

Discontinuous Gas Exchange in Insects: A Clarification of Hypotheses and Approaches*

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ABSTRACT

Many adult and diapausing pupal insects exchange respiratory gases discontinuously in a three-phase discontinuous gas exchange cycle (DGC). We summarize the known biophysical characteristics of the DGC and describe current research on the role of convection and diffusion in the DGC, emphasizing control of respiratory water loss. We summarize the main theories for the evolutionary genesis (or, alternatively, nonadaptive genesis) of the DGC: reduction in respiratory water loss (the hygric hypothesis), optimizing gas exchange in hypoxic and hypercapnic environments (the chthonic hypothesis), the hybrid of these two (the chthonic-hygric hypothesis), reducing the toxic properties of oxygen (the oxidative damage hypothesis), the outcome of interactions between O₂ and CO₂ control set points (the emergent property hypothesis), and protection against parasitic invaders (the strolling arthropods hypothesis). We describe specific techniques that are being employed to

measure respiratory water loss in the presence or absence of the DGC in an attempt to test the hygric hypothesis, such as the hyperoxic switch and H₂O/CO₂ regression, and summarize specific areas of the field that are likely to be profitable directions for future research.

Introduction

Discontinuous gas exchange is often regarded as the quintessential characteristic of insect respiratory physiology. In most textbooks on animal and insect physiology, it is selected for special treatment (Chapman 1998; Willmer et al. 2000; Nation 2002), and, depending on the text, it is not unusual to come away thinking that it is the only gas exchange pattern typical of insects at rest. However, to date, discontinuous gas exchange has been recorded from only 51 species of insects, the large majority of which are either beetles (55%) or other holometabolous insects (a further 33%). Indeed, of the 31 orders of insects, published information has, until recently, been available on the gas exchange patterns of only eight of these, and for the most part, data are sparse on the apterygotes and exopterygotes (Marais et al. 2005). Moreover, it is clear that not all insects that are assumed to be resting show discontinuous gas exchange. Some species are characterized only by the continuous exchange of gases (such as certain tenebrionid beetles; Lighton 1998), others by cyclic gas exchange (Shelton and Appel 2001a, 2001b), and yet others by a wide variety of gas exchange patterns ranging from continuous to cyclic to discontinuous (Marais and Chown 2003; Gibbs and Johnson 2004). Nonetheless, of all the gas exchange patterns characteristic of insects, discontinuous gas exchange has enjoyed by far the most attention (reviews in Miller 1981; Kestler 1985; Lighton 1994, 1996, 1998; Chown and Nicolson 2004).

Not only has this interest provided a wealth of information on the physiological and ecological aspects of discontinuous gas exchange, but, to paraphrase Eiseley (1970, p. 92), it has also generated controversy "in a genuinely confusing ratio." The debate largely concerns the reasons for the evolutionary origin and subsequent maintenance of discontinuous gas exchange cycles (DGCs), that is, whether these cycles are adaptive and, if so, how natural selection brought them about and how it might be responsible for their current retention and modification in insects and in the few other arthropods where they have also been found (Lighton and Fielden 1996; Klok et al.

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2002; Lighton and Joos 2002). Although early work settled on water savings as a plausible hypothesis for the adaptive value of discontinuous gas exchange (Buck et al. 1953; Buck and Keister 1955; Burkett and Schneiderman 1974*a*, 1974*b*; Kestler 1985), later studies questioned this idea for a variety of reasons (summarized in Lighton 1998; Chown 2002). These studies also led to the development of at least two alternative adaptive hypotheses (Lighton and Berrigan 1995; Bradley 2000; Hetz and Bradley 2005) and a nonadaptive one (Chown and Holter 2000). At present, there is no consensus on which of these hypotheses might be correct (see, e.g., Lighton 1998; Shelton and Appel 2000; Duncan et al. 2002; Gibbs and Johnson 2004; also see a review in Chown and Nicolson 2004). Moreover, as discussions at two recent symposium sessions (“Breathing without Lungs: Respiratory Strategies in Tracheate Arthropods,” Third International Conference of Comparative Physiology and Biochemistry, 2004, and “Oxygen, Water and Oxygen Radicals in Insects: Understanding the Balance,” XXII International Congress of Entomology, 2004) devoted to gas exchange in insects have revealed, there is also limited agreement not only on the formulation of these hypotheses but also on their predictions and the ways in which these should be tested.

In this paper, we therefore provide a concise description of the characteristics of DGCs, updating previous ideas with new information on tracheal pressures and gas flows; provide consensus formulations of the major adaptive and nonadaptive hypotheses that have been proposed to explain the evolutionary origin and maintenance of DGCs; set out the various predictions of these hypotheses within a strong inference approach; outline continuing debates; and briefly describe methods that might be used to distinguish between competing hypotheses.

