

Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp.

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Summary

For natural selection to take place several conditions must be met, including consistent variation among individuals. Although this assumption is increasingly being explored in vertebrates, it has rarely been investigated for insect physiological traits, although variation in these traits is usually assumed to be adaptive. We investigated repeatability (r) of metabolic rate and gas exchange characteristics in a highly variable *Perisphaeria* cockroach species. Although this species shows four distinct gas exchange patterns at rest, metabolic rate ($r=0.51$) and the bulk of the gas exchange characteristics ($r=0.08$ – 0.91 , median= 0.42) showed high and significant repeatabilities. Repeatabilities were generally lower in those cases where the effects of body size were removed

prior to estimation of r . However, we argue that because selection is likely to act on the trait of an animal of a given size, rather than on the residual variation of that trait once size has been accounted for, size correction is inappropriate. Our results provide support for consistency of variation among individuals, which is one of the prerequisites of natural selection that is infrequently tested in insects.

Supplementary material available on-line

Key words: adaptation, body size, discontinuous gas exchange, metabolic rate, variation, cockroach, *Perisphaeria* sp.

Introduction

For a trait to be considered an adaptation, natural selection must have been responsible for its origin and must be responsible for its maintenance, or, if one is prepared to blur the distinction between exaptation and adaptation, must be responsible for its maintenance (Coddington, 1988; Baum and Larson, 1991; Ketterson and Nolan, 1999). In turn, for natural selection to take place there are three prerequisites (Endler, 1986; Bech et al., 1999; Dohm, 2002). First, the trait in question must show consistent variation among individuals. Second, there must be a consistent relationship between that variation and variation in fitness. Third, the trait must be heritable. Whilst these assumptions, and particularly that of consistent among-individuals variation, are being increasingly well explored for physiological traits in vertebrates (e.g. Huey and Dunham, 1987; Hayes and Chappell, 1990; Hayes et al., 1992; Chappell et al., 1995; Berteaux et al., 1996; Chappell et al., 1996; Bech et al., 1999; Fournier and Thomas, 1999; Koteja et al., 2000; McCarthy, 2000), the same is not true of invertebrates.

In insects, the genetic variability and heritability of several physiological traits have been investigated, mostly in *Drosophila* (usually *melanogaster*) and often in the context of selection experiments (e.g. Parsons, 1980; Hoffmann and Parsons, 1989a; Graves et al., 1992; Gibbs et al., 1997; Gibert

et al., 1998; Hoffmann et al., 2003). Likewise, variation in traits among populations and as a consequence of acclimation has also been well explored, especially for thermal tolerance and desiccation resistance (Hoffmann, 1990; Hoffmann et al., 2001; Klok and Chown, 2003). Whilst these studies provide evidence that adaptation has probably been responsible for variation in thermal tolerance and desiccation resistance (see also Chown et al., 2002), explicit exploration of the assumptions underlying the hypothesis of adaptation remains scarce for most traits. This is especially true of metabolic rate and gas exchange characteristics. The few explicit studies that have been undertaken have generally demonstrated a metabolic response to laboratory selection for desiccation resistance in *Drosophila melanogaster*, which implies that the conditions for selection must have been met (Hoffmann and Parsons, 1989a,b, 1993; Gibbs et al., 1997; Djawdan et al., 1998; Williams et al., 1998). Nonetheless, it is widely assumed that among-species and among-population variation in whole-organism metabolic traits in most insect taxa is adaptive (for reviews and examples, see Lighton, 1996; Chown and Gaston, 1999; Addo-Bediako et al., 2001, 2002; Gibbs et al., 2003). Metabolic rate is of particular significance in this regard. Not only is it thought to be closely linked to variation in life history characteristics and body size (Hoffmann and Parsons, 1991;

Graves et al., 1992; Kozłowski and Gawelczyk, 2002), but variation therein apparently also has a profound influence on broad-scale variation in diversity (Allen et al., 2002).

