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Notes and Comments

Discontinuous Gas Exchange in Insects: Is It All in Their Heads?

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abstract: Some insects display an intermittent pattern of gas exchange while at rest, often going hours between breaths. These discontinuous gas exchange cycles (DGCs) are known to have evolved independently within five insect orders, but their possible adaptive benefit and evolutionary origin remain an enigma. Current research is primarily concerned with testing three adaptive hypotheses: that DGCs originally evolved or are currently maintained to (1) limit respiratory water loss, (2) enhance gas exchange in subterranean environments, or (3) limit oxidative damage. These adaptive explanations fail to unite a range of apparently contradictory observations regarding the insects that display DGCs and the conditions under which they occur. Here we argue that DGCs are explained by circadian, developmental, or artificially induced reductions in brain activity. We conclude that this pattern results from the thoracic and abdominal ganglia regulating ventilation in the absence of control from higher neural centers, and it is indicative of a sleeplike state.

Keywords: DGC (discontinuous gas exchange cycle), periodic ventilation, brain, evolution, sleep, insect respiration.

Introduction

Insects maintain the highest mass-specific rates of oxygen consumption found in the animal kingdom by eschewing the complex pulmonary and circulatory systems used by most other animals (Suarez 2000). Instead, they use an extensive network of air-filled tubes, called tracheae, which branch and ramify throughout all parts of their body, providing an air-filled pathway for the rapid movement of oxygen and carbon dioxide directly between the insect's tissues and the surrounding atmosphere. Since the insect's cuticle is largely impermeable to gas diffusion, this "tracheal system" opens to the environment through spiracles, small pores located along the lateral margins of the insect's body. Spiracles contain muscular valves that permit or restrict gas exchange by opening and closing, thus enabling insects to display a range of respiratory gas exchange patterns from continuous to periodic. The discontinuous gas exchange cycle (DGC) is perhaps the best known, most intensively researched, and most vigorously debated of these respiratory patterns (Chown et al. 2006). The typical DGC is characterized by a period of negligible gas exchange with the atmosphere when the spiracles are tightly shut (closed phase) followed by a period of intermittent carbon dioxide release and oxygen uptake as the insect's spiracles "flutter" open and shut (flutter phase), which is terminated when the spiracles open fully, releasing a burst of carbon dioxide while oxygen freely enters the tracheal system (open phase). While this respiratory pattern is described in most biology textbooks (e.g., Schmidt-Nielsen 1997), it is far from ubiquitous among insects. In fact, it is a pattern that is known to occur among only a few insect orders (ants, bees and wasps, butterflies and moths, beetles, grasshoppers, and cockroaches; Marais et al. 2005) and then only while at rest or during diapause as a pupa. Most current research on insect DGCs is driven by three adaptive hypotheses explaining the origin or maintenance of DGCs among insects. They propose that discontinuous gas exchange evolved or is currently maintained in order to (1) reduce respiratory water-loss (hygric hypothesis; Buck and Keister 1955), (2) enhance gas exchange in subterranean atmospheres (chthonic hypothesis; Lighton 1998), and (3) ameliorate the toxic effects of near-ambient intratracheal oxygen levels (oxidative damage hypothesis; Bradley 2000).

However, the wide range of environments occupied by insects that display DGCs (from mesic to hyperarid, subterranean to terrestrial), as well as their diverse life strategies (flying and flightless, adult and pupal), makes identifying a common adaptive benefit difficult. As each of these hypotheses predicts a different relationship between DGC duration and ambient temperature, humidity, oxygen partial pressure, or some combination of these variables, a recent study by White et al. (2007) used a strong inference approach to determine which of these hypotheses is best supported by the current data. While this study found support for the hygric hypothesis and some support for the oxidative damage hypothesis on the basis of longer DGC cycles among insects from higher temperature and lower rainfall environments, this conclusion assumes explicitly that of the three hypotheses tested, one must be correct. This is not

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necessarily the case (O'Donohue and Buchanan 2001). Given that DGCs appear to have evolved independently five times (Marais et al. 2005), there are potentially between one and five explanations for their origin and perhaps many more adaptive hypotheses explaining their current function or functions. This would certainly account for the equivocal support that these hypotheses receive. As has been pointed out, insect DGCs could result from multiple causal mechanisms among the various insect groups, making a universal adaptive explanation for their occurrence unrealistic (Chown 2002). Thus, before we continue to conceive and test adaptive hypotheses, there is a fundamental question that must first be addressed: What is the underlying mechanistic basis driving DGCs and is this mechanism the same among all insects?

