

Insect gas exchange patterns: a phylogenetic perspective

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Accepted 12 October 2005

Summary

Most investigations of insect gas exchange patterns and the hypotheses proposed to account for their evolution have been based either on small-scale, manipulative experiments, or comparisons of a few closely related species. Despite their potential utility, no explicit, phylogeny-based, broad-scale comparative studies of the evolution of gas exchange in insects have been undertaken. This may be due partly to the preponderance of information for the endopterygotes, and its scarcity for the apterygotes and exopterygotes. Here we undertake such a broad-scale study. Information on gas exchange patterns for the large majority of insects examined to date (eight orders, 99 species) is compiled, and new information on 19 exemplar species from a further ten orders, not previously represented in the literature (Archaeognatha, Zygentoma, Ephemeroptera, Odonata, Mantodea, Mantophasmatodea,

Phasmatodea, Dermaptera, Neuroptera, Trichoptera), is provided. These data are then used in a formal, phylogeny-based parsimony analysis of the evolution of gas exchange patterns at the order level. Cyclic gas exchange is likely to be the ancestral gas exchange pattern at rest (recognizing that active individuals typically show continuous gas exchange), and discontinuous gas exchange probably originated independently a minimum of five times in the Insecta.

Supplementary material available online at
<http://jeb.biologists.org/cgi/content/full/208/23/4495/DC1>

Key words: adaptation, discontinuous gas exchange, periodic breathing, phylogeny.

Introduction

Discontinuous gas exchange cycles are one of the most striking gas exchange patterns shown by resting or quiescent insects. They were originally described in lepidopteran pupae (by Heller, 1930; cf. Buck, 1962) and the adults of several species (Punt et al., 1957; Wilkins, 1960). However, it was the extensive investigation of discontinuous gas exchange cycles in diapausing saturniid pupae by Schneiderman and his colleagues that resulted in the first comprehensive description of this pattern of gas exchange, and elucidation of the mechanisms underlying it (e.g. Schneiderman, 1960; Levy and Schneiderman, 1966a,b; Schneiderman and Schechter, 1966). This work, as well as later studies on adult cockroaches, ants and beetles, provided strong support for the idea that discontinuous gas exchange cycles (DGCs) evolved to reduce respiratory water loss (Kestler, 1985; Lighton, 1994; Chown, 2002).

Subsequent investigations cast doubt on this hypothesis, and six competing explanations have now been formulated to account for the evolution of DGCs (reviewed in Chown et al., 2005). A prominent feature of virtually all tests of the competing hypotheses is that they have been based either on small-scale, manipulative experiments (Lighton and Berrigan, 1995; Chown and Holter, 2000), or comparative investigations

of a few closely related species (e.g. Lighton, 1991a; Duncan et al., 2002; Chown and Davis, 2003). These approaches have provided many valuable insights into the evolution of insect gas exchange patterns, and especially the mechanisms underlying them. However, broader comparative analyses can also be informative. Indeed, Huey and Kingsolver (1993) have cogently argued that a combined approach involving mechanistic investigations, laboratory selection and comparative methods is essential if an integrated understanding of the evolution of physiological traits, and their broader ecological implications, is to be achieved (see also Kingsolver and Huey, 1998; Feder and Mitchell-Olds, 2003). Moreover, comparative analyses undertaken in a phylogenetic context can provide useful information on the history of a given trait, including its origin, whether or not it should be considered adaptive (in the strict sense, such that natural selection is responsible for its origin and maintenance; Coddington, 1988; Baum and Larson, 1991), and the likelihood of repeated and/or convergent evolution (Brooks and McLennan, 1991). In the context of gas exchange patterns, the value of such a phylogeny-based comparative approach has already been established by Klok et al. (2002), who demonstrated that discontinuous gas exchange probably arose

independently at least four times in the Arthropoda. Nonetheless, no phylogeny-based comparative analysis of the occurrence of gas exchange patterns, and particularly discontinuous gas exchange, in insects (which form a monophyletic unit; Giribet et al., 2001) has been undertaken.

This situation at first appears remarkable, given that published investigations of gas exchange patterns are available for 99 insect species, and it is known that these patterns vary considerably among, and sometimes within, species at rest: from continuous, to cyclic, to discontinuous (Lighton, 1998; Marais and Chown, 2003; Gibbs and Johnson, 2004). However, on closer inspection it is clear that there are probably several reasons why no phylogeny-based analysis has been undertaken, amongst which two are perhaps most significant. First, there is probably a file-drawer problem (Csada et al., 1996), such that in instances where species do not show discontinuous gas exchange the data are not published, thus biasing the literature in favour of reports of discontinuous gas exchange cycles (Lighton, 1998; Chown, 2001). Second, and possibly as a consequence of the file-drawer problem, the taxa for which gas exchange patterns are available is highly skewed towards the holometabolous insects. Thus, of the approx. 100 insect species for which information on gas exchange patterns is presently available, 83 are holometabolous, and of these, 44 are Coleoptera. By contrast, the Exopterygota is comparatively under-represented in the literature, with published information available for six cockroach species, six termite species, and five species of Orthoptera. No Apterygotes (*Zygentoma* and *Archaeognatha*) have been investigated.

In this paper, we address some of the above problems and provide the first, phylogeny-based comparative analysis of the distribution of insect gas exchange patterns at the order level. Whilst we cannot resolve the file-drawer issue, we comprehensively review patterns documented by the existing literature, provide information on several exemplar taxa representing orders of insects that have not previously been investigated (*Archaeognatha*, *Zygentoma*, *Ephemeroptera*, *Odonata*, *Mantodea*, *Mantophasmatodea*, *Phasmatodea*, *Dermaptera*, *Neuroptera* and *Trichoptera*), and add to the data on little studied groups (*Blattodea*, *Orthoptera*, *Hemiptera* and *Diptera*). In undertaking this work we realise that a comprehensive comparative analysis of insect gas exchange patterns is still some way off. The 118 species included here represent less than 0.003% of the estimated global insect fauna (May, 2000). However, because much of the variation in insect physiological traits is partitioned at higher taxonomic levels (Chown et al., 2002), we begin by using exemplar taxa at the Order level. Thus, our aim is to provide a working phylogenetic framework within which the evolution of gas exchange patterns in insects can be discussed. To date, such a framework has been missing.

Materials and methods

Data from the literature

The literature concerning insect gas exchange patterns was

reviewed as far back as 1950, and all studies in the Anglophone literature reporting gas exchange patterns were included. Where authors provided figures of the gas exchange patterns of the species they studied, these were used for assessments of the type of gas exchange pattern (either DGC, cyclic or continuous). DGCs were identified on the basis of the presence of C and F periods (Lighton, 1996; Chown et al., in press) in the figures presented by the authors. For the other patterns our assessments were based on the protocol described in the next section. In those instances where figures were not available, the authors' view on the type of pattern was accepted as correct, although confidence in the pattern assessment was marked lower (confidence in the data was ranked either as high, medium or low, which reflects our access to original data, rather than the abilities of the original authors). These assessments were made independently by E.M. and C.J.K., and in instances of disagreement a consensus was reached following discussion or analysis. The data were then tabulated. In two instances original data from published (Shelton and Appel, 2000) and unpublished works (B. A. Klein, K. M. F. Larsen and A. G. Gibbs) were obtained to verify these assessments. Each species was also scored for whether it is winged or wingless, from a mesic or xeric habitat, or expected to have a subterranean lifestyle, based on comments provided by the authors in the original works, and/or information on the species or higher taxon available elsewhere in the literature.

