluted, 1.5% KOH) shows a continuous consumption of O_2 (54 nl/min, = 3200 µl O_2/g/h). Due to some metabolic change, these aphids do not show a predominant release of CO_2. The curves of the differential records (CO_2–O_2) indicate a very small or zero pressure change, i.e. the released CO_2 is more or less equal to the O_2 consumed (see lower record in Fig. 4). Typically, equal amounts of the consumed and released gas are rather common and indicate pure carbohydrate metabolism (R.Q. = 1). However, the R.Q. recorded for winged aphids that had fasted for some time was 0.7 (lipid metabolism).

Occasionally, as shown in Fig. 5, the differential (CO_2–O_2) records of winged aphids in dry air showed distinctive micro-cycles in CO_2 release. In this case, one large production of gaseous CO_2 was replaced by a series of smaller and short CO_2 bursts, which is not uncommon in other insects. The small winged aphids (1 mg) appeared to be real masters in preventing respiratory acidaemia, which is essential during flight when the respiratory exchange and formation of carbonic acid are greatly increased. These statements are exemplified by the respirographic record in Fig. 6, which shows the common respirographic patterns of a winged adult female of *A. pisum*, with one large burst of CO_2. The record can be used to illustrate certain physiologically important respirometric measures. These are: 1. The rate of O_2 consumption (determined before the measurements shown in Fig. 6), was 50 nl/min (= 2727 µl O_2/g/h); 2. The curve of the differential record of (CO_2–O_2) decreases before the CO_2 burst (= increased CO_2 production?); 3. The curve increases sharply after the burst (= temporary arrest of CO_2 production?); 4.The course of the curve successively declines again during each inter-burst period, indicating marked variations in CO_2 output; 5. During this 10 min

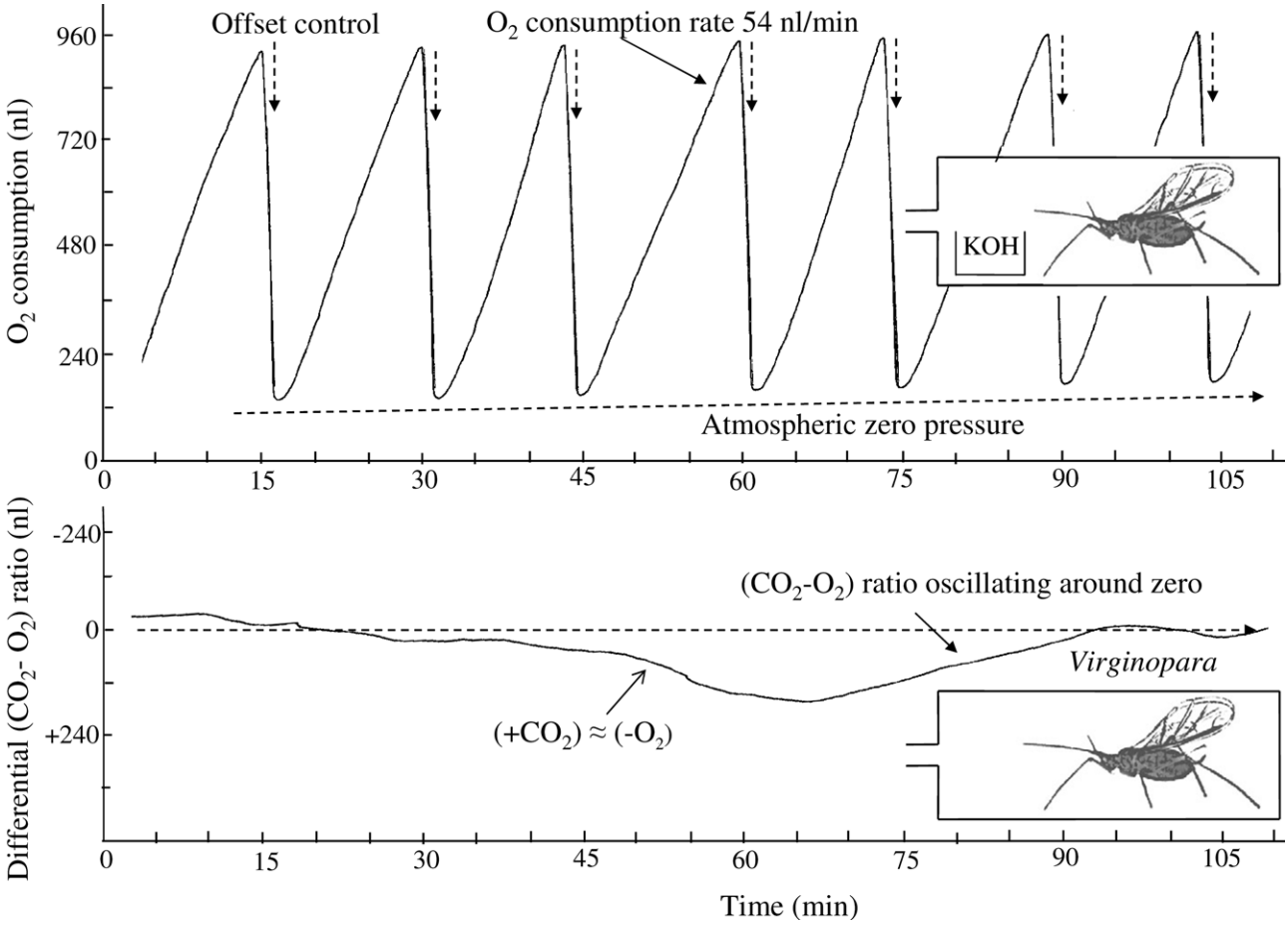

Fig. 4. Scanning respirographic records of O_2 consumption (upper panel) and (CO_2–O_2) ratio for a winged asexual adult aphid (virginopara) of 1 mg body mass immediately after its removal from a plant recorded at 25°C. A more or less constant rate of O_2 consumption (~ 54 nl/min) indicates standard metabolic rate of 3200 µl O_2/g/h. Respiratory quotient (CO_2 / O_2) close to 1 indicates metabolism of a carbohydrate substrate.

burst, the aphid exhaled 2.62 µl of CO_2 (corrected for reciprocal O_2 consumption), which was almost 3 times the volume of the aphid's body, and; 6. The initial velocity of the exhalation of CO_2 at the onset of the burst was very high but difficult to estimate and then decreased successively towards the end of the burst. The described course of events indicates a feed-back homeostatic mechanism regulating excess or deficiency in CO_2 production.