DGCs

The first DGCs were observed by Heller in lepidopterous pupae in 1930 (cf. Kestler 1971; Miller 1974) and in adult insects by Punt (1944) and were attributed to oxygen debt accompanied by lactate accumulation, leading to a cyclic opening of the spiracular valves to repay oxygen debt. Later, Schneiderman and coworkers (Schneiderman 1953; Schneiderman and Williams 1953) showed in moth pupae—soon to become the “classic” DGC preparation—that oxygen uptake was continuous despite the cyclic release of CO₂ in “bursts.” Subsequent investigations have shown that the oxygen uptake is continuous over the fluttering and opening (but not constriction) phases, although the rate of that uptake differs between these phases such that Po₂ remains low and constant over the fluttering phase but rises during the opening phase (Lighton 1988*b*; Hetz and Bradley 2005), and the significance of the information provided by measurement of oxygen uptake over the DGC remains contentious (see Lighton 1988*b*, 1996, 1998).

The classical DGC in moth pupae consists of three periods or phases, originally termed CFO according to the spiracular

activity (Schneiderman 1960): constriction (C), fluttering (F), and opening (O; during which the CO₂ emerges in a burst). The terms were deduced from the spiracular activity that was observed during the CO₂ release; another term in popular use, CFB, mixes spiracular states (C, F) with the burst (B) of CO₂ emission, as does CFV, which emphasizes ventilation (V) during CO₂ emission, and these uses are defended by Kestler (1985) because in some larger insects such as cockroaches, the O phase may consist of several subsequent opening and closing events accompanied by convective gas exchange and leading to a saw-tooth-like CO₂ release trace (Kestler 1985). The terms DGC and (C)FB are just simplifying terms for these different patterns of gas exchange or control.

In recent literature, the distinctions between the three phases of the DGC have been made mostly according to the CO₂ traces from flow-through respirometry (for examples, see Lighton 1996; Davis et al. 1999; Duncan et al. 2002), and these interpretations have in some cases been validated by direct spiracular observation (Lighton et al. 1993*a*). However, without knowing the physiological state of the spiracles (O, C, or F), it is often not possible to distinguish correctly between the three phases, and in many cases, it may be more accurate to term the period between two O phases the “interburst” period (Duncan and Byrne 2002; Lighton and Joos 2002). Simultaneous records of tracheal pressure and CO₂ release may be necessary to detect the phases exactly (Levy and Schneiderman 1966*a*; Kestler 1985; Wobschall and Hetz 2004). The accurate detection is essential if the duration of the C and F phases is used to indicate effects of external parameters on the DGC because in some cases the F phase starts before the CO₂ measurement can detect it (Hetz et al. 1994; Wobschall and Hetz 2004). Pressure measurements are not infallible; simultaneous recordings of tracheal pressure did not always guarantee appropriate designation of the phases within the DGC of lepidopterous pupae (Hetz et al. 1994). However, Kestler (1985) found that in large adult insects, with their coordination of ventilation and spiracular control, tracheal pressure measurements distinguish clearly the VC, CF, and FV transitions. Thus, pressure measurements are at least a useful adjunct to gas exchange measurements. Wobschall and Hetz (2004) provide an example of how a combination of data on CO₂ release, intratracheal pressure, volume changes, and direct measurement of Po₂ within the tracheal system can be used to understand interactions between spiracle behavior and gas exchange. We will now deal with the three phases of the DGC in turn.

The C Phase

The C phase is most pronounced at low metabolic rates and is the only phase of the DGC around which little if any controversy swirls. Within the C phase, a negative pressure is set up (Levy and Schneiderman 1966*b*) much as in the later F phase, when oxygen is taken up from the tracheal system by

tissue respiration while CO₂ is extensively buffered in tissues and hemolymph (Buck and Friedman 1958). The negative pressure leads to a shrinking of the whole pupa as a result of the compliance of the tracheal system, which can be measured as a shortening of the abdomen (Schneiderman and Schechter 1966; Brockway and Schneiderman 1967). Despite the curvilinear tracheal pressure decrease, the oxygen uptake from the tracheal system has been found to be constant, leading to a linear decrease in tracheal volume (Wobschall and Hetz 2004).

