

Discontinuous gas exchange in insects[☆]

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Abstract

Insect respiratory physiology has been studied for many years, and interest in this area of insect biology has become revitalized recently for a number of reasons. Technical advances have greatly improved the precision, accuracy and ease with which gas exchange can be measured in insects. This has made it possible to go beyond classic models such as lepidopteran pupae and examine a far greater diversity of species. One striking result of recent work is the realization that insect gas exchange patterns are much more diverse than formerly recognized. Current work has also benefited from the inclusion of comparative methods that rigorously incorporate phylogenetic, ecological and life history information. We discuss these advances in the context of the classic respiratory pattern of insects, discontinuous gas exchange. This mode of gas exchange was exhaustively described in moth pupae in the 1950s and 1960s. Early workers concluded that discontinuous gas exchange was an adaptation to reduce respiratory water loss. This idea is no longer universally accepted, and several competing hypotheses have been proposed. We discuss the genesis of these alternative hypotheses, and we identify some of the predictions that might be used to test them. We are pleased to report that what was once a mature discipline, in which the broad parameters and adaptive significance of discontinuous gas exchange were thought to be well understood, is now a thriving and vigorous field of research.

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1. Overview

It has been known for over 50 years that many insects exhibit a remarkable pattern of respiration now referred

to as discontinuous gas exchange (DGE). As the name suggests, DGE appears to the observer as periods of low or non-existent gas transfer punctuated at intervals by enormous pulses or bursts of CO₂ release. Oxygen uptake generally tracks CO₂ release, though respiratory exchange ratios may vary through the cycle. DGE was originally thought to be an adaptation to conserve water, and this hypothesis remains the most prevalent one in the literature (Edney, 1977; Hadley, 1994a,b; Lighton, 1996, 1998; Chown, 2002; Chown et al., 2006). Our view of insect gas exchange began

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to change in the 1980s when new, highly sensitive means of measuring CO₂ and water loss were applied to insect systems (reviewed in Hadley, 1994a,b). These methodological innovations vastly improved the temporal resolution of gas exchange measurements in small organisms. In addition, the issue of undetected activity, always a problem in immersed closed-vessel systems, was conquered during the same period by the development of inexpensive motion detectors (Lighton, 1988a).

Empowered with this new technology, researchers began to examine a wide range of insect species. DGE was detected in many terrestrial arthropods, though not all, and the accepted adaptive explanation—water conservation—remained unchallenged. In the early nineties, however, new work began to cast doubt on the notion that DGE was effective as a water-conservation measure in many insects (Hadley, 1994a; Lighton, 1998; see Chown, 2002 for review). These challenges were based on several observations, which are discussed later. New hypotheses have emerged from this recent work, and it is gratifying to report that the area of insect respiration remains as vital today as it was 50 years ago. Several excellent reviews have been published during the last few years (Hadley, 1994a; Lighton, 1994, 1996, 1998; Chown, 2002; Chown and Nicolson, 2004; Chown et al., 2006), so the intent here is to provide updates where possible, and some suggestions about the direction of future studies.

2. Respiratory systems in insects

Insects generally lack respiratory pigments, and hence the blood plays little part in the transport of O₂ (Chapman, 1998). Instead, O₂ is transported directly via a system of tubes called tracheae that arise as ingrowths of the body surface. The insect tracheal system consists of the semi-rigid, gas-filled tracheae that penetrate the body and tissues, and the spiracles, which connect the tracheae to the exterior. The morphology and physiology of insect respiratory systems have been comprehensively reviewed on several occasions (Kaars, 1981; Miller, 1981; Kestler, 1985; Nation, 1985; Chapman, 1998).

Spiracles are usually located laterally in pairs (8–10) along the thorax and abdomen. Structurally they are highly variable. The spiracular opening generally leads

to a cavity (atrium) from which tracheae arise. Spiracles of most terrestrial insects have movable valves, which may be located in the opening itself or at the junction of the atrium and a trachea. Valves may be operated by an opener and a closer muscle, or by a closer muscle that acts against cuticular elasticity. From the atria, the main tracheae branch to give rise to smaller diameter tubes (tracheoles), the smallest of which may penetrate the tissues and become intimately associated with individual cells (Miller, 1974; Chapman, 1998). In some insects, higher-order tracheae may give rise to air sacs in the thorax and/or abdomen (Nation, 1985; Chapman, 1998).