Adaptive explanations for variation in metabolic rate and the patterns in exchange underlying oxygen delivery and CO₂ removal in insects generally take two major forms. First, variation in metabolic rate is thought to take place in response to either dry conditions, when it is reduced to conserve water, or to short seasons, when it is elevated to enable more rapid development (for reviews and discussion, see Chown and Gaston, 1999; Addo-Bediako et al., 2002; Chown, 2002). Second, alterations in gas exchange patterns are thought to have taken place to effect a respiratory water savings under dry conditions. In particular, it has long been thought that discontinuous gas exchange, which is present in many insect species at rest (Lighton, 1996, 1998), evolved as a means to limit respiratory water loss and that it continues to serve this major function (Levy and Schneiderman, 1966; Kestler, 1985; Sláma and Coquillaud, 1992; Lighton et al., 1993a; Duncan et al., 2002a). Discontinuous gas exchange is typically cyclic with each cycle consisting of a Closed (C) period, during which the spiracles are tightly closed, a Flutter (F) period, during which the spiracles partly open and close in rapid succession, and an Open (O) period, during which the spiracles are open (Lighton, 1996). The principal explanations for the contribution of discontinuous gas exchange cycles (DGCs) to water economy are that spiracles are kept closed for a portion (the C-period) of the DGC thus reducing respiratory water loss to zero, and that a largely convective F-period restricts outward movement of water (Kestler, 1985). Moreover, it has also been argued that there is adaptive variation in the durations of the C-, F-, and O-periods to further reduce water loss. That is, a reduced O-period, and prolonged C- and F-periods are likely to further restrict respiratory water loss (Lighton, 1990; Lighton et al., 1993b; Davis et al., 1999; Bosch et al., 2000; Duncan et al., 2002a; Duncan, 2003). Whilst several other hypotheses for the evolution and maintenance of DGCs have been proposed (Lighton and Berrigan, 1995; Lighton, 1998; Bradley, 2000), these are also largely adaptive in nature (though for an exception, see Chown and Holter, 2000).

Nonetheless, with the exception of the laboratory selection experiments on *D. melanogaster*, there have been few explicit attempts to investigate the assumptions underlying these claims for adaptation, which have largely been made on the grounds of comparative studies, of which the majority have not been undertaken in an explicitly phylogenetic context (for discussion, see Chown, 2002; Chown and Gaston, 1999; for recent studies, see Davis et al., 1999; Duncan and Byrne, 2000; Addo-Bediako et al., 2001; Gibbs et al., 2003). Whilst such comparative studies are useful, they are not without their problems (e.g. Leroi et al., 1994), and it is widely accepted that comparative work should be supported by more explicit investigations of the extent to which selection is responsible for variation in physiological traits (e.g. Huey and Kingsolver, 1993; Kingsolver and Huey, 1998). We therefore undertook this study to investigate the repeatability of metabolic rate and

the characteristics of discontinuous gas exchange cycles in an insect species that not only exchanges gases intermittently, but also shows considerable variation in its gas exchange pattern. We reasoned that if these traits show significant repeatability in this species, then it is likely that repeatability will be even more pronounced in most other insect species, which are generally not as variable (see Chown, 2001).

Materials and methods

Experimental animals and design

Repeatability of metabolic rate, and cyclic gas exchange and its components were examined in the cockroach, *Perisphaeria* sp. (Blattodea, Blaberidae). In short-term pilot studies this species showed not only a clear DGC typical of many other insect species, but also several other gas exchange patterns that were not associated with activity, which generally disrupts typical DGC patterns (Lighton, 1994). Seventeen females and three males (males of species within this genus are rarely found; Picker et al., 2002) were collected at altitudes higher than 950 m above sea level on Stellenbosch Mountain (33°57'S, 18°53'E) and returned to the laboratory in Stellenbosch. Here they were held for 2 months before experiments commenced to exclude possible variation associated with microhabitat differences (Huey and Berrigan, 1996). Throughout this period and over the course of the experiments, the cockroaches were kept individually in marked Petri dishes in a humidified incubator at 22±1.0°C (on a 12 h:12 h L:D cycle). They were fed dry dog- and horse-feed pellets *ad libitum* and sliced apple was provided on a regular basis. Subsequent culturing has shown that this species is able to reproduce successfully under these conditions (E. Marais, unpublished data).