More than 50 years of research into insect respiratory control provides a wealth of evidence that strongly suggests there is one thing that all insects displaying DGCs have in common: reduced or absent brain activity. Experiments have demonstrated that decapitated cockroaches spontaneously display DGCs (Edwards and Miller 1986), as do both decapitated and anesthetized ants (Lighton et al. 1993b; Lighton and Garrigan 1995; Duncan and Newton 2000) and diapausing pupa with or without their brains removed (Levy and Schneiderman 1966). Evidently, incapacitating or removing the brain reveals a periodic pattern of ventilation. That headless insects can continue to ventilate at all is due to the decentralized nature of their central nervous system, which consists of a chain of nerve cell clusters (ganglia) in the head, thorax, and abdomen connected by a paired ventral nerve chord. Three fused ganglia in the head comprise the cephalic ganglion or "brain," while the abdominal and thoracic ganglia either occur individually or are fused into various larger groups, depending on the species (Niven et al. 2008). The brain is responsible for integrating and controlling higher functions (e.g., memory, learning, vision, and olfaction; Howse 1975), but many critical functions are controlled by the thoracic and abdominal ganglia and occur with minimal or no input from the brain (e.g., locomotion, calling, mating; Howse 1975). As a result, insects can continue to perform many activities even when the brain is incapacitated. The prominent DGCs displayed by diapausing moth pupae are a case in point. The electrical activity of the moth larva's brain ceases completely a day before the pupal molt, and from then on it neither displays nor responds to electrical stimulation (Van Der Kloot 1955). For the duration of diapause moth pupae exhibit a pronounced DGC. It is relevant to note that this electrical inactivity is confined solely to the pupa's brain—all other ganglia of the ventral nerve cord maintain spontaneous activity during diapause (Van Der Kloot 1955). Consequently, control of the insects' gas exchange pattern reverts to their thoracic and abdominal ganglia in the absence of active regulation

from the brain (Miller 1960; Myers and Retzlaff 1963). In various grasshoppers and cockroaches the thoracic and abdominal ganglia have been shown to possess both spontaneous rhythm generators (Bustami and Hustert 2000) and chemosensitivity to O_2 and CO_2 (Case 1957; Miller 1960; Edwards and Miller 1986; Bustami et al. 2002). It is then the interplay between their CO_2 and O_2 "set points" and how they each stimulate spiracular opening, that results in the emergence of DGCs (Förster and Hetz 2009).

The importance of brain activity in controlling the emergence of DGCs can also be seen among the different castes in ant colonies. All queen ants investigated so far have been shown to display pronounced DGCs, while members of their worker caste generally do not (Lighton et al. 1993a; Lighton and Berrigan 1995). This difference was considered to be evidence of adaptation by the queens to the potential respiratory challenges of their subterranean environment (Lighton 1998). However, morphological studies have found that while virgin queen ants have larger brains than the worker caste (Ehmer and Gronenberg 2004), their brains undergo a significant reduction in size immediately following mating and the onset of the underground, egg-laying phase of their life cycle (Julian and Gronenberg 2002). Thus, queen ants may display DGCs not because of any subterranean respiratory stress but because of a reduction in brain size and activity associated with the reduced behavioral repertoires and a lack of visual stimulation typical of their claustral condition (Julian and Gronenberg 2002).