3. Gas exchange and ventilatory patterns

Gas exchange in terrestrial arthropods is generally investigated by measuring CO₂ release. Oxygen uptake is much more difficult to measure, and thus its measurement is often omitted. Several types of respiratory behavior have been observed in quiescent insects, based on the patterns of CO₂ release. Some insects exhibit a more-or-less steady release of CO₂ (Fig. 1), a pattern that has been described as continuous (Hadley, 1994a; Marais and Chown, 2003; Gibbs and Johnson, 2004). Other species show a much more organized mode of gas exchange that is characterized by periodic releases of CO₂ (“bursts”) separated by intervals of very low gas exchange (discontinuous gas exchange; Fig. 2).

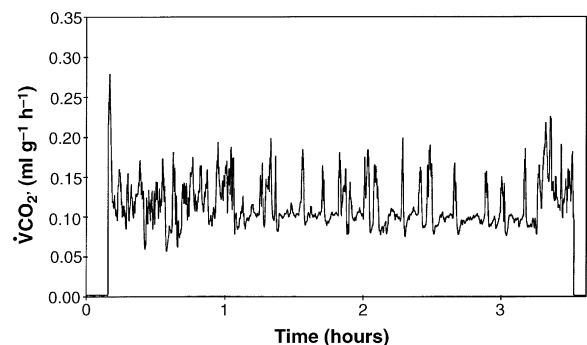


Fig. 1. Carbon dioxide excretion from the tenebrionid beetle *Eleodes armata* (Quinlan and Lighton, unpublished data). Gas exchange and animal activity (data not shown) were determined using techniques described in Quinlan and Lighton (1999). Animal activity was minimal throughout the run. Carbon dioxide loss during the first hour is continuous, but some periodicity is evident in later hours.

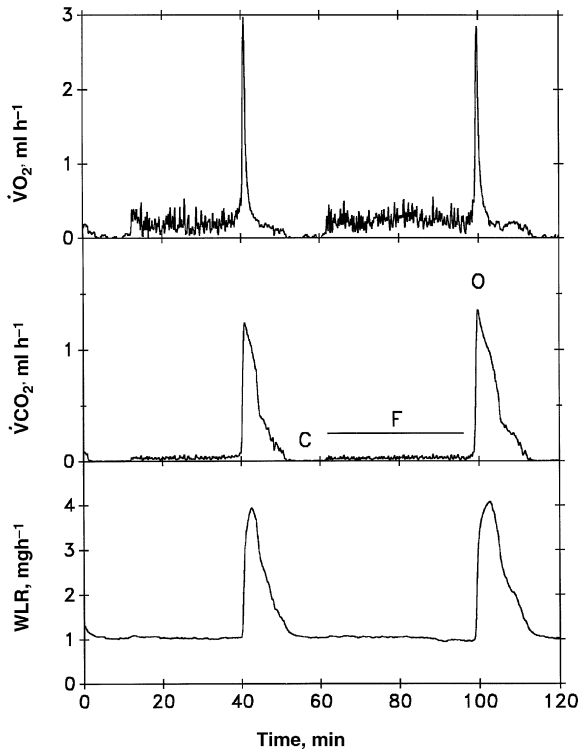


Fig. 2. Oxygen uptake (upper trace), CO₂ excretion (middle trace) and water loss (WLR, lower trace) from a diapausing *Hyalophora* pupa at 20 °C (Quinlan and Hadley, unpublished data). The techniques used to measure these parameters are described in Hadley and Quinlan (1993). Note the closed (C), flutter (F) and open (O) periods in each cycle of discontinuous ventilation. The small excursions in the O₂ and CO₂ traces during the F period represent spiracular fluttering.

Finally, CO₂ release from some insects oscillates in a regular fashion, but without extended periods of very low gas exchange between bursts. This pattern has been called cyclic gas exchange, and an example is shown in Fig. 3 (Quinlan and Lighton, 1999; Shelton and Appel, 2001a,c; Gibbs and Johnson, 2004; Chown et al., 2006).

Discontinuous gas exchange was first reported in the early 1950s (Punt, 1950; Punt et al., 1957; Buck and Keister, 1955; Schneiderman and Williams, 1953, 1955), but a comprehensive physiological explanation was not elucidated until the work of Buck (1958a,b), and later Schneiderman and his colleagues (Schneiderman, 1960; Levy and Schneiderman, 1966a,b,c; Schneiderman and Schechter, 1966; Brockway and Schneiderman, 1967). DGE has attracted attention over the years for sev-