Extensive monitoring of respiratory metabolism of wingless and winged virginoparous aphids kept in humid conditions revealed constant rates of O_2 consumption suitable for determining the standard metabolic rate (SMR). The wingless virginoparous aphids had average rates of O_2 consumption ranging from 1142 µl to 4800 µl of O_2/g/h, with an average of 2571 µl (n = 18; S.D. = 1016). The winged virginoparous aphids had higher average rates of O_2 consumption per unit mass ranging from 2040 µl to 6780 µl of O_2/g/h, with an average of 3343 µl (n = 11; S.D. = 1126).

The differential (CO_2–O_2) records in dry air revealed a plethora of various respiratory patterns (n = 32 records for wingless; n = 19 records for winged aphids). There were numerous respiratory changes depending on the extant physiological state of the aphid, such as trophic situation, water content, temperature and humidity. In order to illustrate this we selected a few records showing short micro-cycles in CO_2 release (Fig. 5), enormous sudden bursts of CO_2 at intervals of several minutes or several hours (Fig. 6) or a relatively slow exhalation of CO_2 over 30 min (Fig. 7). In these records periods of predominantly O_2 consumption alternated with those of CO_2 release. Starved asexual winged *A. pisum* survived occasionally for several days. Under humid or dry conditions, they showed a very constant rate of O_2 consumption and CO_2 release (Fig. 8).

Respiratory metabolism of males and sexual females (oviparae)

The apterous sexual females of *A. pisum* (oviparae) are relatively large aphids, 3 to 3.5 mg in body mass. Their respiratory metabolism shows similar patterns to those recorded for the summer forms (virginoparae). The respiratory quotient of oviparae varied from 1 to 0.7, with an average of 0.75 (n = 5), indicating mainly lipid metabolism. The enormous bursts of CO_2 exhaled within a rather short interval of 5 min recorded in the respirographic records in Fig. 9 confirm these statements. When corrected for reciprocal O_2 consumption, the volume of CO_2 exhaled during the few minutes of the burst surpassed the total body volume of the aphid by about 2-fold (5.2 µl

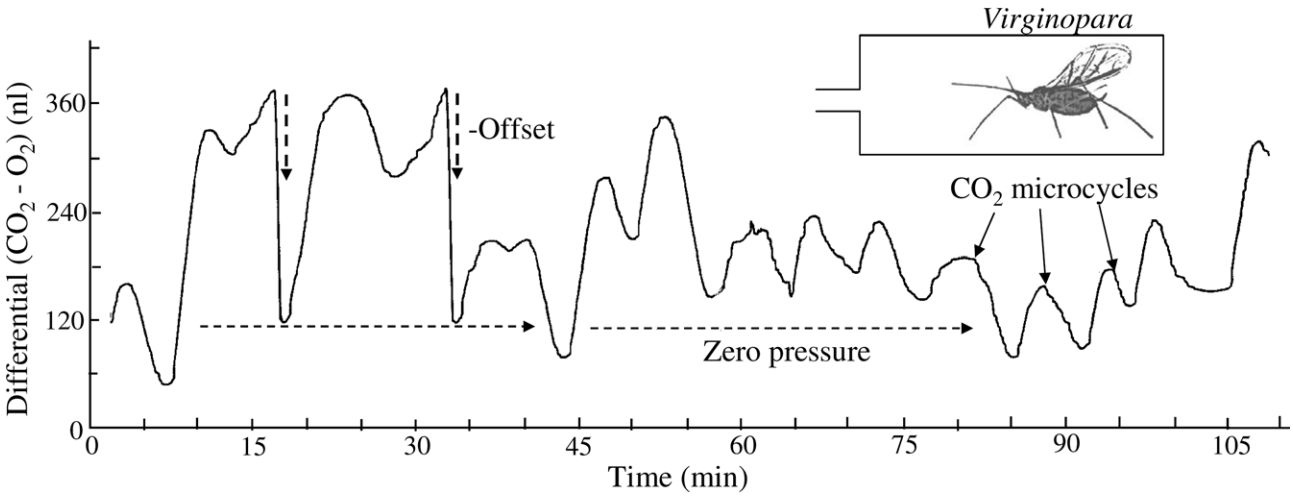

Fig. 5. Differential scanning respirographic record of (CO_2–O_2) ratio for a winged asexual adult aphid (virginopara) of 1 mg body mass recorded at 25°C. The record shows micro-cycles in CO_2 production with a periodicity of some 5 to 8 min.

CO_2, 3.3 mg body mass). The volume of the entire, air filled tracheal system was surpassed almost 20-fold, which provides good evidence that CO_2 leaves the body by a bulk outflow. Before the burst, O_2 consumption and CO_2 release rates were equal, whereas after the burst, the differential respirographic curve showed a temporary arrest in CO_2 production, which indicates a homeostatically regulated physiological system.