Experimental investigations

The additional species collected for investigation were chosen based on Order-level deficiencies in the literature on gas exchange patterns. Adult individuals of 19 species representing the *Archaeognatha* (1 sp.), *Zygentoma* (3 spp.), *Ephemeroptera* (1 sp.), *Odonata* (2 spp.), *Blattodea* (1 sp.), *Mantodea* (1 sp.), *Mantophasmatodea* (1 sp.), *Phasmatodea* (1 sp.), *Orthoptera* (1 sp.), *Dermaptera* (1 sp.), *Hemiptera* (2 spp.), *Neuroptera* (1 sp.), *Diptera* (1 sp.), *Trichoptera* (1 sp.) and *Lepidoptera* (1 sp.) were collected from several localities in South Africa (Table 1) and returned to the laboratory within 1 week of collection. Most experiments started within 12 h of the arrival of the insects at the laboratory because little is known about how long they survive in captivity. Insects were held in an incubator at $22\pm 1^\circ\text{C}$ (12 h:12 h L:D photoperiod), with access to water but not to food (with the exception of the hemipterans, mantophasmatodeans, cockroaches and the stick insects, where food was provided, but where a period of starvation preceded respirometry), before their gas exchange patterns were examined. Assessments were made in dry air for technical reasons and because under these conditions discontinuous gas exchange would seem most likely as a means to conserve water (Duncan et al., 2002). Each individual was weighed using an analytical balance (0.1 mg resolution; Mettler Toledo AX504, Columbus, OH, USA), and placed into a cuvette kept at $20\pm 0.2^\circ\text{C}$, using either a water bath (Grant LTD20, Cambridge, UK) or a temperature-controlled cabinet (Labcon, Johannesburg, South Africa). This slightly lower temperature was selected because it improved quiescence and

Table 1. *Species examined for gas exchange patterns in this study*

	Locality	Response time, lag time (s)
Archaeognatha		
Meinertellidae, sp.	Helderberg Nature Reserve, Somerset West, South Africa (34°02.579'S, 18°52.472'E)	6, 120
Zygentoma		
Lepismatidae		
Lepismatidae sp. 1	Sutherland, South Africa (32°34.105'S, 20°57.747'E)	6, 120
Lepismatidae sp. 2	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	6, 120
<i>Ctenolepisma longicaudata</i> (Echerich, 1905)	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	6, 120
Ephemeroptera		
Heptageniidae sp.	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	9, 210
Odonata		
Coenagrionidae		
<i>Ischnura senegalensis</i> (Rambur, 1842)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	7, 130
Libellulidae		
<i>Trithemis arteriosa</i> (Burmeister, 1839)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	18, 270
Blattodea		
Blaberidae, sp.	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	4, 90
Mantodea		
Mantidae		
<i>Sphodromantis gastrica</i> Stål	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	7, 130
Mantophasmatodea		
Austrophasmatidae		
<i>Karoophasma biedouwensis</i> (Klass et al., 2003)	Cederberg, South Africa (32°05'S, 19°15'E)	9, 210
Phasmatodea		
Phasmatidae		
<i>Extatosoma tiaratum</i> (Macleay, 1826)	Butterfly World, Klapmuts, South Africa, but originally from Australia	7, 130
Orthoptera		
Pneumoridae		
<i>Bullacris intermedia</i> (Péringuey, 1916)	Zuurberg, South Africa (33°48'S, 25°14'E)	7, 130
Dermaptera		
Labiduridae		
<i>Euborellia annulipes</i> (Lucas, 1847)	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	6, 120
Hemiptera		
Coreidae sp.	Nigel, South Africa (26°25.422'S, 28°28.349'E)	6, 120
Lygaeidae sp.	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	6, 120
Neuroptera		
Chrysopidae		
<i>Chrysoperla</i> sp.	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	9, 210
Diptera		
Glossinidae		
<i>Glossina morsitans</i> Westwood	FAO/IAEA, Vienna, Austria (Laboratory colony)	6, 120
Trichoptera		
Leptoceridae		
<i>Leptocerina</i> sp.	Olifants River, Citrusdal (32°35'S, 18°40'E)	9, 210
Lepidoptera		
Plutellidae		
<i>Plutella xylostella</i> (Linnaeus, 1758)	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	9, 210

Localities are provided, and species names where these are available. However, the taxonomic impediment in South Africa means that the latter has not always been possible. Response and lag times refer to delay in first detection of CO₂, and time to zero baseline, respectively, of each of the designs used to examine gas exchange patterns. With one exception these times are well within those calculated from designs typically described in the literature for gas exchange analyses in insects.

might have also induced discontinuous gas exchange. Previous work (Chown, 2001; Marais and Chown, 2003) indicated that gas exchange patterns, whilst repeatable, can be variable within individuals and species. In consequence, conditions favourable to the induction of discontinuous gas exchange were used, and particularly temperatures that are typically lower than mean summer microclimate temperatures in the region (which range from 24°C at sea level, to 22°C at the highest inland site of collection, with absolute maxima ranging from 50°C at the sea level site to 53°C at the high altitude site; see also Botes et al., in press).

Air, scrubbed of CO₂ (using soda lime) and water (using silica gel and then Drierite®, Xenia, OH, USA) was passed through the cuvette (see Table 1 for response times, regulated using a Sidetrak Mass Flow Controller, Monterey, USA) and into a calibrated infrared gas analyzer (Li-Cor Li7000 or Li-Cor Li6262; Lincoln, NE, USA) to measure CO₂ production. Flow rates and cuvette sizes varied according to the species and in a manner such that washout was unlikely to be significant (see Results, and Lighton, 1991b). A Sable Systems (Las Vegas, NV, USA) AD-1 activity detector was used to detect any movement of the individual in the cuvette during the experiment, and the output of the detector was fed into the auxiliary channel of the Li7000 or Li6262. The AD-1 registers activity as a value between -5 and +5 V, where little deviation from the mean indicates that the animal is inactive, and a large deviation indicates high levels of activity (for detail see www.sablesys.com/ad1.html). Each experimental assessment lasted for approximately 2 h, which is typically sufficient to detect variation in gas exchange traces (Chown, 2001) without dehydrating animals to such an extent that the gas exchange pattern might switch to continuous, owing to dehydration, as has been found in some species (Quinlan and Hadley, 1993; Chappell and Rogowitz, 2000). The data file generated by the Li7000 software was exported, *via* Microsoft Excel, to DATACAN V (Sable Systems,), whilst the data stream from the Li6262 was captured directly using Sable Systems hardware and software. DATACAN V was used for initial analysis of the respirometry data (corrected to standard temperature and pressure) for periods of inactivity only.