Prior to each gas exchange assessment, the individual in question was starved for 24 h to reduce variability associated with specific dynamic action (McEvoy, 1984; Lighton, 1989; Duncan et al., 2002b). Assessments were made during the day only, in a well-lit room, because we were concerned only with discontinuous gas exchange and standard metabolic rate. The species is nocturnal, and at night activity and metabolic rate are high (Fig. 1A). Assessments were also made in dry air because under these conditions a discontinuous gas exchange cycle would seem most likely as a means to conserve water (Quinlan and Hadley, 1993; Duncan et al., 2002b). Each individual was weighed (to a resolution of 0.0001 g), using an analytical balance (Toledo AX504, Mettler, Columbus, OH, USA), and placed into a 5 ml cuvette kept at 20±0.2°C using a water bath (Grant LTD20, Cambridge, UK). Air, scrubbed of water (using Drierite, Krugersdorp, South Africa) and CO₂ (using soda lime) was pushed through the cuvette at a flow rate of 200 ml min⁻¹ (regulated using a Sidetrack Mass Flow Controller, Monterey, USA) and into a calibrated infrared gas analyzer (Li-Cor Li7000; Henderson, USA) set in differential mode to measure CO₂ production. A Sable Systems (Henderson, USA) AD-1 activity detector was used to detect any movement of the cockroach in the cuvette during the