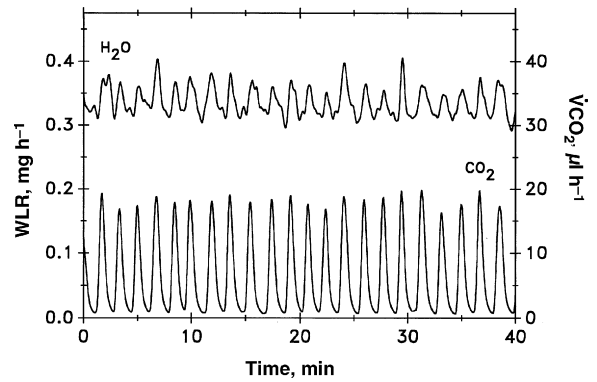


Fig. 3. Water loss (WLR) and CO₂ excretion in the harvester ant *Pogonomyrmex rugosus* (modified from Quinlan and Lighton, 1999). The upper and lower traces are water loss and CO₂ excretion, respectively. Gas exchange in this organism would be termed cyclic as CO₂ release never falls completely to zero, and thus the pattern lacks the C and F phases associated with true discontinuous gas exchange (see text for discussion).

eral reasons. First, the process requires a sophisticated interplay between the nervous system and the mechanisms responsible for spiracular movement (Burkett and Schneiderman, 1974; Kaars, 1981; Miller, 1981; Bustami and Hustert, 2000). Second, measurement of gas fluxes during DGE has yielded significant insights about the relative contributions of diffusion and convection to gas exchange (Lighton, 1996; Chown and Nicolson, 2004). Finally, the adaptive significance of DGE—at first thought to be exclusively water retention—has come into question recently. This reassessment of the adaptive value of DGE has stimulated renewed interest in its characteristics. We will now discuss the specifics of DGE, and the current ideas about its function.

4. Discontinuous gas exchange

4.1. Characteristics

The ventilatory pattern of diapausing pupae of the silkworm *Hyalophora* (Fig. 2) is often cited as a “classic” example of DGE (Hadley, 1994b; Chapman, 1998; Chown et al., 2006). Each cycle is composed of three periods. During the spiracular closed (C) period, O₂ consumption by the tissues lowers the endotracheal P_{O₂}, which causes a drop in the total endotracheal pres-

sure as CO₂ is buffered by extracellular fluids (Hetz et al., 1993). When endotracheal O₂ levels fall sufficiently, the spiracular valves open for very short periods (flutter, or “F” period). Spiracular fluttering allows the convective flow of atmospheric air into the tracheae, thereby raising the P_{O₂}. This process continues until CO₂ levels become so high that the spiracular closer muscles relax and the spiracle opens (open, or “O” period). The accumulated CO₂ is discharged during the O period, and the spiracles close again, thereby repeating the cycle. In larger species capable of abdominal ventilation, a period of pumping (ventilation, or “V” period) may occur in place of the O period (Miller, 1981; Kaars, 1981).

The primary controller of spiracular caliber during DGE in insects appears to be CO₂ (Wigglesworth, 1972; Miller, 1974, 1981; Kaars, 1981; Harrison et al., 1995; Harrison, 1997). Oxygen tension can also exert control over spiracular movements, although its influence is usually via the central nervous system (Miller, 1974; Burkett and Schneiderman, 1974; Bustami et al., 2002). The spiracular muscles, whose contractions cause the spiracular apertures to either open or close, are controlled by ganglionic efferents and, in some cases, by local levels of CO₂ (Hoyle, 1960; Kaars, 1981).

Gas movement during the F periods of DGE cycles is often assumed to be convective and inward, rather than diffusive and bidirectional (O₂ in, CO₂ and H₂O out). This assumption has been confirmed experimentally in pupal lepidoptera (Schneiderman, 1960; Brockway and Schneiderman, 1967; Hetz et al., 1993), the ant *Cataglyphis bicolor* (Lighton et al., 1993a), the cockroach *Periplaneta americana* (Kestler, 1985) and the dung beetle *Aphodius fossor* (Chown and Holter, 2000). However, diffusion seems to be the dominant mode of gas transfer during the F period in the beetle *Psammodes striatus* (Lighton, 1988b) and the ant *Camponotus vicinus* (Lighton, 1988a). In fact, Lighton (1996) concluded that diffusion is the dominant mode of gas transfer during the F period in many insect species. A rough measure of the importance of diffusion during the F period is given by the amount of CO₂ excretion occurring during this period, since CO₂ efflux is likely diffusive (Chown and Nicolson, 2004).

The issue of diffusion versus convection during the F period has important implications for water reten-

tion during gas exchange. The original explanation for DGE put forth by Buck, Schneiderman and colleagues proposed that a predominately convective F period would allow uptake of O₂ without a significant loss of water from the respiratory system (Kestler, 1985). On the other hand, a largely diffusive F period would allow simultaneous loss of CO₂ and water, to the detriment of organismal water balance. The ideal F period, from the perspective of water retention, would be one that exhibited no measurable CO₂ excretion, since this implies a convective gas flow into the animal (see Lighton et al., 1993b, for a non-insect example). In contrast, organisms with elevated F period CO₂ efflux should also suffer elevated efflux of water during this period.