The F Phase

Internal Pco₂ rises and internal Po₂ falls during the C phase to a point where partial openings or flutterings of the spiracles occur, allowing O₂ to enter, whereafter its internal partial pressure is regulated at a very low ca. 2–4 kPa by spiracular activity throughout the F phase (Levy and Schneiderman 1966a; Hetz et al. 1994; Wobschall and Hetz 2004) even if ambient oxygen concentration is varied in a relatively wide range (Hetz and Bradley 2005). This is the basis of the ingenious Bradley-Hetz oxidative damage hypothesis for the genesis of the DGC (Hetz and Bradley 2005).

Within the F phase, the spiracle openings are, if inferred from CO₂ release patterns, irregular with respect to frequency and amplitude. Sometimes single flutter events or “micro-openings” occur singly or in volleys (Schneiderman 1960). Single, short (<1 s) flutter events have also been recorded in pressure traces at the beginning of the F phase in diapausing pupae (Hetz et al. 1994) and adult insects (Brockway and Schneiderman 1967; Kestler 1985).

At the end of the C phase, there may be a negative endotracheal pressure, arising from the consumption of oxygen and buffering of CO₂ in the hemolymph and tissues (e.g., Wobschall and Hetz 2004). Uptake of air into the tracheal system at the beginning of the F phase along the negative hydrostatic pressure gradient may initially inhibit CO₂ release from the tracheae (Wobschall and Hetz 2004). At least some CO₂-based interpretations of F phase durations are therefore underestimates, at least in insects, such as lepidopterous pupae, with exoskeletons rigid enough to sustain significant negative tracheal pressures.

Where there is negative endotracheal pressure, as soon as the spiracles open, there is convective inward air movement, the classical “passive suction ventilation” of Kestler (1971, 1985) and Miller (1974). This negative tracheal pressure can reduce respiratory water loss during the F phase and is the keystone of the hygric hypothesis of the evolutionary genesis of the DGC. However, passive suction ventilation depends on the differential storage of O₂ and CO₂ in the tissues and tracheal system; enough O₂ has to be present in the tracheal system so that its consumption leads to a buildup of negative pressure. With every flutter event, the pressure difference between the tracheal system and the outside decreases. Therefore, diffusion will take

over from convection as the chief mechanism of oxygen uptake in the later F phase, where it is clear that conductance of the spiracles increases because of an increase in opening duration, from <1 s during the initial F phase to some 10 s before the next O phase (Wobschall and Hetz 2004).

The paper (Wobschall and Hetz 2004) showed, moreover, that there were small volume and pressure decreases between the microopenings even after the initial pressure rise phase in the F period. This confirmed a small but significant contribution of suction ventilation in each microopening (see also Kestler 1985).

The O and V Phases

During the O phase in moth pupae, the spiracular valves remain fully opened for several minutes and then begin to alternately close and open until they remain fully closed during the closed phase (Schneiderman 1960). The air within the tracheal system is almost completely exchanged with outside air, leading to an increase in endotracheal oxygen content to nearly atmospheric levels (Levy and Schneiderman 1966a; Hetz et al. 1994).

However, the O phase, or CO₂ burst phase, is variable in expression. In large adult insects such as *Periplaneta*, the O phase may be accompanied by active ventilation, leading to CFV cycles where the O phase may consist of several subsequent opening and closing sawtooth-like events, as has now also been demonstrated in *Schistocerca gregaria* (Orthoptera) and *Blaberus discoidalis* (Blattodea) but not in *Carabus coriaceus* (Coleoptera; P. Kestler, personal communication). Data on *Hyalophora* pupae, especially on O phase CO₂, H₂O, and O₂ kinetics, the latter derived gravimetrically, provide support for Kestler's (1985) partial pressure strategy hypothesis for respiratory water loss reduction (see “Hygric Hypothesis”). These data support Kestler's idea that the V phase is a strategy of adults to prevent high diffusive water loss in the burst phase and to use additional diffusion to utilize the maximum partial pressure differences of CO₂ and O₂ for diffusion and convection (see eqq. [6], [7] in Kestler 1985). This strategy apparently shortens the burst phase in adult insects except ants, which appear not to utilize convection during their burst or, properly, O phase (for further discussion, see Kestler 1985).