The males of *A. pisum* are small and delicate (0.5 mg body mass). Their metabolic rate, expressed in standard units of O_2 consumption per g of body mass, was higher than that of female aphids. Occasionally, we recorded extremely high values of O_2 consumption, close to 9000 µl of O_2 / g / h, both by winged and wingless males. The course of the differential respirographic changes in Fig. 10 shows a distinctive pattern of discontinuous cycles in CO_2 output by a miniature male of this aphid.

DISCUSSION

This study documents that insects, even those as small as aphids, can efficiently control respiratory acidaemia by homeostatic control of CO_2 emissions, which is extremely

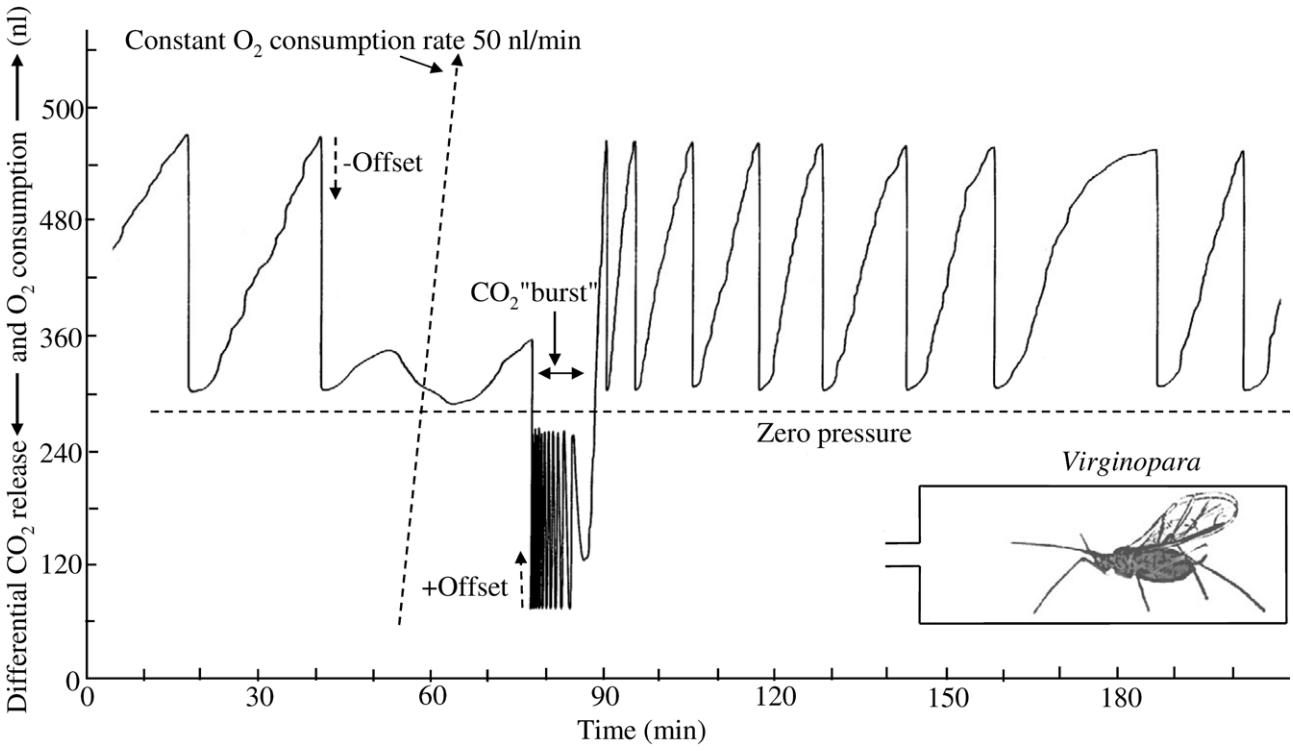

Fig. 6. Differential scanning respirographic record of (CO_2–O_2) ratio for a winged asexual adult aphid (virginopara) of 1.1 mg body mass recorded at 25°C. The record shows a large, 2.12 µl "burst" of CO_2 released within a 10 min period. After correction for the reciprocal course of O_2 consumption (50 nl/min = 2727 µl O_2/g/h), the aphid actually released 2.62 µl of CO_2 in 10 min, which is almost 3 times greater than the total volume of its body.