As more species have been examined, it has become apparent that the specific details of ventilatory patterns differ widely. For example, the F period is often lacking (Quinlan and Lighton, 1999; Rourke, 2000; Duncan and Byrne, 2000; Shelton and Appel, 2001a,b; Gibbs and Johnson, 2004). In some cases, spiracular fluttering may also occur during the O period (Duncan and Byrne, 2000). Another confounding factor is intra-specific variation. The regularity, or even presence, of DGE can vary between conspecifics of some taxa (Hadley and Quinlan, 1993; Rourke, 2000; Chown, 2001; Marais and Chown, 2003). Furthermore, individual insects may exhibit respiratory behaviors that range from regular DGE cycles to continuous gas exchange patterns in the same experiment (Chown, 2001; Marais and Chown, 2003; Gibbs and Johnson, 2004). In some cases this variability may be related to measurement temperature (Chappell and Rogowitz, 2000; Rourke, 2000; Vogt and Appel, 2000) or developmental stage (Mbata et al., 2000), but in many cases the cause of the variation is unclear (Chown, 2001).

4.2. Distribution of DGE in terrestrial arthropods

The insect respiration literature (including textbooks) would suggest that DGE is the dominant form of gas exchange in terrestrial insects. However, the current data set is dominated by species from two orders (Hymenoptera and Coleoptera), and many orders remain unexamined. Recently, Marais et al. (2005) published a comprehensive analysis of the insect respiration literature from 1950 onward (99 species from eight orders). Their analysis revealed

that 59 species generally exhibited DGE, 35 exhibited a cyclic pattern of respiration, and 31 respired continuously. It is important to note that many species exhibited more than one respiratory pattern; 19 species showed two patterns, and four species demonstrated all three. This study also included new information for 19 species of insects from 10 orders not previously examined (18 orders total). Gas exchange in the new species was predominately cyclic (14 species); four respired continuously while one exhibited DGE. Discontinuous gas exchange was noted in only five orders (Blattodea, Orthoptera, Coleoptera, Lepidoptera and Hymenoptera), each of which also include species that respired continuously or via cyclic gas exchange. The authors concluded that cyclic gas exchange is likely to be the ancestral condition in insects, and that DGE is the derived state.

As Marais et al. (2005) point out, the 100 or so insect species that have been examined thus far represent a very small fraction of the total. In addition, the literature likely suffers from a systematic bias related to a “file drawer” problem (Csada et al., 1996). Put simply, species that do not exhibit DGE are less likely to attract the attention of researchers and are thus under-reported in the literature (Lighton, 1998; Chown, 2001). Fortunately, this problem is now recognized by most in the field, and the hope is that future work will take care to note all of the gas exchange patterns observed in the study organisms, as Marais and Chown (2003) and Gibbs and Johnson (2004) have done in their recent works. In any case, it is clear that DGE is far from universal in insects, and that more data are required before generalizations can be made about the distribution of this behavior.

4.3. Adaptive significance of DGE—water conservation?

The ability of insects to regulate spiracular caliber is unquestionably important to their water balance. Tracheal gas is thought to be nearly saturated with water vapor, and water loss increases, often dramatically, when spiracles are kept open experimentally (Edney, 1977). However, the question at hand is whether DGE per se plays a significant role in the water economy of insects. Buck and colleagues suggested early on that DGE evolved as a way of reducing respiratory water loss in *H. cecropia* (Buck et al., 1953; Buck, 1958a,b).

Others have likewise asserted, with varying degrees of empirical support, that DGE benefits the water economy of several insect species (Kestler, 1985; Lighton, 1991, 1994; Duncan and Dickman, 2001; Duncan et al., 2002). Some of the most interesting recent work is that by Chown and Davis (2003), who tested three null hypotheses on five African dung beetles. They showed that species from arid regions had the lowest rates of water loss, and that both cuticular and respiratory water losses were important to the overall water balance of the study organisms. In addition, they found that species from more arid areas had lower metabolic rates, shorter O periods and longer F periods. All of these findings are consistent with the water conservation hypothesis of DGE.