Evolution of Discontinuous Gas Exchange

Demonstrating Adaptation

If DGCs are to be considered adaptive, then evidence must be provided that natural selection was responsible for both their origin and their maintenance, or, at the very least, their present maintenance (Coddington 1988; Baum and Larson 1991; Ketterson and Nolan 1999). For natural selection to take place, several conditions must be met. These are as follows: (1) variation among individuals in the trait(s) of interest (i.e., significant repeatability; Bech et al. 1999; Dohm 2002), (2) a con-

sistent relationship between the trait(s) and fitness, and (3) a consistent relationship for the trait(s) between offspring and parents that is independent of common environmental effects (Endler 1986). Most investigations of DGCs that invoke adaptive explanations either for them or for variation in their constituent parts have assumed that these conditions are met. For condition 1, such an assumption is probably fair, given that the few studies that have investigated repeatability in metabolic rate and the components of DGCs have found significant and often high repeatability (Buck and Keister 1955; Chappell and Rogowitz 2000; Marais and Chown 2003; Nespolo et al. 2003). However, conditions 2 and 3 have not been rigorously assessed, although responses of gas exchange patterns to laboratory natural selection (Gibbs 1999) for desiccation resistance (see Williams et al. 1997, 2004) indicate that selection can act on gas exchange patterns and thus that conditions 2 and 3 might be satisfied, at least in one species.

Conclusions regarding the adaptive nature of DGCs have typically been based on experimental or comparative work on one or a few species and often involve qualitative comparisons with expected environmental conditions (such as aridity). The experimental work has tended to focus on specific predictions of the adaptive hypotheses, often demonstrating that they are not supported (e.g., Lighton and Berrigan 1995; Chappell and Rogowitz 2000; Chown and Holter 2000). In most instances, these can be considered relatively strong tests, although those that make simple statements about the likely nature of gas exchange patterns without considering that animals must regulate the entire internal environment and not just water balance are overly simplistic, as has been pointed out (Lighton 1996; Harrison 2001). The statement that discontinuous gas exchange should be retained under dry and warm conditions is a good example because it ignores the fact that metabolic rate, the pool of water available to insects, and pH will all be affected by these changes (Chown and Nicolson 2004).

Comparative work usually examines patterns of CO₂ emission and compares them with the conditions under which animals are thought to exist (e.g., Lighton 1988a, 1990; Davis et al. 1999; Duncan and Byrne 2000). Rarely are comparisons phylogeny based (see Felsenstein 1985; Harvey and Pagel 1991 for rationale), and often the trait of interest (such as water loss) is not measured at all. Moreover, in only a few instances are specific predictions made and tested with regard to measured environmental conditions and the traits of interest (e.g., Chown and Davis 2003). Thus, at present, comparative assessments must be considered relatively weak tests of any of the adaptive hypotheses proposed for DGCs (see also Davis et al. 2000) even though they are a perfectly legitimate way of exploring them (see Baum and Larson 1991; Harvey and Pagel 1991; Kingsolver and Huey 1998; Freckleton et al. 2002; but also see Leroi et al. 1994).

The current situation, therefore, is that much needs to be done, especially in comparative physiological subsystem re-

search, before statements regarding the adaptive nature of DGCs and variation in their constituent parts can be made with the same sort of confidence that can be used when referring to interpopulation and interspecific variation in traits such as desiccation resistance (see Gibbs et al. 2003). Providing clear expositions of the adaptive and nonadaptive hypotheses for the origin and maintenance of DGCs, their predictions, and the ways in which they might be tested is an essential part of addressing this shortcoming.

The Hypotheses

At present, there are five major hypotheses that have been proposed to account for the origin of DGCs. Four of these are adaptive and one nonadaptive. In addition, one of the adaptive hypotheses has at least two interpretations, and we subdivide it for clarity.

Hygric Hypothesis. DGCs have long been thought to provide a means by which insects might restrict respiratory water loss. The idea can be traced back to Buck et al. (1953, p. 541), who argued that “discontinuous CO₂ release is a consequence of the cycle of spiracular activity which reduces transpiratory water loss.” Buck and his colleagues reiterated this idea in several other papers (“the retention of CO₂ is not the prime objective in itself, but a consequence of provisions for minimizing transpiratory water loss”; Buck and Keister 1955, p. 161; Buck 1958, p. 127), and it was subsequently also espoused by Schneiderman and coworkers (e.g., Levy and Schneiderman 1966a, 1966b; Burkett and Schneiderman 1967, 1968, 1974a, 1974b). Many authors have since argued that restriction of respiratory water loss should be regarded as the main adaptive function of DGCs or as a likely candidate for this role (Kestler 1985; Lighton 1994, 1996; Vogt and Appel 2000; Duncan et al. 2002) and/or that variation in the constituent parts of these cycles should also be regarded as an outcome of the adaptive process (Lighton 1988a, 1990, 1991; Lighton et al. 1993b; Davis et al. 1999; Duncan and Dickman 2001; Chown and Davis 2003).