The emergence of DGCs in other adult insects may also be attributed to reduced brain activity. It has often been noted that insects display DGCs only while quiescent (Kestler 1985; Lighton 1998). Quiescence in some insects is associated with reduced optical sensitivity, heightened arousal thresholds, and postural changes suggestive of a sleeplike state (Kaiser and Steiner-Kaiser 1983; Tobler and Neuner-Jehle 1992). Thus, the nocturnal cockroach *Blaberus giganteus* enters into a distinct resting state during the light period of its circadian cycle, characterized by a marked increase in its disturbance threshold and a prostrate body posture, and displays DGCs only while resting (Miller 1973; Tobler and Neuner-Jehle 1992). Similarly, a study on the diurnal grasshopper *Romalea guttata* found that it tended to display DGCs during nighttime hours and not during the day, even if the animal was kept in a darkened condition (Hadley and Quinlan 1993). These examples suggest that DGCs emerge as a result of a circadian reduction in the excitability of the insects' brains.

Given that DGCs have a neurological basis, it might be expected that the disparate insect orders that display them possess similar nervous systems. In fact, those insects that display DGCs as adults all possess enlarged mushroom bodies, outgrowths of the insect brain generally associated with more complex behaviors (Howse 1975). While all

insects possess these structures (Farris 2005), large mushroom bodies characterized by doubled, convoluted calyces have evolved only among three distantly related insect orders, Dictyoptera (Blattodea), Hymenoptera, and Coleoptera (Farris 2008), all of which contain species known to display DGCs. In contrast, those groups with reduced mushroom bodies, for example, the Hemiptera (Farris 2005), do not display DGCs (Marais et al. 2005). Orthoptera is the only order in which adult insects known to display DGCs do not possess the doubled calyces described above. However, they do share a similarity with the other insect groups that display DGCs, relating to brain size. Those orthopterans so far identified as displaying DGCs are gregarious acridids, a group commonly known as locusts (Marais et al. 2005). Interestingly, it has been demonstrated that when desert locusts begin to swarm and change from solitary into gregarious morphs, they show a dramatic 49% increase in the volume of their mushroom body calyces associated with a 30% increase in their overall brain size (Ott and Rogers 2010).

A plausible explanation for the link between those insects that display DGCs and those that possess large, complex brains can be made by considering the cost of maintaining such energetically expensive neuronal circuitry. Brains are metabolically costly to run, a fact that is particularly relevant to humans. Our brains occupy 2% of our body mass but account for an estimated 20% of our resting metabolic rate (Rolfe and Brown 1997). This substantial cost is reduced by light sleep, which decreases the brain's metabolic rate by 10%–30%, while cessation of almost all electrical activity reduces it by up to 60% (Erecinska and Silver 1989). From this it can be hypothesized that insects, particularly those with larger brains or long-term energetic constraints, would substantially reduce their metabolic costs simply by downregulating brain function during periods of quiescence. In the absence of sensory feedback from an active brain, control of ventilation then reverts to the central pattern generators in the thoracic and abdominal ganglia: the result is a DGC.

An Alternative Neural Hypothesis for the Evolution of DGCs

While the hygric, chthonic, and oxidative damage hypotheses are based on the assumption that DGCs evolved as a result of natural selection acting on the gas exchange pattern itself, we suggest an alternative neural hypothesis, namely, that DGCs are a nonadaptive consequence of the downregulation or complete cessation of brain activity. From this perspective, it is the reduction in brain activity associated with a quiescent, sleeplike state or the absence of brain activity during diapause that is adaptive (Siegel 2009). Thus, DGCs are predicted to occur where energetic constraints favor the downregulation of neural activity, especially but not exclusively in those insects possessing larger, more complex brains. DGCs occur only in this state of cerebral inactivity, arising spontaneously as a consequence of the thoracic and abdominal ganglia regulating gas exchange using separate hypoxic and hypercapnic thresholds to trigger spiracular opening (Förster and Hetz 2009).