While the work of Buck, Schneiderman and colleagues remains persuasive regarding the function of DGE in moth pupae, more recent observations on other insect taxa cast doubt on the general applicability of the water conservation hypothesis. For example, DGE tends to break down at higher temperatures in grasshoppers and beetles, despite the fact that vapor-pressure gradients for water loss are greater (Quinlan and Hadley, 1993; Quinlan and Lighton, 1999; Chappell and Rogowitz, 2000; Rourke, 2000). Thus, insects appear to lose spiracular control when it would be most advantageous for water conservation. Another problem is that even modest activity generally disrupts DGE, so that only completely quiescent animals are likely to benefit from water conservation associated with DGE. In the few cases where desiccated arthropods have been examined, all have abandoned DGE when total body water was reduced (Hadley and Quinlan, 1993; Hadley, 1994b). In addition, the F-period is reduced or absent in many insects (Quinlan and Lighton, 1999; Rourke, 2000; Duncan and Byrne, 2000; Shelton and Appel, 2001a,b; Gibbs and Johnson, 2004). Recall that the F period is a critical feature of the water-conservation model, since it is during this period that convective air flow allows O₂ uptake with minimal water loss. Finally, improvements in flow-through respirometry (Hadley and Quinlan, 1993; Lighton et al., 1993c; Hadley, 1994a,b) and other analytical approaches (Gibbs and Johnson, 2004; Lighton et al., 2004; Schilman et al., 2005) have made it possible to quantify spiracular water loss by simultaneously measuring CO₂ release and water loss from test organisms. Water loss rates do increase when the spiracles

open, but in many cases the increase is comparatively small (see Chown, 2002 for a review). Only when cuticular transpiration has been drastically reduced do respiratory losses begin to constitute a significant fraction of total water loss (Zachariassen et al., 1987; Zachariassen, 1991; Hadley, 1994a; Chown, 2002; Chown and Davis, 2003). Taken together, these data do not support the hypothesis that DGE is an adaptation for water conservation in many insects (Chown, 2002; Chown et al., 2006), and several alternatives have been proposed.

4.4. Alternative models for DGE cycles

Although water conservation has been the dominant explanation for DGE for decades, it has not been the only one. An early alternative was the “strolling arthropod” hypothesis (Miller, 1974). This proposes that spiracles may have originated to prevent infection by parasites (e.g. mites, fungal or bacterial spores, etc.) via tracheal openings. This hypothesis has received occasional mention, but little experimental testing. For example, Harrison et al. (2001) found that reserve capacity for flight performance of honeybees is compromised by the presence of tracheal mites. Although this observation does show that such infections can hurt performance, it does not demonstrate that spiracular control either originated as a means to repel invaders or that it serves this function today.

The renewed interest in DGE has stimulated several additional hypotheses. The one receiving the most attention to date is the chthonic hypothesis (Lighton and Berrigan, 1995; Lighton, 1996). These authors proposed that DGE cycles originated in insects from subterranean habitats, where CO₂ levels can be relatively high (Anderson and Ultsch, 1987). Closure of the spiracles would allow tracheal P_{CO₂} to rise high enough above ambient levels to allow efficient diffusive gas exchange when the spiracles open. Burrows may be relatively hypoxic as well, so Lighton (1996) suggested that DGE may also aid in reducing internal P_{O₂} levels to enhance the gradient for O₂ influx. In either case, the value of DGE lies in better matching of CO₂ and O₂ gradients to foster rapid gas exchange (Lighton, 1998). Note also that the chthonic hypothesis proposes that DGE cycles originated as an adaptation to hypercapnia. This does not preclude their later use in descendants inhabiting other environments,

e.g. as an exaptation for water conservation above ground.

The first exposition of the chthonic hypothesis (Lighton and Berrigan, 1995) proposed that an alternative to DGE cycles would be to allow the spiracles to remain open and enhance diffusion of gases, but that “this strategy may impose an unacceptable [respiratory] water loss penalty”. Thus, the chthonic hypothesis may be considered an extension of the traditional water conservation model that postulates an adaptive role for DGE in hypercapnic habitats. Other discussions of the chthonic hypothesis have concentrated on gas exchange as an issue separate from water conservation (e.g. Lighton, 1996). This has led to some confusion about exactly what the chthonic hypothesis is and what predictions can be derived from it. Chown et al. (2006) have therefore recently distinguished the chthonic-hygic hypothesis (sensu Lighton and Berrigan, 1995) from a more general chthonic hypothesis that does not require water conservation. The latter version focuses on DGE as a mechanism to increase gradients for gas exchange.