Lighton (1996, p. 319) first termed this water savings hypothesis the hygric genesis hypothesis, noting that “the evolutionary genesis of the DGC ... may be hygric (derived from selective pressures to minimize respiratory water-loss rates).” In keeping with early work and with Lighton’s (1996) discussion, here we define the hygric hypothesis as follows: DGCs are an adaptation to reduce respiratory water loss. If the focus were exclusively on the adaptive origins of DGCs, then “hygric genesis” may have been more appropriate a term than “hygric hypothesis.” However, we note that at a minimum, a trait should be considered an adaptation if natural selection is responsible for its maintenance (i.e., its current utility; see Reeve and Sherman 1993; Schluter 2000). If natural selection were responsible only for its origin, then that trait might very well be a plesiomorphy rather than an adaptation, although from

a functional perspective, it might still be essential (for additional discussion, see Schluter 2000).

In this respect, Kestler (2003) has demonstrated the physical mechanisms by which the different subsystems of the cycles contribute to different strategies of water retention. He emphasized that increasing the partial pressure difference of O₂ and CO₂ over the constant one of H₂O (the partial pressure strategy) is a specific strategy solely for water retention. The second specific strategy is the replacement of diffusive O phases by convective V phases wherever possible to avoid the higher diffusivity of water and to make use of the higher O₂ and CO₂ partial pressures both for diffusion and convection to maximize closure and fluttering.

Chthonic Hypothesis and Chthonic-Hygric Hypothesis. On the basis of their work on *Messor* ants and comparative data on discontinuous gas exchange in other insects, Lighton and Berrigan (1995, p. 528) argued that a hypothesis of water savings could account neither for their data nor for the distribution of DGCs among insects from different environments. As an alternative, they proposed the following: "An insect can solve the conundrum of diffusive gas exchange in significantly hypoxic and hypercapnic environments in two distinct ways. First, it may open its spiracles maximally for long periods, thus increasing net diffusive flux and compensating to some extent for a shallow partial pressure difference. However, unless the insect's environment is perfectly water-saturated, this strategy may impose an unacceptable water loss penalty; overall water loss rates can increase several-fold while the spiracles are open. The second alternative is to sequester CO₂ and deplete O₂ within the tracheal system for as long as physiologically feasible. In this case, the partial pressure gradient will increase until a brief period of spiracular opening can effect the required net flux. Hence discontinuous ventilation." These sentences can readily be interpreted as indicating that DGCs are an adaptation to effect gas exchange in hypoxic or hypercapnic environments in such a way that there is not an unacceptably high respiratory water loss, and indeed such an interpretation has already been made more than once (Chown and Holter 2000; Gibbs and Johnson 2004). It should be noted, however, that in the latter paper, the actual hypothesis being tested was the hygric hypothesis, not the chthonic hypothesis, because it was a test of the water retention properties of the DGC under normoxic and acapnic conditions. Thus, the chthonic hypothesis has not yet been rejected.

In the abstract to their paper, Lighton and Berrigan (1995, p. 521) wrote, "We hypothesize ... that discontinuous ventilation occurs primarily in insects that may experience hypoxic and hypercapnic conditions ... that discontinuous ventilation is not necessarily essential to reduce respiratory water loss." Moreover, in reviewing the field a year later, Lighton (1996, p. 319) proposed that this idea be termed the chthonic genesis hypothesis, noting that "the evolutionary genesis of the DGC

may be chthonic (derived from gas-exchange contingencies in underground conditions characterized by hypercapnia and hypoxia)" and later (p. 320) that "the DGC acts primarily to facilitate gas exchange in hypoxic and hypercapnic environments." No mention was made of the water savings argument that was elaborated by Lighton and Berrigan (1995). Lighton (1998, pp. 487–488) pursued a similar, though somewhat more complex, argument (separating diffusive and convective gas exchange), arguing that "the diffusion-based DGC is a functional and viable gas exchange strategy in hypercapnic and hypoxic environments" and that "in practice, the high water loss rate during the O phase could be mitigated by the relatively high ambient water vapor pressure in most underground environments." It is therefore clear that in works postdating that of Lighton and Berrigan (1995), Lighton indicates that the chthonic genesis hypothesis is one of gas exchange facilitation under hypoxic and hypercapnic environmental conditions, without reference to water loss restriction. This interpretation has subsequently been followed by other workers (e.g., Shelton and Appel [2000]; Duncan et al. [2002]). In consequence, the chthonic genesis hypothesis has two distinct meanings in the literature.

In keeping with the idea that we are concerned not only with the origin of DGCs and in following Lighton's (1996) discussion, we suggest that the chthonic genesis hypothesis be renamed the chthonic hypothesis and stated as follows: DGCs are an adaptation to ensure that either metabolic oxygen requirements are met under environmental hypoxia or requirements for CO₂ release are met under environmental hypercapnia or both. By contrast, Lighton and Berrigan's (1995) hypothesis, as described in their discussion, should be termed the chthonic-hygric hypothesis: DGCs are an adaptation to reduce respiratory water loss under environmental conditions of either hypoxia or hypercapnia or both. The term "chthonic-hygric" acknowledges that elements of both the chthonic and hygric hypotheses are contained within this idea and that it takes into account the likely flows of all the gases (O₂, CO₂, H₂O vapor) that are important to insects.