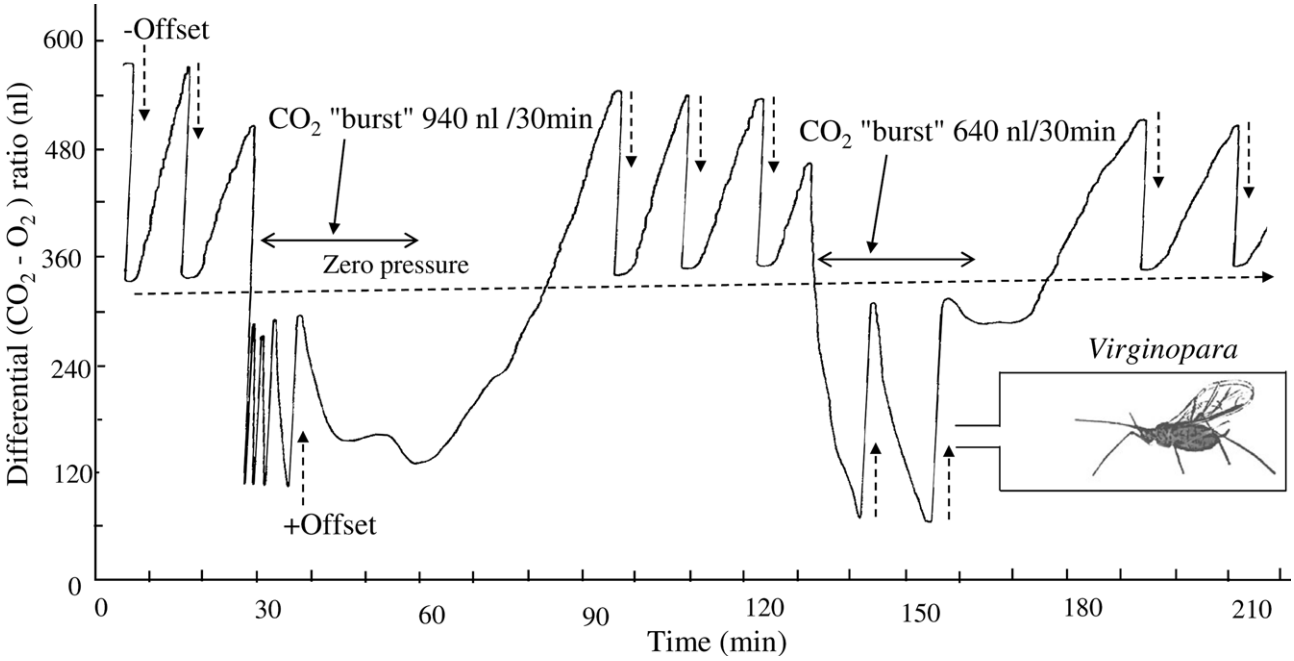

Fig. 7. Differential scanning respirographic record of (CO_2–O_2) ratio for a winged asexual adult aphid (virginopara) of 1.2 mg body mass recorded at 25°C. Foregoing measurements revealed O_2 consumption of 55 nl/min (= 2750 µl O_2 / g / h). There are two relatively long (30 min) CO_2 "bursts"; 940 nl and 640 nl (2.59 µl and 2.29 µl of CO_2 released during the bursts, corrected for reciprocal O_2 consumption).

important in all insects that have a very high metabolic rate. The respirographic records of individual aphids presented can be used to evaluate our statements. Previous studies give only averages obtained by measuring the respiration of large groups of aphids (Salvucci & Crafts-Brandner, 2000). Nevertheless, the reported values of

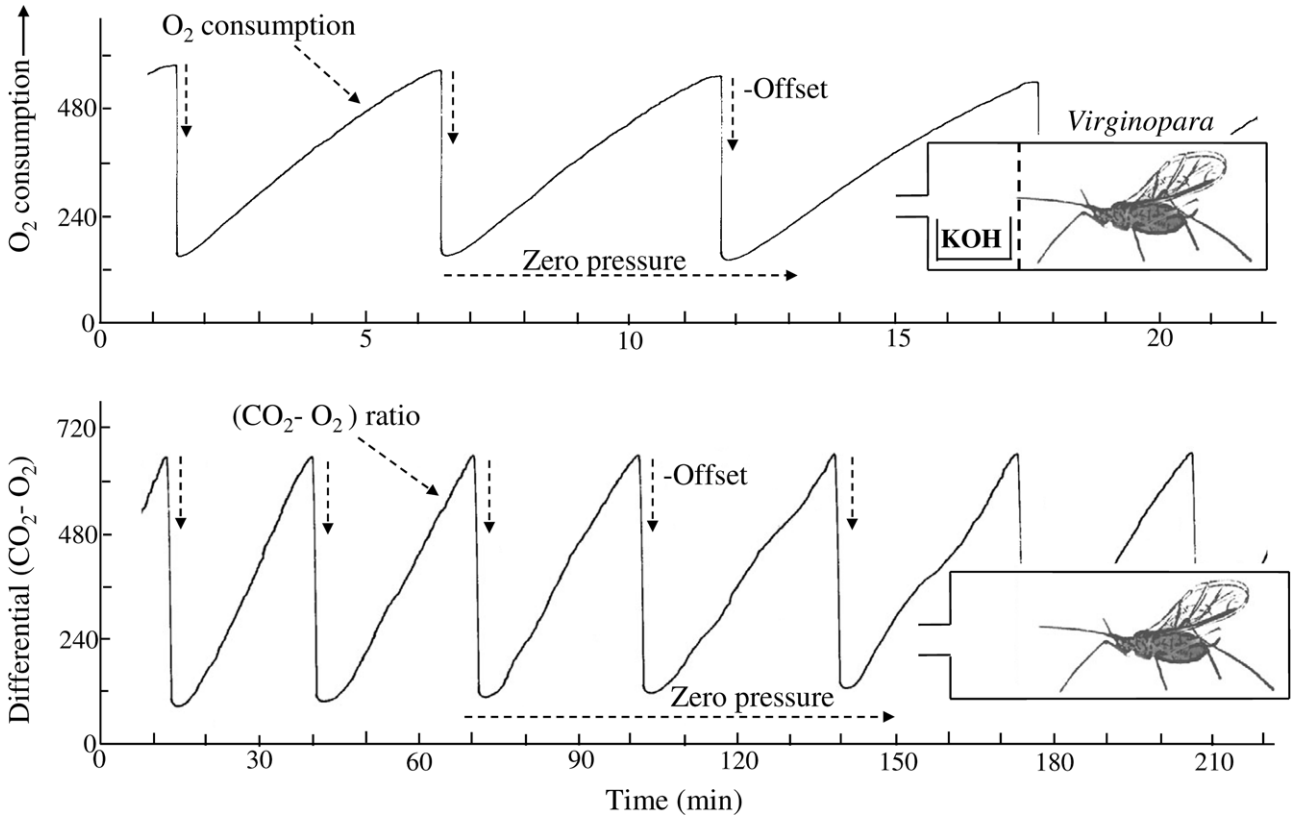

Fig. 8. Scanning respirographic records of O_2 consumption (upper panel) and differential (CO_2–O_2) ratio for a winged asexual adult aphid (virginopara) recorded after one day of starvation. The rate of O_2 consumption is 120 nl/min (= 3600 µl O_2 / g / h) and (CO_2–O_2) ratio is 16 nl/min (= 3100 µl CO_2/g/h), respiratory quotient = 0.86.