While the chthonic hypothesis proposes that DGE cycles serve to improve gas exchange, the oxidative stress hypothesis (Hetz and Bradley, 2005) proposes instead that DGE cycles limit gas exchange, specifically O₂ uptake, so as to minimize damage from O₂-derived free radicals (superoxide, peroxides, etc.; see Landis and Tower, 2005). Under this model, spiracles must open to allow CO₂ release, but this allows potentially toxic amounts of O₂ to enter. Oxygen levels decline during the C phase, then are regulated at levels that are low, but sufficient for metabolic demand during the F phase. Consistent with this model, endotracheal P_{O₂} values are tightly regulated during the F phase in the face of varying external P_{O₂} by adjusting the fluttering rate of the spiracles (Hetz and Bradley, 2005).

Chown and Holter (2000) put forth a non-adaptive hypothesis, the interacting setpoints model. This notes that spiracular opening is regulated by both CO₂ and O₂ levels (Miller, 1974; Bustami et al., 2002; see Section 4.1). Thus, DGE cycles may simply be an emergent property of the sensory mechanisms regulating spiracular muscles, and may have no adaptive significance. This can be treated as the null hypothesis for DGE: insects exhibit DGE cycles because that is how they are wired.

5. Testing hypotheses

5.1. Within and among species

Studies of DGE in insects have generally presumed that this breathing pattern is adaptive, i.e. increases the fitness (survival, fecundity) of the organism. For adaptation to hold, respiratory patterns must differ consistently among individuals, this variation must be heritable, and it must result in differential reproductive success. The first criterion has been met in a few cases (Buck and Keister, 1955; Chappell and Rogowitz, 2000; Marais and Chown, 2003). Heritable variation has not been rigorously demonstrated, although gas-exchange characteristics differ consistently between *Drosophila* (Williams and Bradley, 1998) and cockroach strains (Dingha et al., 2005). The third criterion, differential reproductive success, has not been rigorously examined either. Instead, physiological correlates of DGE (reduced water loss rates, reduced oxidative stress, etc.) have been used as proxies for fitness.

It is critical to note that insects use a wide variety of respiratory patterns, both within and among species. For DGE to be adaptive under a given hypothesis, this pattern must be better at reducing water loss or oxidative stress, matching CO₂ and O₂ exchange, or keeping out strolling arthropods, than alternative modes of breathing. With few exceptions (e.g. Kestler, 2003; Gibbs and Johnson, 2004), discussions have concentrated on classical DGE as opposed to no spiracular control at all. Discontinuous gas exchange has received by far the most attention, but any general understanding of insect respiration will ultimately require consideration of patterns besides continuous and discontinuous gas exchange.

Gas-exchange patterns in insects have been examined at two general levels: within and among species. Investigations in single species have tested specific hypotheses about DGE and its effects on water balance, its occurrence under specific environmental conditions, etc. For example, several studies have challenged the water conservation hypothesis by noting that respiratory water loss comprises such a small fraction of total water loss, even during the O phase, that DGE would have little effect on the overall water budget (e.g. Hadley and Quinlan, 1993). Furthermore, in several studies (e.g. Williams and Bradley, 1998; Shelton

and Appel, 2001b; Gibbs and Johnson, 2004) water loss was not lower during cyclic respiration than during acyclic patterns.

Other studies have considered whether the focal species either displays the breathing pattern one might expect, based on habitat, or whether DGE cycles respond appropriately to environmental conditions. For example, under the chthonic hypothesis, one would expect insects exposed to hypoxia or hypercapnia to maximize gradients for gas exchange by lengthening the closed period of the cycle. In general, they do not; instead insects respire more frequently (Chown and Holter, 2000), as would be expected given current knowledge of how spiracles are controlled. Such studies highlight a general limitation of the single-species approach: one can learn more about how insect respiration works, but this does not rigorously tell us why some insects exhibit DGE and others do not.

An alternative approach has been to use species from different habitats to test hypotheses regarding the benefits of DGE in different ecological contexts. Habitat-based predictions about gas exchange patterns are usually based on presumed environmental conditions rather than actual microclimatic data. It is also important to incorporate phylogenetic information in statistical analyses (Garland et al., 2005). Relatively few studies have attempted to incorporate phylogenetic information, and in each case it has been difficult to tease apart phylogeny and habitat. For example, Gibbs et al. (2003) found that two desert-dwelling species of *Drosophila* were more likely to exhibit a cyclic pattern of CO₂ release (not DGE) than four mesic species, but these were also closely related to each other. Chown and Davis (2003) studied five dung beetles and found that those from drier regions were more likely to exhibit DGE cycles. However, the two arid-living species were again closely related.