Oxidative Damage Hypothesis. This idea was first articulated by Bradley (2000, p. 952) in the abstract of a symposium paper, where he stated: "I propose that the DGC may serve to reduce the supply of oxygen to the tissues during periods of low metabolic rate. Oxygen is toxic to cells, even at ambient concentrations, and insects may use the DGC to provide adequate gas exchange while reducing oxidative damage during periods of reduced oxygen demand." Kestler (1985, p. 153) had previously suggested that liquid filling in tracheoles might serve to prevent oxidative damage, excessive loss of CO₂, and water vapor more or less simultaneously and speculated that a "rise in tissue partial pressure of oxygen should occur especially in those insects ... which have either no or open spiracles." However, Kestler did not propose that DGCs per se could function as an

adaptation to prevent oxidative damage but rather focused almost exclusively on their role in controlling respiratory water loss (Kestler 1985, p. 161 onward). The oxidative damage hypothesis (Bradley 2000) has subsequently been set out in detail by Hetz and Bradley (2005) and can be stated as follows: DGCs are an adaptation to provide adequate gas exchange while reducing oxidative damage to tissues at rest that would otherwise result from a tracheal system that has evolved to maximize oxygen availability during activity.

Emergent Property Hypothesis. Chown and Holter (2000) found little support for either the chthonic-hygric hypothesis (they termed it the chthonic genesis hypothesis) or the hygric hypothesis in the dung beetle they examined. While they did not assess the oxidative damage hypothesis, they concluded that the former hypotheses could not explain the change in DGCs they observed when *Aphodius fossor* was subjected to increasing environmental hypoxia. In consequence, they argued that DGCs might be nonadaptive, arising as an epiphenomenon of the interaction of two feedback systems regulating a single function with minimal demand. They provided no support for this idea, but it is clear that there are two feedback systems (one based on O₂ and the other on CO₂) that interact to regulate spiracle opening and gas exchange (Schneiderman 1960; Burkett and Schneiderman 1974b; Miller 1974; Kaars 1981).

Their idea of the origin of the DGC as a consequence of interacting feedback systems should be termed the emergent property hypothesis, which can be stated as follows: DGCs are a nonadaptive outcome of interactions between the O₂ and CO₂ set points that regulate spiracle opening and closure.

Strolling Arthropods Hypothesis. Several early papers suggested that sieve plates, spiracular bristles, and spiracular valves may have evolved at least in part to prevent dust and/or parasites from entering the tracheal system. By contrast, Miller (1974) argued that the spiracles were not designed solely to keep out “small strolling arthropods,” as had previously been assumed, and the theme of parasite exclusion has largely been neglected in investigations of the reasons for spiracle closure. Nonetheless, this is an idea that merits consideration. For example, Harrison et al. (2001) demonstrated that tracheal mite infestations can substantially reduce the safety margin for oxygen delivery in flying honeybees, thus affecting their survival and ultimately colony fitness. Therefore, the role of the DGC in ensuring spiracular closure and parasite exclusion must be taken seriously and, as in recognition of Miller’s (1974) delightful phrase, we have called it the strolling arthropods hypothesis, defined as follows: DGCs are an adaptation to increase the frequency of spiracle closure to reduce the risk of parasitic infestation of the tracheae.

At this point, it is worth reiterating that one of the most significant aspects of the adaptive definitions is that they do not specify whether natural selection should have been re-