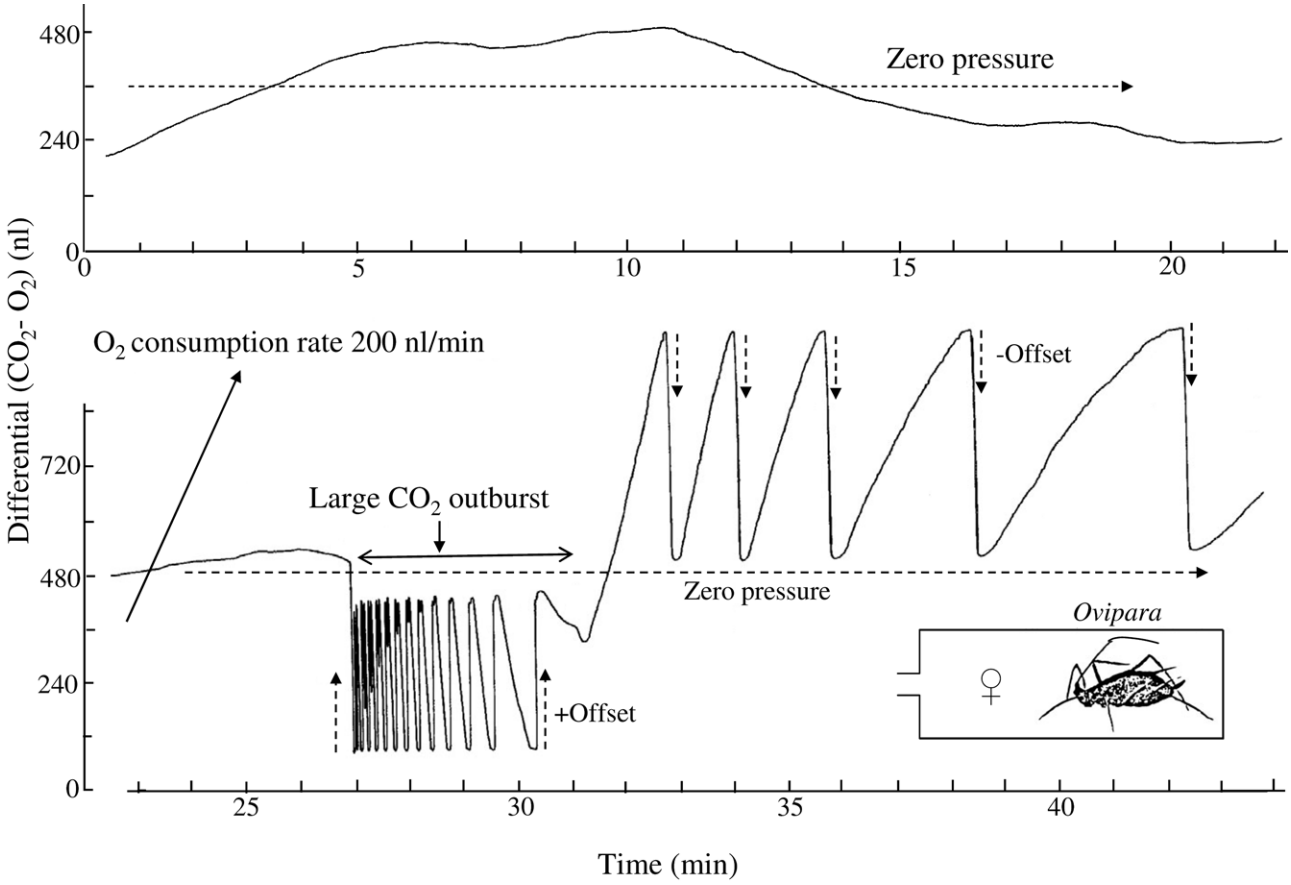

Fig. 9. Differential scanning respirographic records of (CO_2–O_2) ratio for a wingless female aphid (ovipara) of 3.5 mg body mass recorded at 27°C. Upper panel shows an example of prolonged periods when (CO_2–O_2) ratio oscillated around zero (R.Q. close to 1).The rate of O_2 consumption (200 nl O_2 per min; = 3428 µl O_2/g/h) was determined in advance. Lower panel shows a large CO_2 "burst", averaging 4.4 µl of CO_2 released in 4 min (5.2 µl of CO_2 corrected for simultaneous O_2 consumption).

5040 µl CO_2/g/h for the cotton aphid are similar to the range of CO_2 released (5140 µl CO_2/g/h) and O_2 consumed (3085 µl O_2/g/h) recorded in this study for virginoparous adults of *A. pisum*. More recently, Castañeda et al. (2009, 2010) reports respirometric data for the grain aphid (*Sitobion avenae*) obtained by a combination of constant volume and flow-through techniques. They report values of CO_2 release one order of magnitude smaller (from 250 to 630 µl CO_2/g/h) than recorded in this study and by Salvucci & Crafts-Brandner (2000) for *A. pisum*. For the first time, however, our data was based on multiple recordings of O_2 consumption and CO_2 output of individual aphids of different morphs.

Due to the technical problems associated with small size, measurements of aphid metabolism were usually based on their utilization of specific food substrates. For instance, the metabolism of *A. pisum* was indirectly estimated using radio-labelled glucose (Rhodes et al., 1996; Douglas et al., 2006). There are similar metabolic studies of other species of aphids, *Megoura viciae* (Ehrhardt, 1962) and *Myzus persicae* (Kunklel & Hertel, 1975). In the case of *Aphis fabae* the metabolic rate was indirectly determined by monitoring its utilization of dietary amino acids (Wilkinson et al., 2001). After conversion of the data into standard units of metabolic activity, these authors report extremely high rates, equivalent to 10,000 µl of O_2/g/h. Our preliminary respirographic measurements of *Aphis fabae* revealed, surprisingly, that the data of Wilkinson et al. (2001) could be correct. As a matter of fact, these minute aphids consumed incredible amounts of gas with peaks in O_2 consumption of as high as 20,000 µl O_2/g/h.