Recent work by Marais et al. (2005) provides an excellent initial step in comparative phylogenetic analyses, but more rigorously designed studies are needed to test specific predictions related to DGE hypotheses. For example, several predictions can be made regarding the water conservation hypothesis for DGE. An obvious one is that DGE cycles should be more prevalent in xeric than mesic species. Although much early work tended to support this hypothesis, many xeric species do not appear to perform DGE cycles (e.g. North Amer-

ican and Australian tenebrionid beetles; Lighton, 1998; Duncan and Dickman, 2001; Fig. 1), whereas many mesic species do (e.g. lubber grasshoppers; Hadley and Quinlan, 1993). In comparing species which do perform DGE, water conservation should also be enhanced by minimizing the length of the O phase. This appears to be the case in certain dung beetles (Chown and Davis, 2003), but as noted above the species from arid habitats were also closely related.

Another prediction from the water conservation hypothesis is that xeric species should accumulate more CO₂ during the C and F phases than mesic species, creating a higher gradient for outward diffusion during the O phase. In contrast, tracheal water vapor pressure (at a given temperature) is generally presumed to be constant. Xeric species should therefore lose more CO₂ relative to water loss during the O period.

Discontinuous gas exchange will also cause large fluctuations in P_{CO_2} . Resultant fluctuations in pH can be moderated by increased tissue buffering capacity. Because most internal CO₂ will be in the form of bicarbonate, high levels of carbonic anhydrase are also expected, in order to allow rapid CO₂ release during the O phase. Unfortunately, tissue buffering and carbonic anhydrase have received little attention in the context of insect respiratory patterns.

The oxidative damage hypothesis is based on dissolved oxygen's ability to spontaneously form superoxide free radicals that can then damage proteins, nucleic acids and membrane lipids (Sohal, 2002; Barja, 2004). Reducing internal P_{O_2} would reduce the possibility of damage. If this hypothesis is correct, one would predict that insects performing DGE cycles would suffer less damage than non-DGE breathers. Alternatively, continuous breathers could have higher levels of antioxidant systems, such as superoxide dismutase and catalase. Oxidative damage and antioxidants in insects remain poorly studied, with the exception of aging research performed using *Drosophila* (e.g. Golden et al., 2002; Sohal, 2002; Landis and Tower, 2005).

6. Testing hypotheses using a strong-inference approach

Each adaptive hypothesis for DGE posits an organismal benefit that is specific to certain environmental conditions, such as low humidity or hypercapnia. As

described above, these models can be used to derive predictions regarding the effects of environmental stress on respiratory patterns, including the presence, prevalence or characteristics of DGE cycles, which are not necessarily relevant to alternative hypotheses. Experimental support for such predictions is consistent with a particular model, but does not necessarily disprove others. It is important to be able to distinguish between competing hypotheses. This can only be achieved if two models make different predictions regarding outcomes (Huey and Berrigan, 1996; Huey et al., 1999; Chown, 2002).

We discuss below a few potential circumstances in which alternative DGE models make conflicting predictions, and therefore should allow strong-inference tests of these hypotheses. We emphasize that these studies need to account for phylogenetic relationships, and that the environmental conditions actually faced by insects also need to be understood. The latter factor is a serious weakness in most work on insect respiration, but these problems are beginning to be addressed more frequently. Good recent examples include work by Davis et al. (1999, 2000), Duncan and Byrne (2000), Duncan and Dickman (2001) and Chown and Davis (2003). We applaud these efforts and hope that the hypotheses discussed in previous sections will be illuminated by insights gained from phylogenetic and microclimatic information.

Unfortunately, it is not always feasible to obtain microclimate data. An alternative approach involves correlation of macroclimatic variables with breathing patterns on a broad scale. Recent years have seen an increasing interest in understanding large-scale patterns in physiological traits (i.e. "macrophysiology"; Chown et al., 2004). This approach has been particularly effective in the case of insect thermal biology and water balance (e.g. Addo-Bediako et al., 2001, 2002; Chown et al., 2002, 2003; Sinclair et al., 2003) and may prove useful in understanding breathing patterns as well. Obviously, these comparisons need to be done with care. For example, even in arid deserts, relative humidities increase rapidly with soil depth and are usually nearly saturating within centimeters of the surface. Thus subterranean environments are not particularly arid. The same is likely to hold true for other putatively hypercapnic/hypoxic habitats (e.g. those of wood-borers), contradicting a basic assumption of the chthonic-hygic hypothesis.

A macrophysiological approach may allow the little-studied strolling arthropods hypothesis to be tested against the well-studied hygric hypothesis. The hygric hypothesis predicts that DGE cycles should be most prevalent in insects from deserts and other arid environments. On the other hand, tropical forests and other moist habitats have a very high abundance and diversity of endophytic fungi and their spores (Arnold et al., 2000). Assuming this is also true for insect pathogens, spiracular regulation may be particularly beneficial in mesic environments. The strolling-arthropod hypothesis therefore predicts that DGE cycles will be more prevalent in these habitats. Unfortunately, the historical focus on water conservation has left mesic species (especially tropical ones) woefully understudied in terms of breathing patterns.