sponsible for both the origin and the maintenance of DGCs, just for their origin, or just for their maintenance. It would be difficult to argue in favor of the adaptive nature of DGCs in all species if these cycles are basal among the insects, even if natural selection was responsible for their original origin. The fact that DGCs are typically not found among the basal insect orders (e.g., Archaeognatha, Zygentoma, Odonata, Mantophasmatodea, Phasmatodea, Mantodea) and indeed seem to be polyphyletic in origin (Marais et al. 2005; see also Klok et al. 2002) partially resolves this problem. Maintaining the strict requirement of both origin and maintenance would certainly aid in distinguishing between the alternative hypotheses, but it is also restrictive in the sense that a single hypothesis, such as the hygric hypothesis, would be required to account for both the origin and the maintenance of DGCs. Because insects respond to a variety of conditions in their environments (Chown 2002), it seems much more plausible, at least from an adaptive perspective, that DGCs might arise for one reason and then be subsequently pressed into service for another. It seems equally likely that DGCs might have arisen many times for a variety of reasons (and differences between the mechanisms regulating DGCs in insects and pseudoscorpions suggests that this may be the case; see Lighton and Joos 2002). Therefore, the current utility perspective (Schluter 2000) seems a more plausible one to adopt, though acknowledging that understanding the ultimate origin of DGCs would also be highly informative. “Predictions and Tests,” which sets out the predictions of the hypotheses and the ways in which these might be tested, mostly adopts the current utility perspective but also provides a guide to the way in which questions about origins might be addressed. In this context, it is worth noting that the extent of phenotypic plasticity that is typical of many insects, especially when tests involve exposure to different environmental conditions for prolonged periods such as during development (see Loudon 1988; Frazier et al. 2001), might also considerably affect the conclusions based on the experimental approaches described below. Such phenotypic flexibility has not been sufficiently well explored during investigations of the hypotheses proposed to account for the evolution of DGCs despite early knowledge thereof (see Wigglesworth 1935), and it deserves further consideration.

Predictions and Tests

Most studies to date have been designed to address only one of the competing DGC hypotheses. Indeed, alternative hypotheses appeared only when a series of papers challenged the hygric model. It is important to be able to distinguish between competing models. This can be achieved only if two models make different predictions regarding outcomes (Huey and Berrigan 1996; Huey et al. 1999; Chown 2002; Chown and Davis 2003). The hygric, chthonic, and chthonic-hygric hypotheses are all based on adaptation to different environmental condi-

tions. Thus, properly designed comparative studies correlating DGC characteristics with habitat should allow these hypotheses to be tested using a strong inference approach. We emphasize that these studies need to account for phylogenetic relationships and that the microclimatic conditions actually faced by insects also need to be understood.

The chthonic and chthonic-hygic hypotheses propose that DGCs 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 DGCs will be favored in exposed environments. Thus, the oxidative damage and chthonic/hybrid genesis models yield different predictions about the prevalence of DGCs in enclosed versus open habitats. The available data are somewhat contradictory: the chthonic/hybrid genesis hypothesis was based primarily on the existence of DGCs in burrowing ants and beetles, but several grasshoppers also use DGCs (Hadley and Quinlan 1993; Quinlan and Hadley 1993; Rourke 2000). Clearly, more thorough phylogenetically based research is needed.

A corollary of the hygric hypothesis is that respiratory patterns should vary between environments differing in water availability in an adaptive fashion. In many areas of the world, high-altitude environments are drier than low-altitude ones. Thus, the hygric hypothesis predicts that DGCs 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 DGCs should be less prevalent at higher altitudes. Elevational patterns in DGCs may therefore allow us to distinguish between the oxidative damage and hygric hypotheses. However, the strong response of insect tracheal systems to alterations in oxygen availability (see Loudon 1988; Frazier et al. 2001) provides a cautionary note that the role of phenotypic flexibility needs to be taken into consideration in such investigations. In any event, methods of determining respiratory water loss rates in the presence and absence of DGCs are a primary requirement for experimental testing of the hygric genesis hypothesis.

Novel Methods to Assess the Importance of DGCs for Respiratory Water Loss

The hygric and hybrid exchange hypotheses both propose that the DGC is a mechanism to control water balance. A major experimental problem in testing these is quantifying respiratory water loss. This is relatively easy to do using flow-through respirometry, as long as the experimental animals are performing DGC. Cuticular water loss can be measured during the C phase and subtracted from total loss to obtain respiratory water loss. For insects breathing in other patterns, however, this approach does not work.

Two new methods have been developed to estimate respiratory water loss, regardless of respiratory mode. One is Light-

ton's hyperoxic switch method (Lighton et al. 2005), which was validated in three insects (*Drosophila melanogaster* and two species of ants), all of which exchanged respiratory gases continuously under the conditions of the experiment. When ambient air in a flow-through respirometry system is switched from 21% to 100% O₂, marked spiracular constriction took place, causing a transient but substantial—up to 90%—reduction in CO₂ output. A reduction in water loss rate occurred simultaneously. Their results—respiratory water loss estimates of 23% of total water loss rate in *Drosophila* and 7.6% and 5.6% of total water loss rates in the two ant species—are reasonable in light of literature estimates. The hyperoxic switch may therefore allow straightforward estimation of respiratory water loss rates in arthropods lacking discontinuous gas exchange. Significantly, though, especially in terms of the hygric hypothesis, in one ant species (*Pogonomyrmex californicus*) that the authors were able to measure with and without a DGC, presence or absence of a DGC did not affect respiratory versus total water loss rates. However, as the authors acknowledge, the presence/absence measurements did not take place under the same circumstances or with the same animal.