The records in Figs 2, 3, 6, 7 and 9 show that an adult *A. pisum* can suddenly exhale relatively large volumes of CO_2. This important physiological feature was previously recorded for other species using electronic microrespirographic methods (Sláma, 1994; Sláma & Coquillaud, 1992; Sláma et al., 2007). The relatively short exhalations of CO_2 cannot be properly displayed by the commonly used flow-through methods because they become blurred or disappear in the air stream. Augustus Krogh who proposed the diffusion theory (Krogh, 1920) was unaware of that insects can exhale CO_2 in very short bursts. Using recently developed electronic methods (see Figs 9 and 10 for an example) it is possible to show that even a relatively small insect can within a millisecond suddenly inspire or exhale large amounts of gas (Sláma, 1988, 2010).

The problem remains, why do aphids and other insects produce large, discontinuous exhalations of CO_2 in dry air but not under humid conditions (Sláma et al., 2007). Obviously, insects exposed to dry air are in danger of

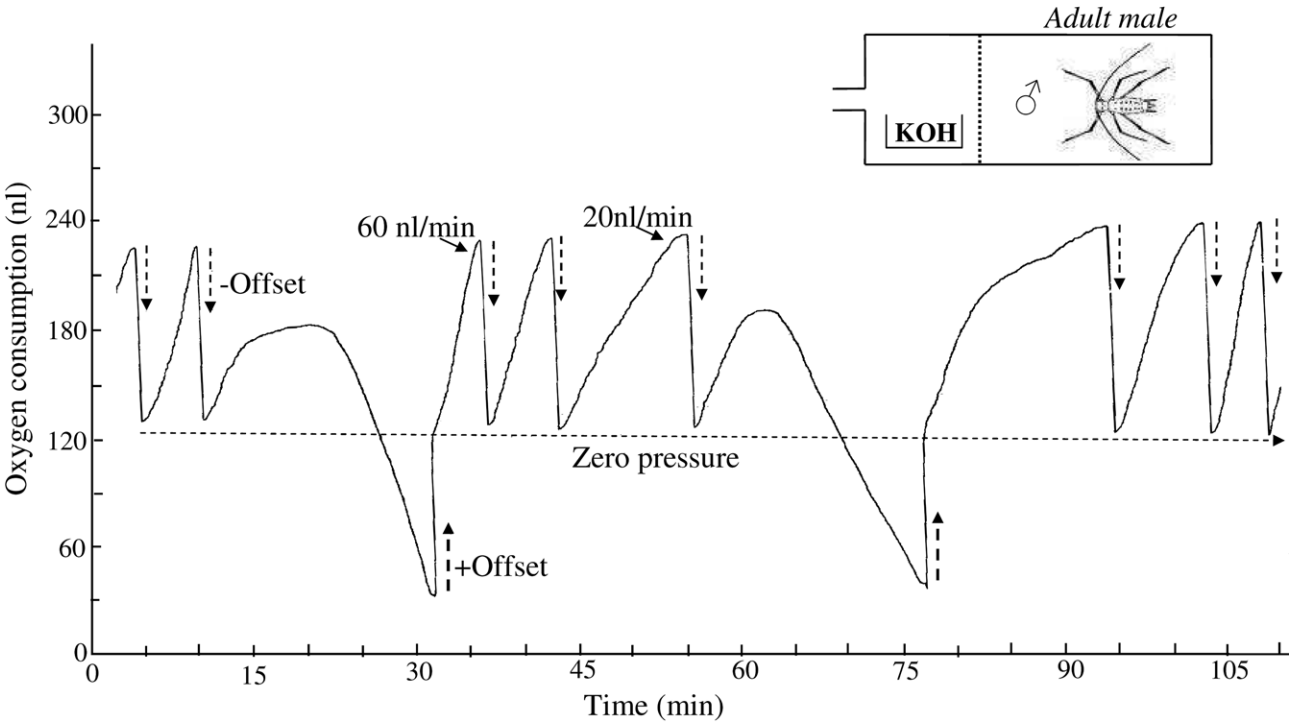

Fig. 10. Scanning respirographic record of O_2 consumption recorded for a very small wingless adult male aphid of *A. pisum* (0.5 mg body mass). The course of the respirographic curve of O_2 consumption indicates a rate varying between 60 nl /min (= 7200 µl O_2 / g / h) and 20 nl/ min (= 2400 µl O_2 / g / h). In spite of the presence of CO_2 absorbent within the respiratory vessel, the record shows incomplete or delayed absorption due to the very small amounts of CO_2.

dessicating and should close their spiracular valves as much as possible, sometimes 97% of the time (Sláma, 2009, 2010). It is illusory to assume that insects dispose of CO_2 through wide open spiracles (Lighton, 1996; Klok & Chown, 2005; Chown et al., 2006; Bradley, 2007) and bearing in mind the 36-fold greater solubility of CO_2 in body liquids also unrealistic to assume that CO_2 could exit the body by simple diffusion through narrow spiracular apertures without a serious loss of water. In addition, the tentative diffusion pathway of gaseous CO_2 is very complicated. The gas needs to pass at first from haemolymph through tracheal epithelium with chitinized taenidia into the air-filled tracheal lumen. Then, it needs to pass through the more or less constricted spiracular valves into a narrow, sub-integumental atrium. Finally, it needs to pass through mesh-like spiracular foam in some insects, or diffuse through highly attenuated chitinous cuticular sieves of external orifices . Krogh (1920) did not consider these obstacles to diffusion. He simplified his calculations by taking into account only the internal diameter of the spiracular orifice. Ironically, at that time there was good experimental evidence of convective gas exchange by ventilatory movements (Babák, 1921) but this was ignored for 90 years (Chown et al., 2006).