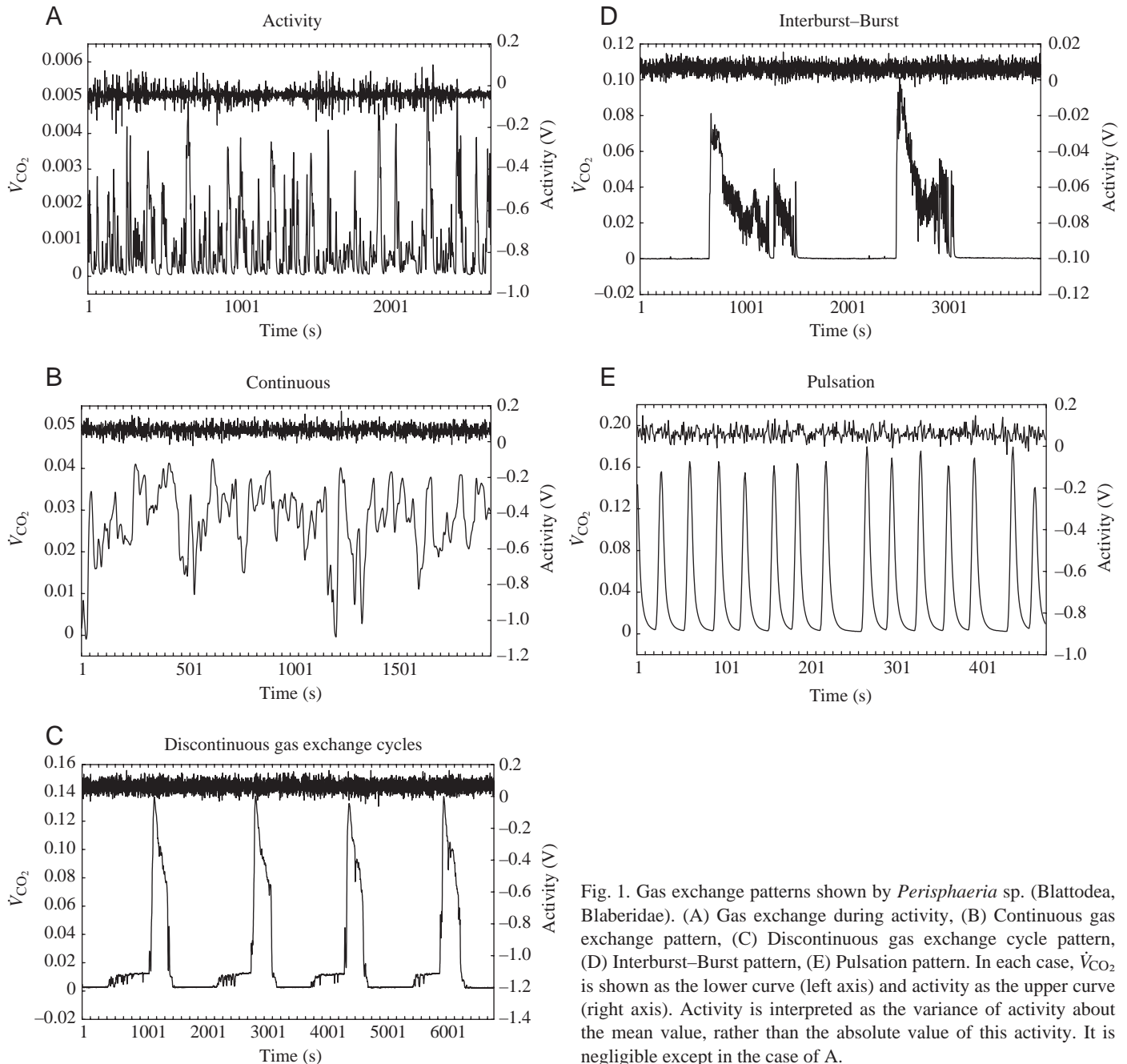


Fig. 1. Gas exchange patterns shown by *Perisphaeria* sp. (Blattodea, Blaberidae). (A) Gas exchange during activity, (B) Continuous gas exchange pattern, (C) Discontinuous gas exchange cycle pattern, (D) Interburst-Burst pattern, (E) Pulsation pattern. In each case, \dot{V}_{CO_2} is shown as the lower curve (left axis) and activity as the upper curve (right axis). Activity is interpreted as the variance of activity about the mean value, rather than the absolute value of this activity. It is negligible except in the case of A.

experiment, and the output of the detector was fed into the auxillary channel of the Li7000. The AD-1 presents activity as a value between -5 V and 5 V, where 0 V is an accurate indication that the specimen is inactive (for more detail, see www.sablesys.com/ad1.html). Inspection of several individuals confirmed lack of activity detected by the AD-1. To avoid the potential influence of pheromones on the behaviour of individuals, the cuvette was cleaned thoroughly with ethanol after each experimental trial. Each experimental assessment also lasted for at least 3 h (for rationale, see Chown, 2001). The data file generated by the Li7000 software was exported, *via* Microsoft Excel, to DATACAN V (Sable Systems), which was

used for initial analysis of the respirometry data (corrected to standard temperature and pressure).

Each individual was assessed five times: twice between 07:00 h and 11:00 h, once between 11:00 h and 14:00 h and twice between 14:00 h and 18:00 h. This was done because circadian patterns in metabolic rate have been found in other insect species (Takahashi-Del-Bianco et al., 1992). Typically, at least 5 days elapsed between each assessment of an individual, and the order of assessment with regard to time of day was randomised. The interval between repeated measures is important because the shorter it is the greater the likelihood that a high repeatability will be found (Chappell et al., 1996;

Table 1. *CO₂ emission volumes, period durations, emission rates, total metabolic rate and mass for each of the four gas exchange patterns identified for Perisphaeria sp. (Blattodea, Blaberidae)*

Pattern	C-period	F-period–Interburst	O-period–Burst	N	Metabolic rate ($\mu\text{l h}^{-1}$)	Mass (g)
Emission volumes (μl)						
DGC	0.3989 \pm 0.034	0.877 \pm 0.078	6.607 \pm 0.333	13		
Interburst–Burst		1.516 \pm 0.307	5.585 \pm 0.153	13		
Pulsation		0.0312 \pm 0.010	0.298 \pm 0.0001	9		
Continuous				13		
Period duration (min)						
DGC	11.667 \pm 0.588	6.504 \pm 0.400	7.919 \pm 0.358	13		
Interburst–Burst		13.039 \pm 0.474	8.986 \pm 0.793	13		
Pulsation		0.466 \pm 0.007	0.367 \pm 0.047	9		
Continuous				13		
Emission rate ($\mu\text{l h}^{-1}$)						
DGC	2.148 \pm 0.120	8.400 \pm 0.600	50.940 \pm 1.500	13	20.717 \pm 2.314 ^A	0.3148 \pm 0.0179
Interburst–Burst		9.060 \pm 2.160	46.140 \pm 1.080	13	19.811 \pm 2.751 ^A	0.2846 \pm 0.0185
Pulsation		10.680 \pm 0.780	48.900 \pm 0.600	9	20.721 \pm 1.891 ^A	0.3083 \pm 0.0112
Continuous				13	36.178 \pm 3.425 ^B	0.3887 \pm 0.0148

Values are means \pm S.E.M. (N = number of individuals), calculated using the values from each trial.