Traces of rates of CO₂ production (\dot{V}_{CO_2}) were categorized as continuous, cyclic or discontinuous gas exchange by inspection. The DGCs were readily identified based on the presence of C-periods and F-periods. However, identification of gas exchange patterns in the absence of the C- and F-periods is less straightforward. Several statistical approaches were explored for distinguishing continuous from cycling patterns objectively. These included spectral analysis and the modification thereof that has been used to identify population cycles (Cohen et al., 1998). Unfortunately, these methods typically did not allow continuous and cyclic gas exchange to be distinguished, most notably because even continuous gas exchange has some periodicity. The variance approach adopted by Williams et al. (1997) is also unsuitable because it does not take temporal autocorrelation into account. In consequence, any comparison of variances between species would be

confounded. Nonetheless, it is essential that some objective criterion has to be developed to allow traces to be classified or distinguished in a repeatable manner.

Therefore, we developed a simple, alternative convention based on the principle of the presence of regular bursts. We assumed that when a line is drawn through the centre of the \dot{V}_{CO_2} trace a cyclic trace should have fewer data points above this line than below it. By contrast, more continuous traces should show the converse. We applied this convention to the traces we recorded by adhering to the following steps. First, subtract the minimum point of the time series that has to be analysed from all the data points. Second, shift the data series down by 50% to ensure that the zero line passes through the centre of the trace. Third, calculate the percentage of data points that lie above the zero line. We adopted a conservative approach here and assumed that if the percentage of data points above the zero line was <30% the trace was cyclic, whilst if the percentage was >30% the trace was more likely to be continuous (Fig. 1). This technique is sensitive to traces that show drift and/or outliers (e.g. electronic glitches, baseline measurements) in the time series that result in artificial minima or maxima. However, such errors can routinely be corrected using modern analytical software. Although it might be argued

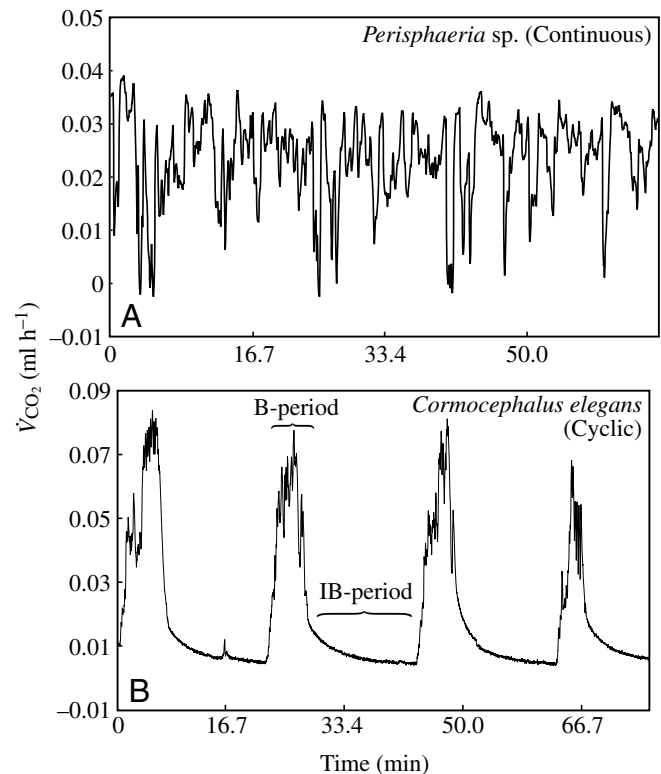


Fig. 1. Continuous gas exchange patterns (A; from a cockroach) are defined here as those in which the fluctuations between high and low \dot{V}_{CO_2} release are not regular, while cyclic patterns (B; from a centipede) are defined as those which show bursts at more regular intervals. The cyclic patterns are characterised by interburst periods (IB) and burst periods (B).

that an alternative set of criteria should be used, the approach we adopted makes any decision on cyclic *vs* continuous traces explicit, repeatable, and more objective than simple inspection. Here, once this method had been applied to the traces, summary statistics for the data were calculated, based on the approach to cyclic and discontinuous gas exchange patterns adopted by Marais and Chown (2003).

Analyses

Based on the data from the literature and the data generated in this study we assigned gas exchange patterns (continuous, cyclic and discontinuous) to all of the insect orders that have been investigated to date, and these were plotted onto the phylogeny of the orders provided by Gullan and Cranston (2005). In those orders where species showed different gas exchange patterns, or where a single species showed more than one pattern, all gas exchange patterns were listed. A formal parsimony analysis (see Brooks and McLennan, 1991) was undertaken and used to assess the likely evolution of gas exchange patterns [see Scholtz and Chown (1995) for use of this approach to investigate the evolution of scarabaeoid diets]. In cases where both unknown patterns (orders not yet investigated) and known patterns were present on shallower nodes, preference was given to the known patterns at the deeper nodes. It should be noted that although the tree provided by Gullan and Cranston (2005) indicates some controversy in interpretation of the branching patterns, it was not presented with likelihood values for these alternatives. Therefore, a single parsimony analysis for one character (gas exchange pattern, with three states) based on the given tree was undertaken. Adopting the same approach with the consensus phylogeny presented by Grimaldi and Engel (2005) did not change our conclusions.

Results

Reliable assessments of gas exchange patterns could be made for 99 species for which published data are available (and in one case we used unpublished information; see supplementary material, Appendix 1). Of these species, 59 typically showed discontinuous gas exchange cycles, 35 showed cyclic gas exchange, and 31 continuous gas exchange. Four species showed all three patterns, 19 species showed two of them, and in 76 species the work in which the outcome was reported suggested that the focal species showed only a single pattern. Indeed, in many published investigations the authors discussed the most common gas exchange pattern without providing explicit information on whether other gas exchange patterns were also evident in the species being examined. Thus, our characterization reflects the reporting convention of the previous literature, and probably under-represents multiple gas exchange patterns (especially the frequency of continuous gas exchange). It is now more common to find reporting of all gas exchange patterns (e.g. Gibbs and Johnson, 2004).

Our own investigations added 19 species to the list of those that have been investigated, and importantly most of these

were Exopterygotes or Apterygotes: groups that have enjoyed little attention to date (Fig. 2). In a few instances, sample sizes were low, but sufficient to indicate which kinds of gas exchange patterns were present. In these instances repeated measures of the individuals at hand were also undertaken (for rationale, see McNab, 2003). Likewise, although the response-time of one of our designs was slow (280 ml cuvette with 150 ml flow rate, to accommodate dragonflies), in none of the cases did Z-transformations (Bartholomew et al., 1981), using empirically derived response-time information from the experimental set-ups (Table 1), suggest that evidence for a lack of spiracle closure was a consequence of the experimental design. Moreover, the empirically derived times were well within those that would be typical of the majority of published studies, based on the cuvette sizes and flow rates reported in those studies (e.g. Lighton, 1990; Harrison et al., 1991; Duncan and Lighton, 1997).