The virginoparous adults of *A. pisum* usually consume 2000 to 4000 µl O_2/g/h, which is similar to that recorded for other small insects, such as ants or termites (Sláma et al., 2007). The smallest insect reported to exhale burst of CO_2 is an adult ant (*Monomorium pharaonis*; 0.14 mg body mass), which emits 2.5 nl CO_2 at regular intervals of 3.5 to 4 min (O_2 consumption 2143 µl O_2 / g / h; Sláma, 1999). Metabolic formation of carbonic acid might represent a rate limiting factor determining survival of insects that lack an efficient mechanism for neutralizing respiratory acidaemia. The importance of these mechanisms in the regulation of acid-base relationships have been known for a long time (Harrison, 2001). Importance of homeostatic mechanisms for restraining respiratory acidaemia is most critical when spiracles are blocked (water submersion) or in dry conditions when insects are forced to keep spiracular valves closed as much as possible in order to conserve water. Accordingly, the stereotypic DGC respiration cycles ascribed to opening, closure or "fluttering" of the spiracles (see Lighton, 1996, 2008; Klok & Chown, 2005; Chown et al., 2006; Gibbs & Johnson, 2004; Marais et al., 2005; Gray & Bradley, 2006; Bradley, 2007; Contreras & Bradley, 2009) are viewed as artifacts resulting from keeping the insects in dry or scrubbed air. An alternative explanation of the bursts of CO_2 assumes that during the millions of years of arthropod evolution insects that had the best physiological mechanism for optimizing water retention and CO_2 output were at a selective advantage (Sláma, 2010). The mechanism evolved in response to: (a) Feed-back responses from the environment (food supply, temperature, humidity); (b) Proprioceptive signals received from intermediary metabolism (glycogen, lipids, other reserves); (c) Acid-base conditions associated with neutralization of carbonic acid, and; (d) Feed-back messages on the level of dessication and ionic balance. Our finding of substantially different respiratory scenarios in aphids exposed to

low and high relative humidity confirms the existence of an active physiological control of respiration.

The closed volume, micro-respirometric technique reveals that the sharp bursts of CO_2, which surpass the tracheal volume of the aphid by more than 20-fold (see Figs 2, 6, 9), were made exclusively by a bulk outflow of CO_2 from the body. It is practically impossible that such a burst of CO_2 could be made by instantaneous diffusion of accumulated gas through the spiracles. We know that this statement conflicts with the widely accepted theories on DGC (Lighton, 1996; Hetz & Bradley, 2005; Chown et al., 2006; Bradley, 2007). The argument in favour of a convective bulk outflow of CO_2 is that constant volume respirography cannot display a diffusive transfer of gas between two parts of the same compartment. In other words a tentative diffusion of CO_2 previously accumulated within the tracheal trunks could never appear as a CO_2 burst in our records.

Based on the above technical data, all CO_2 bursts recorded using respirographic techniques for ticks (Sláma, 1991), pseudoscorpions and solifugae (Sláma, 1995), beetles (Sláma & Coquillaud, 1992), ants and cockroaches (Sláma, 1999), *Drosophila* (Sláma, 2007), termites (Sláma et al., 2007), cecropia silkworms (Sláma, 2010) and aphids, (present work), are not diffusive DGC, but convective bulk outflows of CO_2 from the tracheal system. Large insects, such as cockroaches (*Periplaneta*), released CO_2 by continuous diffusion into the air-filled tracheal system. The respirographic records did not record bursts of CO_2, whereas a flow-through IR analysis revealed beautiful CO_2 bursts associated with periodically repeated, vigorous ventilatory movements (Sláma, 1999). We have experimental evidence that indicates that insects as small as aphids use ventilatory movements to exhale CO_2 (the data will be published elsewhere). According to recent investigations, the physiological mechanism moderating respiratory acidaemia by discontinuous bursts of CO_2 depends on hydration of carbonic acid by carbonic anhydrase present in the epithelial layers of tracheal tubes and tracheal sacs (Sláma, 2009). It is a pity that these physiologically important functions remained unresolved for 90 years, due to persistent belief in Krogh's diffusion theory (DGC cycles).

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REFERENCES

Babák E. 1921: Die Mechanik und Innervation der Atmung. D. Tracheaten. In Winterstein H. (ed.): *Handbuch der Vergleichenden Physiologie* Gustav Fischer, Jena, pp. 265–1052.

Bartlett D. & Areson J.G. 1977: Quantitative lung morphology in newborn mammals. *Respir. Physiol.* **29**: 193–200.

Bradley T.J. 2007: Control of the respiratory pattern in insects. In Roach R.C. et al. (eds): *Hypoxia and the Circulation*. Springer, New York, pp. 215–224.

Castañeda L.E., Figueroa C.C., Fuentes-Contreras E., Niemeyer H.M. & Nespolo R.F. 2009: Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, Sitobion avenae. *J. Exp. Biol.* **212**: 1185–90.

Castañeda L.E., Figueroa C.C., Fuentes-Contreras E., Niemeyer H.M. & Nespolo R.F. 2010: Physiological approach to explain the ecological success of "superclones" in aphids: interplay between detoxification enzymes, metabolism and fitness. *J. Insect Physiol.* **56**: 1058–1064.

Chown S.L., Gibbs A.G., Hetz S.K., Klok G.J., Lighton J.R.B. & Marais E. 2006: Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool*. **79**: 333–343.