DGC, discontinuous gas exchange cycle; C, closed; O, open; F, flutter.

The metabolic rate of the continuous gas exchange pattern differed significantly from the cyclic patterns (repeated measures $F_{3,41}=6.79$, $P<0.0008$; Tukey's HSD for unequal sample sizes).

Means with the same symbols do not differ significantly.

Bech et al., 1999). Although we have no data on lifespan for this species, we have cultured adults for more than a year, and other blaberids are known to have an adult lifespan of several years (Scholtz and Holm, 1985). Therefore, an interval of at least 5 days is appropriate for this species, though perhaps biased somewhat in the direction of higher repeatability. The total time taken for the study was approximately 5 months.

Analyses

Because of the small number of males available, we generally restricted our analyses of repeatability to females. Somewhat surprisingly, we found four major patterns of gas exchange, of which three were intermittent and cyclic (see Results), and the fourth was continuous. Data from the continuous pattern were excluded because metabolic rate was significantly higher (approximately twofold) during this pattern of gas exchange than during the others [repeated-measures analysis of variance (ANOVA), $F_{(3,41)}=6.79$, $P=0.0008$, Tukey's HSD for unequal sample sizes, Table 1], even though the individuals were inactive. In consequence, investigations of the repeatability of gas exchange components were undertaken for each of the three major cyclic patterns, and across the dataset as a whole. The variables investigated were duration (s), CO_2 volume (μl) and CO_2 emission rate \dot{V}_{CO_2} ($\mu\text{l h}^{-1}$) for each period, and mean \dot{V}_{CO_2} and mean frequency of the cycles. Where the analyses were done across the three cyclic patterns, the Flutter period typical of discontinuous gas exchange was compared with the 'Interburst' period associated

with the other forms of cyclic gas exchange. In these cases we also included data for males. Repeatability (r) was calculated using the intraclass correlation approach (Berteaux et al., 1996; Falconer and Mackay, 1996), based on analyses of variance and the equations provided by Lessells and Boag (1987). Because variation in body mass affects variation in metabolic rate and DGC characteristics in arthropods (Peters, 1983; Lighton, 1991; Lighton and Fielden, 1995; Davis et al., 1999), and because there was a reasonable range in the body mass of the specimens we examined (females: mass 0.3397 ± 0.0184 g, mean \pm S.E.M., range 0.1795–0.4643 g; males: mass 0.2357 ± 0.0425 g, range 0.1793–0.3189 g) the effects of body size were taken into account in a second round of repeatability analyses. Usually, to do this the residuals from the regression of body mass and the characteristic of interest are used (Berteaux et al., 1996; Fournier and Thomas, 1999). Here, this was not done. Rather, in all cases, body mass was included as a covariate in the initial ANOVAs (for rationale, see Freckleton, 2002). Where mass did not explain a significant portion of the variance in the independent variable, r was not determined including mass as a covariate. Confidence intervals for r were calculated using the formulae provided by Krebs (1999). A significant repeatability value of 1 indicates that individuals are perfectly consistent in their performance over time, whereas a non-significant repeatability value, or one of 0, indicates no consistent variation among individuals. In all cases a sequential Bonferroni test ($\alpha=0.05$) was used to correct table-wide significance values for multiple tests (Rice, 1989).

To further investigate the likely sources of variation in these traits, nested (hierarchical) analyses of variance (Sokal and Rohlf, 1995) were used. This method allows ready identification of the level at which most variation can be explained, and has been used for this purpose in several other studies (Berteaux et al., 1996; Chown et al., 1999; Addo-Bediako et al., 2002). For each of the major gas exchange patterns, variance was partitioned between error nested within (<) trial < time of day < individual < gender. Gender was not used as a level of partitioning in the pulsation pattern because males never showed the pattern. A similar analysis was also undertaken across all three cyclic gas exchange patterns. In the case of frequency and mean metabolic rate, the trial level was excluded because metabolic rate and frequency are calculated across all the cycles, rather than just for each individual cycle as can be done for the characteristics of each of the periods. A sequential Bonferroni correction ($\alpha=0.05$) was also applied here.