In virtually all of the species, substantial among-individual variation in gas exchange pattern at rest was common, such that several individuals showed continuous gas exchange while a few showed cyclic or discontinuous patterns, or *vice versa* (Table 2). In most cases, cyclic gas exchange patterns were clearly identifiable as such, as were discontinuous gas exchange cycles (Fig. 2A–T). Thus, on the basis of these data, and on that available in the literature, gas exchange patterns were assigned to 18 of the 30 orders of insects (Fig. 3). Within all of the orders examined some species show continuous gas exchange, many show cyclic gas exchange, and only five orders contain species that show clear evidence of discontinuous gas exchange cycles. Moreover, within the latter orders where more than one or two species have been investigated substantial variation between species is typical (supplementary material, Appendix 1; Table 2). Nonetheless, the parsimony analysis demonstrated that, at the order level, continuous and cyclic gas exchange are basal, and that discontinuous gas exchange has evolved independently at least five times: in the Blattodea, Orthoptera, Coleoptera, Lepidoptera, and Hymenoptera.

Discussion

Gas exchange patterns have now been reported for 118 species from 18 insect orders. Although this represents an increase of nearly 125% in the number of orders for which data are available, much remains to be done, and in this context data on exemplars from the 12 outstanding orders (see Fig. 3) would be a useful step forward. The question nonetheless remains of what can be learned from the comparative data that are at hand, especially in the context of the adaptive hypotheses that have been proposed to account for the evolution of discontinuous gas exchange cycles (Chown et al., in press). Because only a few species have been examined in most orders, little certainty can be attached to the absence of a particular pattern in a given order. Thus, although it now appears that DGCs have evolved independently five times in the insects at the order level, this situation may change as additional species are investigated in

Table 2. CO₂ emission volumes, period durations, emission rates, total CO₂ emission rates and masses for each of the species investigated in this study

	Mean	Standard deviation	N		Mean	Standard deviation	N
Archaeognatha				<i>Interburst</i>			
Meinertellidae sp. (Fig. 2A)				Emission volume (μl)	3.309	2.881	5
Mass (mg)	12.75	5.18	4	Duration (s)	1160.84	908.49	5
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	4.036×10^{-3}	0.713×10^{-3}	4	Emission rate (μl min ⁻¹)	0.302	0.422	5
Zygentoma				<i>Burst</i>			
Lepismatidae sp. 1 (Fig. 2B)				Emission volume (μl)	1.215	1.106	5
Mass (mg)	23.04	1.99	5	Duration (s)	218.83	104.455	5
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	3.89×10^{-3}	0.884×10^{-3}	5	Emission rate (μl min ⁻¹)	0.291	0.157	5
Frequency (mHz)	3.701×10^3	0.521×10^3	5	Libellulidae			
<i>Interburst</i>				Trithemis arteriosa (Fig. 2G)			
Emission volume (μl)	0.179	0.078	5	Mass (mg)	103.06	37.88	8
Duration (s)	218.43	45.87	5	\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	3.400×10^{-2}	0.828×10^{-2}	8
Emission rate (μl min ⁻¹)	0.046	0.012	5	Frequency (mHz)	4.969		2
<i>Burst</i>				<i>Interburst</i>			
Emission volume (μl)	0.222	0.073	5	Emission volume (μl)	0.702		2
Duration (s)	106.60	9.050	5	Duration (s)	80.0		2
Emission rate (μl min ⁻¹)	0.120	0.032	5	Emission rate (μl min ⁻¹)	0.495		2
Lepismatidae sp. 2 (Fig. 2C)				<i>Burst</i>			
Mass (mg)	17.18	0.72	6	Emission volume (μl)	2.344		2
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	4.450×10^{-3}	1.130×10^{-3}	6	Duration (s)	205.0		2
Frequency (Hz)	5.140	1.240	6	Emission rate (μl min ⁻¹)	0.690		2
<i>Interburst</i>				Blattodea			
Emission volume (μl)	0.143	0.070	6	Blaberidae sp. (Fig. 2H)			
Duration (s)	172.0	35.84	6	Mass (mg)	2.54×10^3	0.94×10^3	6
Emission rate (μl min ⁻¹)	0.052	0.017	6	\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	9.100×10^{-2}	0.004×10^{-2}	6
<i>Burst</i>				<i>DGC</i>			
Emission volume (μl)	0.166	0.041	6	Frequency (mHz)	1.579		2
Duration (s)	108.74	13.34	6	<i>C-period</i>			
Emission rate (μl min ⁻¹)	0.102	0.026	6	Emission volume (μl)	0.306		2
Lepismatidae				Emission rate (μl min ⁻¹)	0.130		2
<i>Ctenolepisma longicaudata</i> (Fig. 2D)				<i>F-period</i>			
Mass (mg)	26.64	5.46	9	Emission volume (μl)	0.800		2
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	2.430×10^{-3}	0.492×10^{-3}	9	Duration (s)	152.40		2
Frequency (mHz)	5.627	3.501	7	Emission rate (μl min ⁻¹)	0.336		2
<i>Interburst</i>				<i>O-period</i>			
Emission volume (μl)	0.046	0.032	7	Emission volume (μl)	11.71		2
Duration (s)	90.86	63.73	7	Duration (s)	261.5		2
Emission rate (μl min ⁻¹)	0.034	0.040	7	Emission rate (μl min ⁻¹)	2.686		2
<i>Burst</i>				<i>Interburst-burst pattern</i>			
Emission volume (μl)	0.137	0.068	7	Frequency (mHz)	5.319	6.081	3
Duration (s)	129.71	30.30	7	<i>Interburst</i>			
Emission rate (μl min ⁻¹)	0.063	0.021	7	Emission volume (μl)	1.237	1.579	3
Ephemeroptera				Duration (s)	113.86	71.01	3
Heptageniidae sp. (Fig. 2E)				Emission rate (μl min ⁻¹)	0.530	0.578	3
Mass (mg)	5.61	5.98	12	<i>Burst</i>			
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	1.425×10^{-3}	1.143×10^{-3}	12	Emission volume (μl)	14.55	12.82	3
Odonata				Duration (s)	340.86	358.38	3
Coenagrionidae				Emission rate (μl min ⁻¹)	2.733	1.705	3
<i>Ischnura senegalensis</i> (Fig. 2F)				Mantodea			
Mass (mg)	21.16	4.58	10	Mantidae			
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	1.265×10^{-2}	0.658×10^{-2}	10	<i>Sphodromantis gastrica</i> (Fig. 2I)			
Frequency (mHz)	0.629	0.331	5	Mass (mg)	335.7	276.7	7

Table continued on next page.