Cockbain A.J. 1961: Fuel utilization and duration of tethered flight in Aphis fabae Scop. *J. Exp. Biol*. **38**: 163–174.

Contreras H.L. & Bradley T.J. 2009: Metabolic rate controls respiratory pattern in insects. *J. Exp. Biol*. **212**: 424–428.

Douglas A.E., Price D.R.G., Minto L.B., Jones E., Pescol K.V., François C.L. & Ehrhardt P. 1962: Untersuchungen zur Stoffwechselphysiologie von Megoura viciae Buckt., einer phloemsaugenden Aphide. *Z. Vergl. Physiol.* **46**: 169–211.

Gibbs A.G. & Johnson R.A. 2004: The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *J. Exp. Biol*. **207**: 3477–3482.

Gray E.M. & Bradley T.J. 2006: Evidence from mosquitoes suggests that cyclic gas exchange and discontinuous gas exchange are two manifestations of a single respiratory pattern. *J. Exp. Biol*. **209**: 1603–1611.

Harrison J.F. 2001: Insect acid-base physiology. *Annu. Rev. Entomol*. **46**: 221–250.

Hetz S.K. & Bradley T.J. 2005: Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**: 516–519.

Klok C.J. & Chown S.L. 2005: Temperature- and body mass-related variation in cyclic gas exchange characteristics and metabolic rate in seven weevil species: broader implications. *J. Insect Physiol.* **51**: 789–801.

Krogh A. 1920: Studien über Tracheenrespiration. II. Über Gasdiffusion in den Tracheen. *Pflügers Arch. Ges. Physiol.* **179**: 95–112.

Kunkel H. & Hertel R. 1975: Erstellung einer Stoffwechselbilanz für beide virginoparen Morphen von Myzus persicae (Sulz.) Larven in dritten Stadium. *Oecologia* **18**: 23–35.

Lighton J.R.B. 1996: Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**: 309–324.

Lighton J.R.B. 1998: Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas exchange in Tracheate Arthropods. *Am. Zool*. **38**: 483–491.

Lighton J.R.B. 2008: *Measuring Metabolic Rates. A Manual For Scientists*. Oxford University Press, Oxford, 201 pp.

Marais E., Terblanche J.S., Klok C.J. & Chown S.L. 2005: Insect gas exchange patterns: a phylogenetic perspective. *J. Exp. Biol*. **208**: 4495–4507.

Pritchard M.J. & Boonham N. 2006: Sweet problems: insect traits defining the limits to dietary sugar utilisation by the pea aphid, Acyrthosiphon pisum. *J. Exp. Biol*. **209**: 1395–1403.

Rhodes J.D., Croghan P.C. & Dixon A.F.G. 1996: Uptake, excretion and respiration of sucrose and amino acids by the pea aphid Acyrthosiphon pisum. *J. Exp. Biol*. **199**: 1269–1276.

Salvucci M.E. & Crafts-Brandner S.J. 2000: Effects of temperature and dietary sucrose concentration on respiration in the silverleaf whitefly, Bemisia argentifolii. *J. Insect Physiol*. **46**: 1461–1467.

Schneiderman H.A. 1960: Discontinuous respiration in insectrs: role of the spiracles. *Biol. Bull*. **119**: 494–528.

Sláma K. 1988: A new look at insect respiration. *Biol. Bull*. **175**: 289–300.

Sláma K. 1991: The presence and functions of the autonomic nervous system in ticks. In Dusbábek F. & Bukva V. (eds): *Modern Acarology. Vol. 2.* Academia, Prague and SPB Academic Publishers, The Hague, pp. 389–395.

Sláma K. 1994: Regulation of respiratory acidemia by the autonomic nervous system (Coelopulse) in insects and ticks. *Physiol. Zool.* **6**7: 163–174.

Sláma K. 1995: Respiratory cycles of Chelifer cancroides (Pseudoscorpiones) and Galeodes sp. (Solifugae). *Eur. J. Entomol*. **92**: 543–552.

Sláma K. 1999: Active regulation of insect respiration. *Ann. Entomol. Soc. Am*. **92**: 916–929.

Sláma K. 2007: Respiratory concerts revealed by scanning micro-respirography in the fruitfly (Drosophila melanogaster). In: *Abstracts of the 20th European Drosophila Research Conference.* Research Institute of Molecular Pathology and Institute of Molecular Biology of the Austrian Academy of Science, Vienna, p. 60.

Sláma K. 2009: Extracardiac haemocoelic pulsations and the autonomic neuroendocrine system (coelopulse) of terrestrial insects. *Terr. Arthr. Rev.* **1**: 39–80.

Sláma K. 2010: A new look at discontinuous respiration in pupae of Hyalophora cecropia (Lepidoptera: Saturniidae): Haemocoelic pressure, extracardiac pulsations and O_2 consumption. *Eur. J. Entomol*. **107**: 487–507.

Sláma K. & Coquillaud M.-S. 1992: Homeostatic control of respiratory metabolism in beetles. *J. Insect Physiol*. **38**: 783–791.

Sláma K. & Denlinger D.L. 1992: Infradian cycles of oxygen consumption in diapausing pupae of the flesh fly, Sarcophaga crassipalpis, monitored by a scanning microrespirographic method. *Arch. Insect Biochem. Physiol.* **20**: 135–143.

Sláma K., Šobotník J. & Hanus R. 2007: Respiratory concerts revealed by scanning microrespirography in termite (Prorhinotermes simplex). *J. Insect Physiol.* **53**: 295–311.

Wilkinson T.L., Minto L.B. & Douglas A.E. 2001: Amino acids as respiratory substrates in aphids: an analysis of Aphis fabae reared on plants and diets. *Physiol. Entomol.* **26**: 225–228.

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