The other approach involves regressing water loss rate against CO₂ release measured at the same time (Gibbs and Johnson 2004). Extrapolation to the intercept provides an estimate of cuticular water loss. Respiratory water loss is calculated by subtracting this estimate from total water loss. In individuals that switch from one pattern to another, estimated rates of cuticular water loss during continuous breathing are usually very close to those measured more directly during the C phase of DGC (A. G. Gibbs, unpublished data). This is not always the case, however, particularly during active pumping of the tracheal system. A second limitation is that this method works best in CO₂-free air. There may also be differential kinetic issues to consider, in that the method assumes that H₂O and CO₂ have identical kinetics in the flow-through system. This is an assumption that requires testing and possible corrective measures.

Another new technique (J. R. B. Lighton and R. Turner, unpublished manuscript) makes use of the fact that the DGC can be gradually turned off by progressive hypoxia and represents a progression of an earlier technique (Lighton et al. 1993b) with the addition of high-resolution water vapor measurement. Using this technique with alates of the ant *Camponotus vicinus*, it can be demonstrated that total water loss (respiratory plus cuticular) does not increase significantly, while the DGC is gradually reduced in expression until it had ceased altogether. Finally, several kilopascals below the Po₂ at which the DGC is abolished, spiracular opening and overall H₂O loss rates increase dramatically. This constitutes a disproof of the hygric hypothesis, which predicts that abolition of the DGC should cause an increase in respiratory water loss.

Outstanding Questions

We pose here just a few of the many questions raised by recent work in insect gas exchange.

Respiratory Water Loss during the F Phase

J. R. B. Lighton and R. Turner (unpublished data) have described the gradual abolition of the DGC via progressive hypoxia in the ant *Camponotus vicinus*. Spiracular area increased to offset the reduced oxygen diffusion gradient, thus elevating CO₂ output during the F phase and delaying the hypercapnic trigger for initiation of the O phase, as previously described (Lighton et al. 1993b). Finally, the DGC failed altogether. Perhaps the most unexpected finding of this study is that the rate of water vapor loss during the F phase was not measurably affected. Because the water vapor detection technique used by Lighton and Turner was about an order of magnitude higher resolution than those used in previous studies, they were confident that the lack of an increase in water vapor output was not an experimental artifact. This leads to the question, What is responsible for the apparent decoupling of CO₂ and H₂O emission kinetics during the F phase?

Buffering of CO₂

The role of the hemolymph and the tissues as a buffer for CO₂ within the interburst period (i.e., combined C and F phases) needs to be addressed. The hygric, chthonic, and hybrid genesis hypotheses propose that rapid CO₂ release from the hemolymph and tissues during the O phase is crucial. The fast unloading of large amounts of CO₂ from the hemolymph and tissues may need enzymatic catalysis by carbonic anhydrase (CA). CA activity has been found in several insects (Edwards and Patton 1967). Recently, Coddington and Chamberlin (1999) found that CA may not be involved in pH regulation in the midgut of larval Lepidoptera. Although the hemolymph of moth pupae is considered to lack CA activity (Buck and Friedman 1958), the tissues showed CA activity. Buck and Friedman concluded that the CA in the tissues supports the rapid interchange into the tracheal system of CO₂ from the tissues where most of it is buffered.

The implications of different gas exchange patterns for acid-base balance have received remarkably little attention (but see, e.g., Harrison et al. 1995). Closure of the spiracles results in the accumulation of CO₂ and consequent acidification of the hemolymph and cells. Because of the high buffer values (e.g., Bridges and Scheid 1982), the pH changes during an O phase within the hemolymph of lepidopteran pupae are small (Hetz and Wasserthal 1993). Interestingly, Buck has stated that hemolymph buffering capacity in insects showing a DGC (Buck 1953; Buck and Friedman 1958; Buck and Keister 1958) is not significantly different from that of insects that do not exhibit

cyclic CO₂ release (Buck 1962). But what is the role of CA? The crucial experiment—blocking CA activity and measuring the resulting effect on the DGC—remains to be done.

Conclusions

A long phase of comparative neglect has ended, and the ways in which tracheate arthropods breathe is once again a thriving area of research. While the field does not propose to mitigate human well-being in the short term, it is a matter of more than idle curiosity to understand how the most abundant and speciose animals on our planet exchange respiratory gases without killing themselves via dehydration. The field offers outstanding opportunities to both theoretical modelers and experimentalists and remains one of biology's best frontiers for engaging in discovery science.

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