Table 2. *Continued*

	Mean	Standard deviation	N		Mean	Standard deviation	N
<i>Sphodromantis gastrica</i> (continued)				Dermaptera			
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	6.440×10 ⁻²	0.005×10 ⁻²	7	Labiduridae			
Frequency (mHz)	5.648	2.519	4	<i>Euborellia annulipes</i> (Fig. 2N)			
<i>Interburst</i>				Mass (mg)	31.76	26.50	12
Emission volume (μl)	0.451	0.810	4	\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	8.727×10 ⁻³	8.004×10 ⁻³	12
Duration (s)	66.65	82.31	4	Frequency (mHz)	1.044×10 ³		2
Emission rate (μl min ⁻¹)	0.531	0.886	4	<i>Interburst</i>			
<i>Burst</i>				Emission volume (μl)	1.398	0.974	3
Emission volume (μl)	3.683	4.027	4	Duration (s)	533.88	341.90	3
Duration (s)	214.35	119.81	4	Emission rate (μl min ⁻¹)	0.172	0.131	3
Emission rate (μl min ⁻¹)	0.806	0.515	4	<i>Burst</i>			
Mantophasmatodea				Emission volume (μl)	0.621	0.571	3
Austrophasmatidae				Duration (s)	306.50	136.13	3
<i>Karoophasma biedouwensis</i> (Fig. 2J)				Emission rate (μl min ⁻¹)	0.107	0.065	3
Mass (mg)	98.62	83.50	14	Hemiptera			
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	2.396×10 ⁻²	1.487×10 ⁻²	14	Coreidae sp. (Fig. 2O)			
Frequency (mHz)	3.450	1.01	14	Mass (mg)	556.1	63.64	4
<i>Interburst</i>				\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	0.164	0.073	4
Emission volume (μl)	0.416	0.239	11	Frequency (mHz)	4.208	2.048	4
Duration (s)	120	69	11	<i>Interburst</i>			
Emission rate (μl min ⁻¹)	0.216	0.010	11	Emission volume (μl)	2.88	3.137	3
<i>Burst</i>				Duration (s)	134.15	120.44	3
Emission volume (μl)	1.254	0.566	11	Emission rate (μl min ⁻¹)	1.486	0.691	3
Duration (s)	192	63	11	<i>Burst</i>			
Emission rate (μl min ⁻¹)	0.394	0.144	11	Emission volume (μl)	12.03	2.164	3
Phasmatodea				Duration (s)	172.92	39.28	3
Phamatidae				Emission rate (μl min ⁻¹)	4.233	0.522	3
<i>Extatosoma tiaratum</i> (Fig. 2K)				Lygaeidae sp. (Fig. 2P)			
Mass (mg)	0.59×10 ³	0.42×10 ³	10	Mass (mg)	19.66	3.94	9
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	0.218×10 ⁻³	0.066×10 ⁻³	10	\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	1.103×10 ⁻²	1.873×10 ⁻²	9
Frequency (mHz)	7.650	0.022	10	Trichoptera			
<i>Interburst</i>				Leptoceridae			
Emission volume (μl)	9.59		1	<i>Leptocerina sp.</i> (Fig. 2S)			
Duration (s)	39.75		1	Mass (mg)	5.20		2
Emission rate (μl min ⁻¹)	1.41		1	\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	3.532×10 ⁻³		2
<i>Burst</i>				Frequency (mHz)	10.21		2
Emission volume (μl)	13.95		1	<i>Interburst</i>			
Duration (s)	80.75		1	Emission volume (μl)	0.054		2
Emission rate (μl min ⁻¹)	1.14		1	Duration (s)	51.5		2
Orthoptera				Emission rate (μl min ⁻¹)	0.045		2
Pneumoridae				<i>Burst</i>			
<i>Bullacris intermedia</i> (Fig. 2M)				Emission volume (μl)	0.141		2
Mass (mg)	1.69×10 ³	1.19×10 ³	5	Duration (s)	83.5		2
\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	0.297	0.135	5	Emission rate (μl min ⁻¹)	0.085		2
<i>Interburst</i>				Lepidoptera			
Emission volume (μl)	17.71		1	Plutellidae			
Duration (s)	160.5		1	<i>Plutella xylostella</i> (Fig. 2T)			
Emission rate (μl min ⁻¹)	6.643		1	Mass (mg)	3.52	0.93	13
<i>Burst</i>				\dot{V}_{CO_2} (ml CO ₂ h ⁻¹)	3.751×10 ⁻³	0.578×10 ⁻³	13
Emission volume (μl)	1.40		1				
Duration (s)	90.75		1				
Emission rate (μl min ⁻¹)	0.926		1				

Note that differences in sample size between mass data and frequency data provide an indication of the frequency of cyclic or DGC patterns relative to continuous gas exchange, thus providing a measure of within-species variation. \dot{V}_{CO_2} , rate of CO₂ production.