There are several possible ways to test whether a reduction in brain activity initiates DGCs. First, anesthesia, surgery, or temperature could be used to directly manipulate brain activity and its effect on gas exchange patterns. Determining whether DGCs can be artificially induced in those insects known to display them by reducing brain activity would indicate whether the discontinuous gas exchange pattern was indeed hardwired into the thoracic and abdominal ganglia and whether the absence of higher neural control is necessary for their manifestation. Simultaneous manipulation of the insect's metabolic rate would enable the effects of low metabolism (considered to be essential for the production of the DGC; Bradley 2007) to be disentangled from an absence of brain activity, since these two conditions co-occur in nature. Second, using a phylogenetically informed comparative approach to examine the distribution of DGCs and brain size among the Insecta would allow a robust test of this hypothesis. Since insects with relatively large brains are more likely to be compelled by energetic considerations to periodically downregulate the activity of their cephalic ganglia, then the presence of DGCs should correlate with an increase in relative brain size. This prediction can be tested by comparing brain sizes and respiratory patterns both within and between insect species, with the examination of polymorphic social insects with variable brain sizes likely to prove informative (Jaffe and Perez 1989; Ott and Rogers 2010). Interspecifically, the comparative approach could be used within the superorder Dictyoptera, where DGCs are displayed by gregarious cockroaches that engage in complex interspecific interactions (e.g., *Nauphoeta cinerea*, *Periplaneta americana*, *Blattella germanica*; Dingha et al. 2005; Woodman et al. 2008; Schimpf et al. 2009) but not by the solitary giant burrowing cockroach *Macropanesthia rhinoceros* (Woodman et al. 2007) or the cockroaches' close relatives, the highly eusocial termites (Lighton and Ottesen 2005). This distribution of DGCs among gregarious and social insects but not the highly eusocial caste insects is particularly relevant to the current hypothesis, since brain size appears to correlate with increasing social complexity until increased behavioral specialization within castes begins to reduce individual neural capacity (Jaffe and Perez 1989; Mares et al. 2005).

In addition to the proposed tests of the neural hypothesis, the hygric, chthonic, and oxidative damage hypotheses should also be further scrutinized. Crucially for these hypotheses, no study has yet demonstrated that DGCs are heritable in insects or that gas exchange patterns respond

to natural or artificial selection. DGCs displayed by individual cockroaches are consistent through time (Marais and Chown 2003) and so may respond to natural selection. However, repeatability is not necessarily equivalent to heritability, since a measurable trait is the result of both genetic factors, which are heritable, and environmental factors, which are not. In order to be heritable, the time-consistency of differences between individuals must be attributable to the genetic rather than the environmental components of phenotypic variance (Falconer and Mackay 1997; Dohm 2002). In the absence of empirical measures of heritability and fitness, the evidence in support of the adaptive hypotheses is largely correlational. In the case of the hygric hypothesis, for example, it has been shown that DGCs are more common in arid than in mesic environments (Marais et al. 2005) and that DGCs are longer in environments where water loss is likely to be most severe (White et al. 2007). However, association with aridity is confounded by association with low primary productivity and energetic constraints beyond simple water availability. Stronger support for the putative adaptive benefits of DGCs would be gleaned from adoption of an experimental evolution approach (e.g., Garland and Rose 2009). If DGCs vary within a population, are heritable, and provide a fitness benefit according to the predictions of either the hygric or oxidative damage hypotheses, then long-term exposure to desiccating or hyperoxic conditions should favor those individuals that display DGCs over those that do not. Therefore, DGCs should become more common and pronounced in populations maintained under such conditions for multiple generations. Only through experiments such as these can an adaptive function be conclusively attributed to the DGC.

Conclusion

From the multiple lines of evidence presented here, it may be argued that the one common factor shared by all insects displaying DGCs is a capacity for the downregulation, or complete cessation, of brain function. If this is the case, then the DGC is merely an emergent property of the insect's nervous system (Chown and Holter 2000; Förster and Hetz 2009) that occurs when ventilatory control is assumed by the thoracic and abdominal ganglia in the absence of control from the insect's brain. While the emergent hypothesis of Chown and Holter (2000) proposed a plausible mechanism for the spontaneous emergence of DGCs due to interactions between CO_2 and O_2 set points that govern spiracular opening, the neural hypothesis explains why only particular insects display DGCs where and when they do, while previous hypotheses could not. Furthermore, the correlation between adult insects with larger, more complex brains displaying DGCs while quiescent presents the intriguing possibility that DGCs are, in fact,

a pattern of ventilation symptomatic of insects in a sleep-like state. Continued research into this area is likely to reveal much about the hierarchy of neural control in insects, as well as the evolution and importance of sleep in animals with complex brains.

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