Breathing patterns may also be affected by altitude. In many areas of the world, high-altitude environments are quite arid. Thus, the hygric hypothesis predicts that DGE should be more prevalent in insects from higher elevations. Oxygen levels decline as altitude increases, suggesting that oxidative damage should be reduced. Therefore, the oxidative damage model predicts that DGE cycles should be less prevalent at higher altitudes. Elevational patterns in DGE may therefore allow us to distinguish between the oxidative damage and hygric hypotheses. However, the strong developmental response of insect tracheal systems to alterations in O₂ availability (see Loudon, 1988; Henry and Harrison, 2004) provides a cautionary note that the role of phenotypic flexibility needs to be taken into consideration in such investigations.

The chthonic and chthonic-hygric hypotheses propose that DGE will be favored in enclosed, hypercapnic habitats. These are often hypoxic, so the danger of oxidative damage would be reduced. The oxidative damage hypothesis, therefore, predicts that DGE will be favored in exposed environments. Thus, the oxidative damage and chthonic models yield different predictions about the prevalence of DGE in enclosed versus open habitats. The available data are somewhat contradictory; the chthonic/chthonic-hygric hypotheses were based primarily on the existence of DGE in burrowing ants and beetles, but several grasshoppers also perform DGE (Hadley and Quinlan, 1993; Quinlan and Hadley, 1993; Rourke, 2000).

The chthonic hypotheses posit that DGE cycles are an adaptation to increase the gradient for out-

ward diffusion of CO₂ in hypercapnic environments, or inward diffusion of O₂ in hypoxic environments. If so, these conditions should trigger DGE in species that do not otherwise exhibit this pattern, particularly in those inhabiting such habitats (e.g. fossorial and wood-boring insects). Alternatively, the interacting-setpoints hypothesis implies that hypercapnia or hypoxia will increase the cycle frequency, no matter what the habitat of origin, potentially causing the spiracles to “stick” in the open mode at high enough CO₂ or low enough O₂ levels. These predictions are difficult to test in the case of hypercapnia, because of the need to detect CO₂ release against a high background level. However, cycles of water loss may be a good indicator of spiracular opening (see Figs. 2 and 3), or high-precision O₂ consumption measurements may be possible. In the case of hypoxia, evidence to date suggests that insects open their spiracles more frequently, consistent with the interacting-setpoints hypothesis.

7. Future directions

Insect respiratory physiology is in the midst of a renaissance of sorts. The field was long dominated by the ground-breaking work of Punt, Buck, Schneiderman and colleagues, and for many years their work was viewed as the alpha and omega of insect gas exchange, at least as far as DGE was concerned. This situation has changed dramatically in recent years, due in large part to three developments. First, technical improvements in CO₂ and water vapor measurement have made it possible to develop very detailed pictures of gas transfer in even small insects. The instrumentation responsible for these technical improvements is accurate, stable and available commercially. The second factor behind the recent efflorescence of insect respiratory work is the increased use of comparative methods that incorporate robust corrections for phylogeny. A corollary of this point is the notion that a good comparative study should include several species with known phylogenetic relationships. The final element is the infusion of ecological perspectives into the interpretation of comparative studies of insect respiration. Recent work by Chown, Duncan and colleagues has used habitat and life history information to great effect in African taxa, and this approach needs to be applied to the insect fauna of other continents. Chown's group has also been suc-

successful at partitioning physiological variation at the individual, species, genus and family levels (Addo-Bediako et al., 2001; Chown, 2001), to the enormous benefit of those engaged in comparative studies.

Those interested in insect respiration have an unparalleled assortment of tools, both physical and conceptual, at their disposal. We hope that future efforts will extend the work of those mentioned above to new groups in different parts of the world. The adaptive value of DGE, if indeed it is adaptive, is unclear at best. It may well be that its original function in ancestral insects is very different from its current function(s) (Chown, 2002; Marais et al., 2005). The point has also been made by Chown and Nicolson (2004) that DGE may have different functions in extant organisms depending on the needs of the particular organism. We believe that answers to these complexities will come from careful comparative studies of related taxa from different ecological settings.

Our understanding of insect respiration, and DGE in particular, may be murkier in some regards than it was 30 years ago despite many significant advances. That said, it remains an area of enormous interest to many people, and we clearly have much to learn before we can “close the book” on insect gas exchange.

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