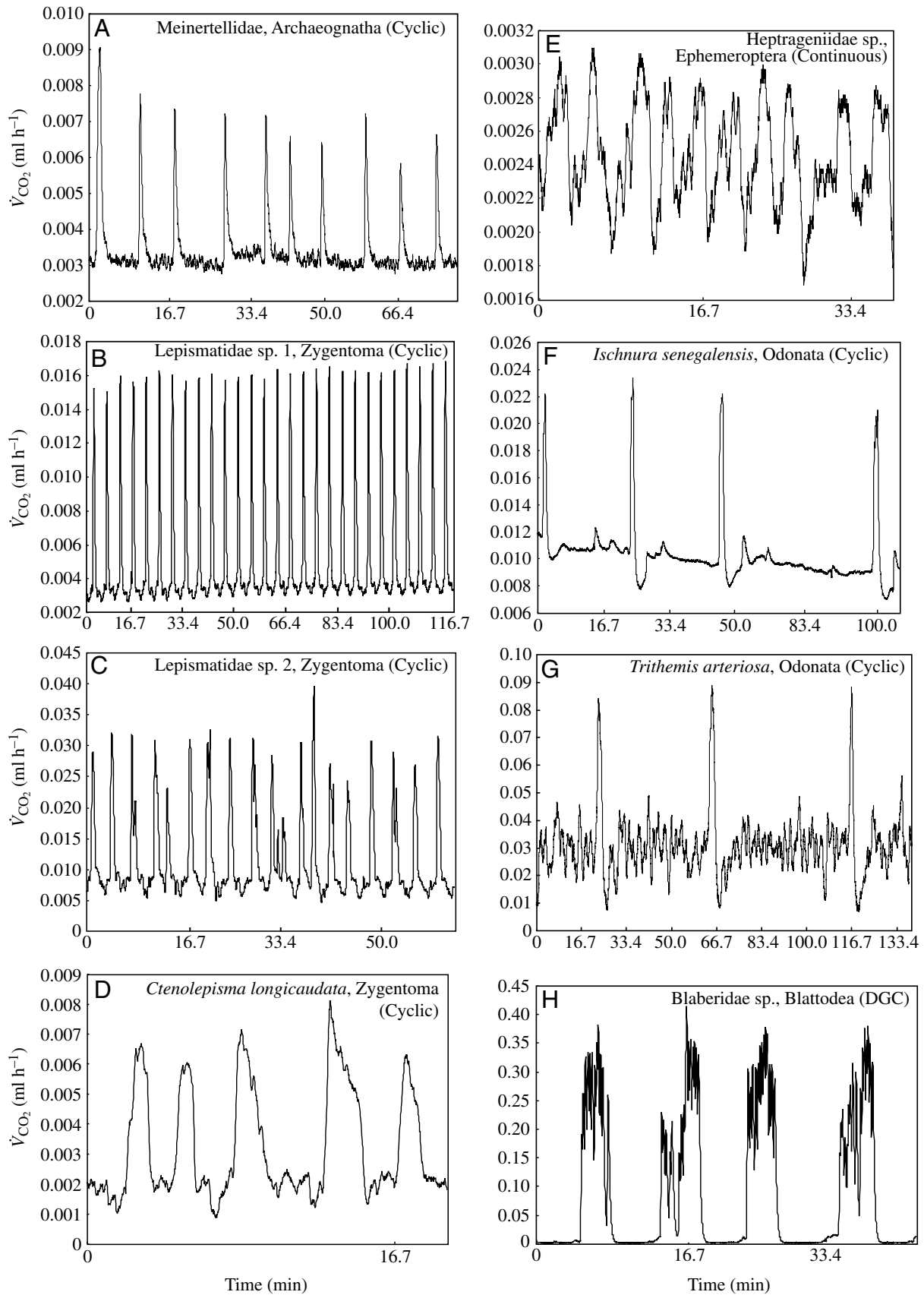


Fig. 2A–H

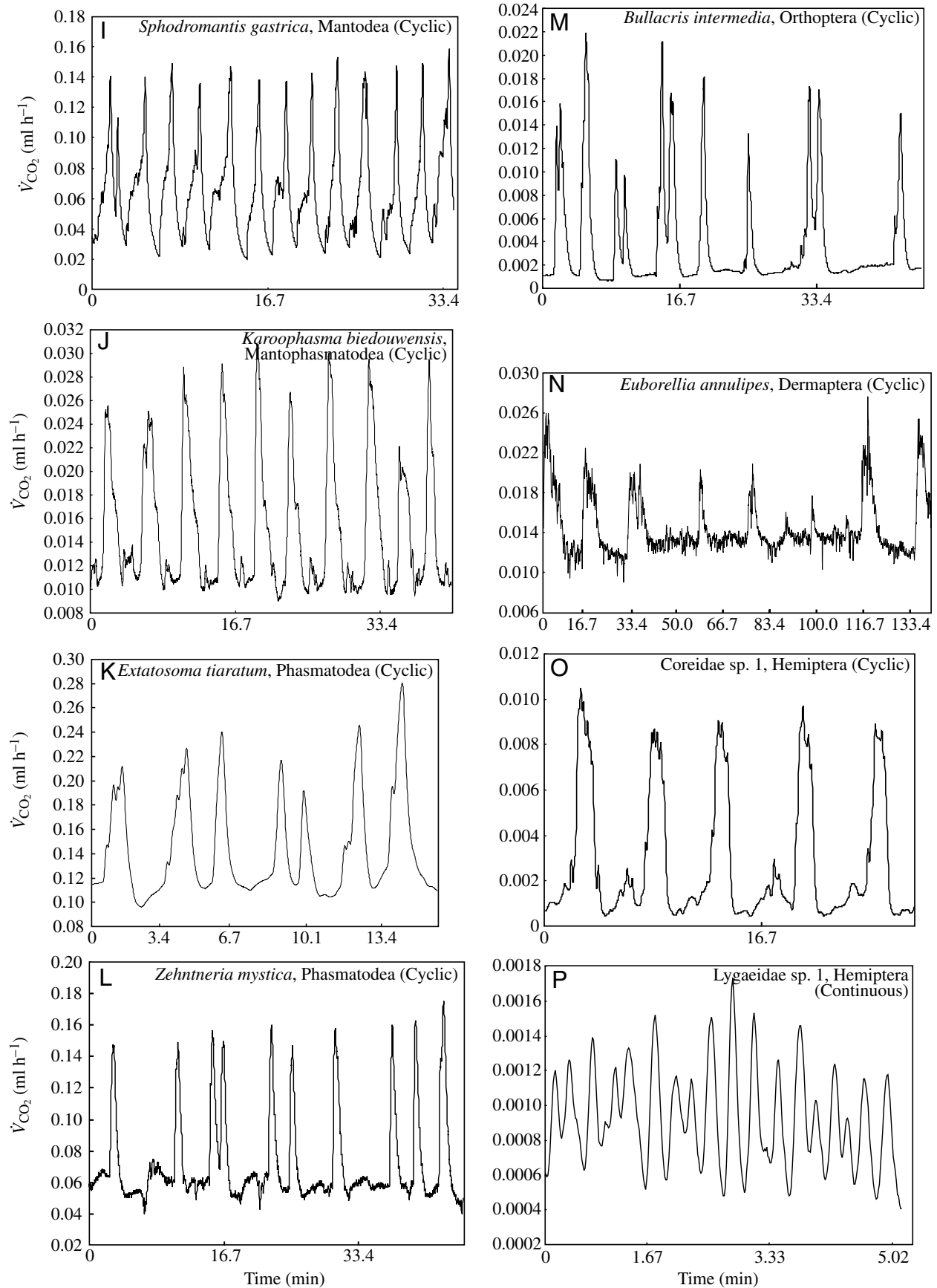


Fig. 2I-P

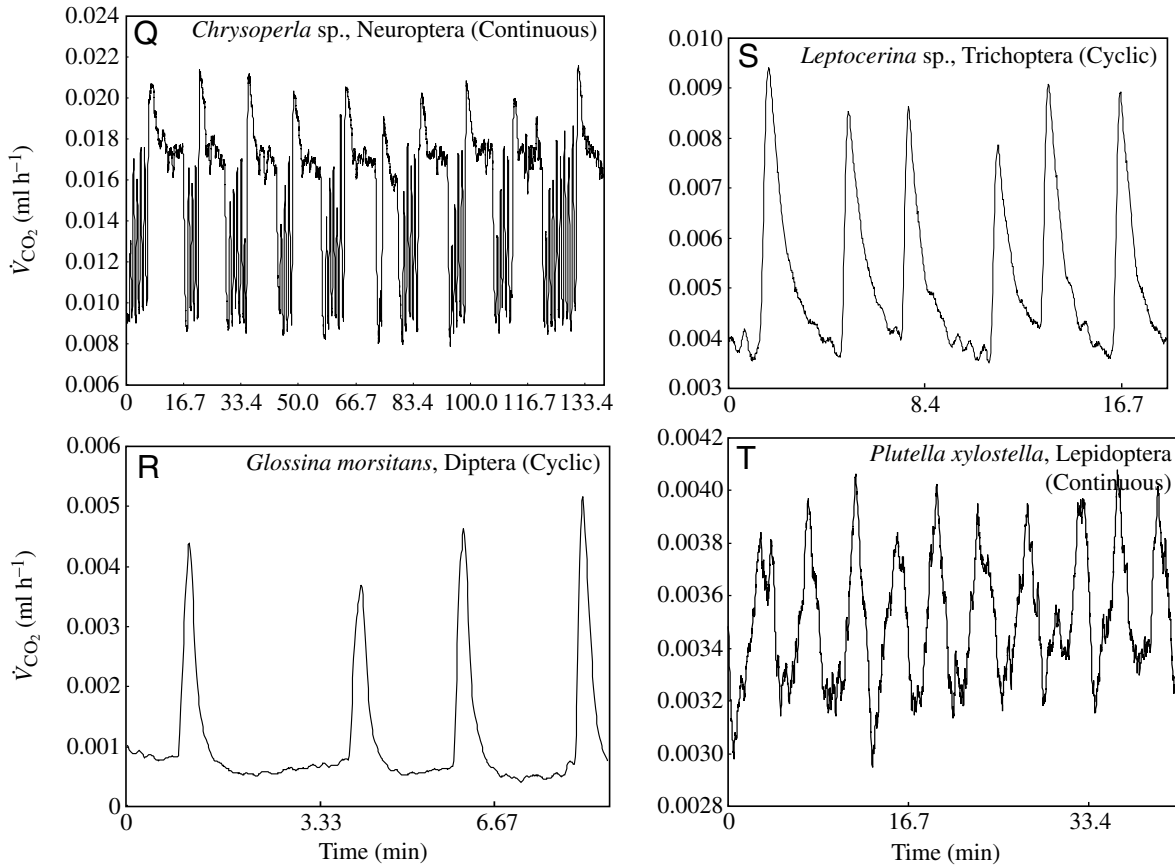


Fig. 2. (A–T) Representative continuous, cyclic or discontinuous gas exchange patterns for all of the species and orders investigated in this study. *Zehntneria mystica* (Phasmatodea, Phasmatidae) (L) is not discussed in the text. However, a single specimen showed a clear cyclic trace, therefore the graph is included. The other phasmid, *Extatosoma tiaratum* (K), is a leaf-mimicking species that shows a swaying response to the slightest air flow, which explains the limited evidence for cyclic gas exchange in this species.

each of the orders and as species from previously unstudied orders are examined. Even so, the independent evolution of DGCs in five insect orders is not unlike the situation amongst the Arthropoda as a whole, where discontinuous gas exchange cycles appear to have evolved independently at least four times, with the Pseudoscorpiones and Solifugae possibly inheriting DGCs from a common ancestor, and in one instance with a control system different to that of the insects (Klok et al., 2002; Lighton and Joos, 2002). Such independent evolution of DGCs, and the basal nature of both continuous and cyclic gas exchange, suggest that discontinuous gas exchange might well be adaptive, rather than an ancestral feature of the insects. The presence in particular families of some species with the trait and others without it (supplementary material, Appendix 1; Table 2) provides additional evidence for this idea. Perhaps more importantly, this independent evolution indicates that there is no *a priori* reason why control of discontinuous gas exchange cycles should be similar in the different orders of insects (see also Lighton and Joos, 2002), and that considerable care must be taken in applying the outcomes of the careful and informative analyses of model preparations (such as Lepidopteran pupae, cockroaches and grasshoppers e.g. Kestler, 1985; Harrison, 1997; Levy and Schneiderman,

1966a,b) more broadly. This caveat has been raised previously by work showing substantial differences between insect species (Harrison, 1997; Lighton, 1998; Davis et al., 1999), and the present study lends further support to it.

Whilst the comparative analysis does indicate which gas exchange characteristics are basal, it does not clearly resolve the reason(s) for the origin and/or maintenance of discontinuous gas exchange. Nonetheless, examination of the data (supplementary material, Appendix 1; Table 2) reveals several interesting patterns to the presence and absence of DGCs. There is no clear pattern of association between DGCs and subterranean vs non-subterranean lifestyles ($\chi^2=1.08$, $P=0.30$). Likewise, both winged and wingless species show DGCs ($\chi^2=0.17$, $P=0.68$), despite their apparently different oxygen demands (Reinhold, 1999; Addo-Bediako et al., 2002), which should mean greater threat of oxidative damage (see Hetz and Bradley, 2005) in flying species at rest because of their highly developed tracheal system that should mean enhanced oxygen access to tissues (Chapman, 1998). However, associations between DGCs and xeric environments ($\chi^2=9.26$, $P=0.002$), as might be predicted from the hygric hypothesis (Lighton, 1998; Chown and Nicolson, 2004), were found. These preliminary analyses broadly suggest that cyclic and

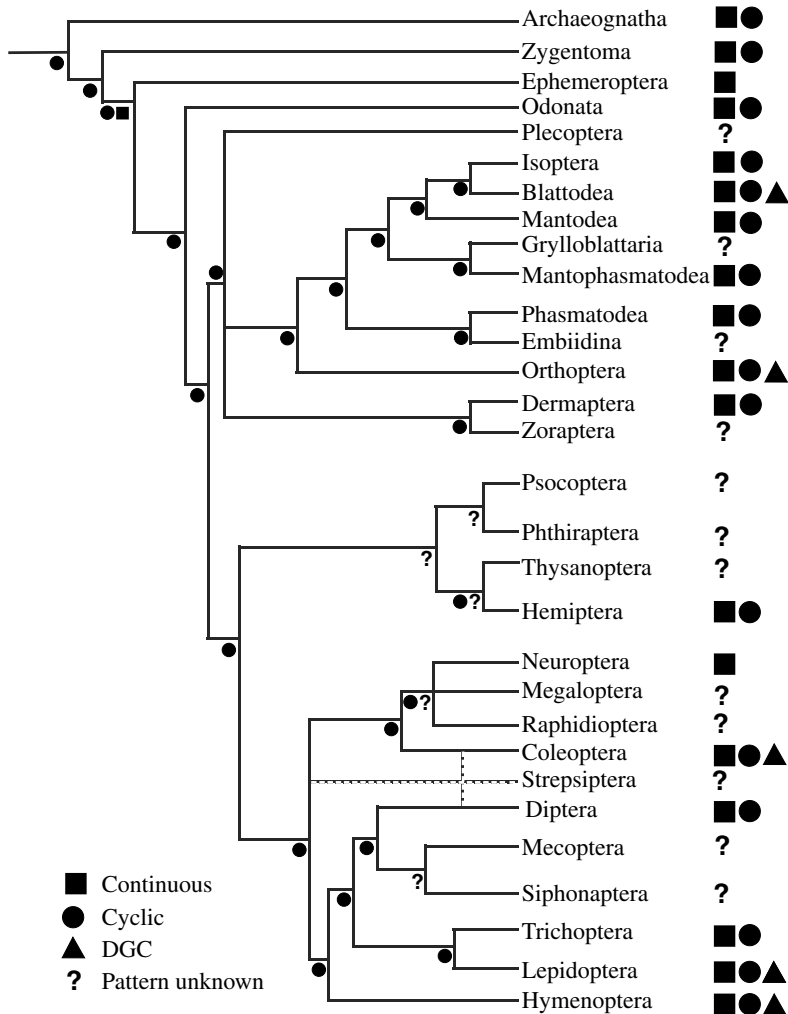


Fig. 3. Phylogeny of insects (redrawn from Gullan and Cranston, 2005) indicating the gas exchange patterns that are shown by species within each of the orders. Symbols for the gas exchange patterns are as follows: squares, continuous gas exchange; circles, cyclic gas exchange; triangles, discontinuous gas exchange. The parsimony analysis is also indicated on the phylogeny and shows that the cyclic pattern is the ancestral condition at rest. Discontinuous gas exchange cycles (DGCs) have been observed in only 5 of the 30 orders of insects, while continuous and cyclic patterns have been observed in all of the orders examined.

continuous gas exchange at rest are more likely in mesic than in xeric environments, than are DGCs, but that DGCs can evolve in both kinds of environments. However, the data have a strong phylogenetic signal such that DGCs are restricted to a few families. If these analyses are repeated within orders (Coleoptera, Hymenoptera, Lepidoptera, Orthoptera) or families (Tenebrionidae, Scarabaeidae, Formicidae) that have sufficiently large sample sizes, in what is essentially then a phylogenetically nested approach to examining these associations (see Harvey and Pagel, 1991), none of the associations are significant (χ^2 , $P > 0.07$ in all cases). Thus, the overall result does not appear to be strongly biased by a single taxon. However, such an approach does not adequately exclude phylogenetic signal (Garland et al., 2005), and does not make

full use of the potentially available environmental data. To this end, conversion of the environmental data to a continuous, rather than categorical form, a phylogeny at the species level for the 118 species that have been studied, and a phylogenetic generalized least-squares analysis (Grafen, 1989) is required. Such work is now underway (C. L. White, S. L. Chown and others, unpublished data).

In the few studies where a tally has been kept of individuals showing DGCs vs other gas exchange patterns (e.g. Gibbs and Johnson, 2004), one of the predictions of the emergent property hypothesis also seems to be supported. That is, DGCs should emerge whenever the gas exchange system has little demand placed on it, but that this might vary given initial conditions (Chown and Holter, 2000). Where there is variation in gas exchange patterns (see also Table 2), considerable variation among individuals in gas exchange pattern has been found. Therefore, DGCs might be an emergent property of the interacting CO₂ and O₂ setpoints, although it is not clear why DGCs emerge in only a few orders. In consequence, there is good reason to undertake modelling work of interactions between the CO₂ and O₂ setpoints, in the context of knowledge of gas exchange regulation (reviewed in Chown and Nicolson, 2004), as well as to examine and, just as importantly, to report variation in gas exchange patterns within and between individuals (for additional discussion, see Lighton, 1998; Chown, 2001).

Another striking outcome of this phylogeny-based analysis of insect gas exchange patterns is that, despite a wide range of studies, undertaken over many years, nothing remains known of gas exchange in 12 of the 30 insect orders, and that of the remaining orders, only the Coleoptera and Hymenoptera have had investigations undertaken on more than ten species. The same is true of the Arthropoda as a whole, where gas exchange investigations of terrestrial groups are restricted to only a handful of species (see e.g. Lighton et al., 1993; Lighton and Fielden, 1996; Lighton, 2002; Lighton and Joos, 2002; Klok et al., 2002; Terblanche et al., 2004). This bias in the data does not mean that a clear understanding of the mechanisms underlying gas exchange, and particularly discontinuous gas exchange, is not emerging (reviewed by in Lighton, 1996, 1998; Chown and Nicolson, 2004). However, it does suggest that investigations of the reasons for the origin and maintenance of particular forms of gas exchange, in other words their likely adaptive value, will be constrained, at least from a comparative perspective, by the absence of appropriate information. To some extent this is true also of comparisons at the species level where, to date, not a single comparative analysis, in the strict phylogenetically independent sense (see Harvey and Pagel, 1991), or using a parsimony style approach

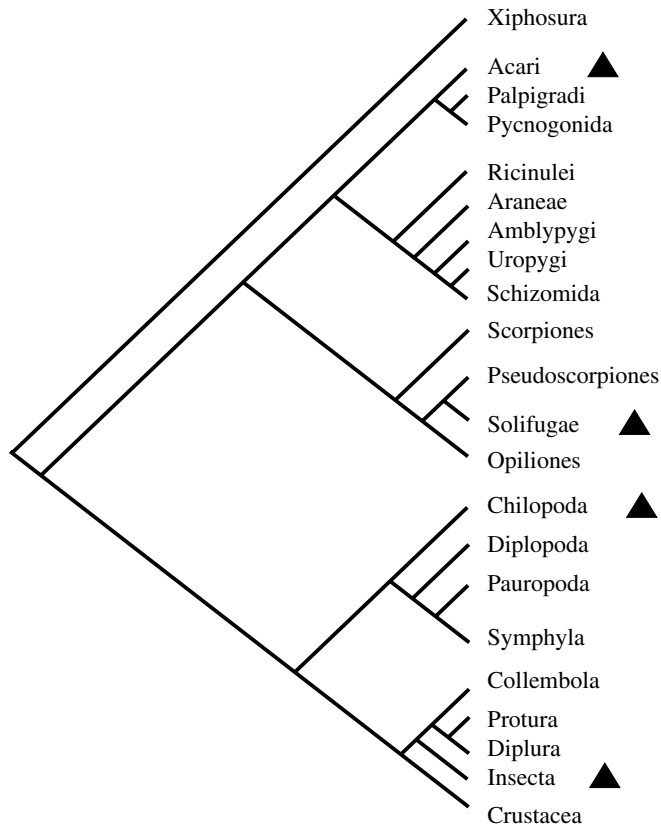


Fig. 4. The distribution of discontinuous gas exchange cycles (DGCs) across the arthropod phylogeny (redrawn from Chown and Nicolson, 2004).

(see Brooks and McLennan, 1991), has been undertaken for a multi-species monophyletic unit. In addition, laboratory selection experiments investigating the response of gas exchange patterns to different conditions have been restricted to a few *Drosophila* species and then only under conditions of starvation and desiccation (Gibbs et al., 1997; Williams et al., 1997, 2004).

Thus, of the approaches that Huey and Kingsolver (1993; see also Kingsolver and Huey, 1998) suggested are essential for providing an integrated understanding of the evolution of physiological traits, insect gas exchange physiology has been successful at the mechanistic investigations (reviewed by Harrison, 1997; Lighton, 1996, 1998; Chown and Nicolson, 2004). However, it has so far performed relatively poorly when it comes to laboratory selection and comparative approaches. Remedying the situation does not mean undertaking 4–8 million studies to assess gas exchange patterns in all of the insects (May, 2000). Rather, there should be a concerted approach to identify gas exchange patterns in a variety of species from the major orders, given that variation in many insect physiological traits is partitioned at the order and family levels (Chown et al., 2002). This work could be combined with smaller scale comparative investigations, laboratory selection experiments (for an excellent example of this combined approach, see Gibbs et al., 1997), and the kinds of mechanistic

studies for which the field is best known, and which have revealed striking differences amongst regulatory systems in the arthropods (Lighton and Joos, 2002).

We thank Antoinette Botes, Henry Davids, Fabian Haas, Ulrike Irlich, Mike Picker, Priya Ranchod, Michael Samways, Krystal Tolley, Ruan Veldtman and Ester van der Westhuizen as well as the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory staff for assistance with field collections, insect identification or data acquisition. Allen Gibbs and Thomas Shelton provided access to data. Johan du Preez of the Signal Processing Unit at Stellenbosch University provided much advice on signal analysis and suggested the method for separating continuous from cyclic patterns. Sue Jackson and three anonymous reviewers provided helpful comments that improved the manuscript. Stefan Hetz is thanked for his ongoing and generous sharing of ideas with us. This work was supported by National Research Foundation Grant FA2004032000006 to S.L.C.

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