Results

This *Perisphaeria* sp. showed four major gas exchange patterns at rest (confirmed by inspection and by the AD-1), here termed Continuous (Fig. 1B), DGC (Fig. 1C), Interburst-Burst (Fig. 1D), and Pulsation (Fig. 1E). Metabolic rate did not differ among the latter three patterns, although this was not the case with Continuous gas exchange (see *Analyses* above). Whilst one individual showed all four patterns over the course of the study, most individuals showed two or three of them. However, neither females nor males preferred a particular pattern (females: log-linear analysis, ML $\chi^2=65.3$, $P=0.05$, d.f.=48; males: log-linear analysis, ML $\chi^2=7.18$, $P=0.31$, d.f.=6), although males never showed the Pulsation pattern. Time of day made no difference to the patterns that were shown (log-linear analysis, ML $\chi^2=3.94$, $P=0.68$, d.f.=6), and no individuals switched from one pattern to another over the course of a recording.

For most of the characteristics examined here, repeatability was significant and large (Fig. 2, Bonferroni correction did not alter significance values appreciably). Within patterns, repeatability tended to be highest, as might be expected, with values for Burst or O-period characteristics generally above 0.3 (with the exception of Burst duration in the Pulsation pattern when mass was included as a covariate). By contrast, Interburst or C-period characteristics tended to have lower repeatabilities (with the significant exception of emission rate, Fig. 2). Across patterns, repeatabilities were also high for the Burst period and somewhat lower for the Interburst period, with emission rate now having the lowest repeatability. This is not surprising because the three patterns differ in the extent to which individuals close their spiracles. In the DGC pattern the spiracles are held closed, whilst this is generally not the case in the other patterns (Fig. 1). There was consistent among-individual variation in metabolic rate (excluding mass: $r=0.51$ for males and females, 0.48 for females only; including mass $r=0.22$ for males and females, 0.29 for females only) and

frequency (excluding mass: $r=0.25$ for females only, 0.31 for males and females; including mass: $r=0.29$ for females only, 0.35 for males and females) (see Supplemental data, Appendix 1A). In general, repeatabilities tended to decline when mass was included as a covariate, but this was not always the case (Supplemental data, Appendix 1B).

The nested analyses of variance generally bore out our repeatability results (Table 2). Moreover, they provided additional insight into the level at which variation that was not a function of individual identity was partitioned. Thus, it is clear that DGC patterns tended to vary much more between trials (the Trial term in Table 2) than within a given trial (the Error term in Table 2), whilst the converse was true of the Pulsation pattern and, to a lesser extent, of the Interburst-Burst pattern. In this context it is important to realize that the error term includes both error and variation between individual cycles in a particular trial. The nested ANOVAs also revealed that there is generally little variation amongst genders in most of the traits examined here. Although this does not appear to be the case when the analyses are undertaken across all three intermittent patterns, this is solely the consequence of the absence of a pulsation pattern in the males.

Discussion

The presence of four, or at the least three, very different patterns of gas exchange at rest is unusual for most insects, but perhaps not entirely so for cockroaches. Although most studies acknowledge that there is some variation in gas exchange characteristics (excluding that associated with body size and/or treatment temperature; see Lighton, 1991; Davis et al., 1999; Rourke, 2000), the majority have not found the range of variation within a single species documented here (for a discussion, see Lighton, 1998; Chown, 2001). The only investigations that have suggested that there might be pronounced variability within a species have been those on *Blaberus* cockroaches by Miller (1973, 1981), who investigated patterns in CNS firing associated with spiracle control, although he thought that some of the variability was probably associated with activity. Nonetheless, among different taxa a range of periodic gas exchange patterns has been found, varying from purely cyclic, with no spiracle closure, to discontinuous gas exchange cycles of the kind originally documented so carefully for lepidopteran pupae (Punt et al., 1957; Levy and Schneiderman, 1966; Lighton, 1988, 1991; Duncan and Byrne, 2000; Shelton and Appel, 2000, 2001). Our findings for *Perisphaeria* indicate either that the variety of gas exchange patterns documented in insects does not represent as much of a major difference between taxa as comparative studies might suggest (Lighton, 1998), or that basal taxa such as cockroaches and termites differ substantially from more derived groups.

Despite this variety in gas exchange patterns, repeatability values for metabolic rate, frequency and the other gas exchange characteristics were generally high and always significant when examined across the three patterns that were typical

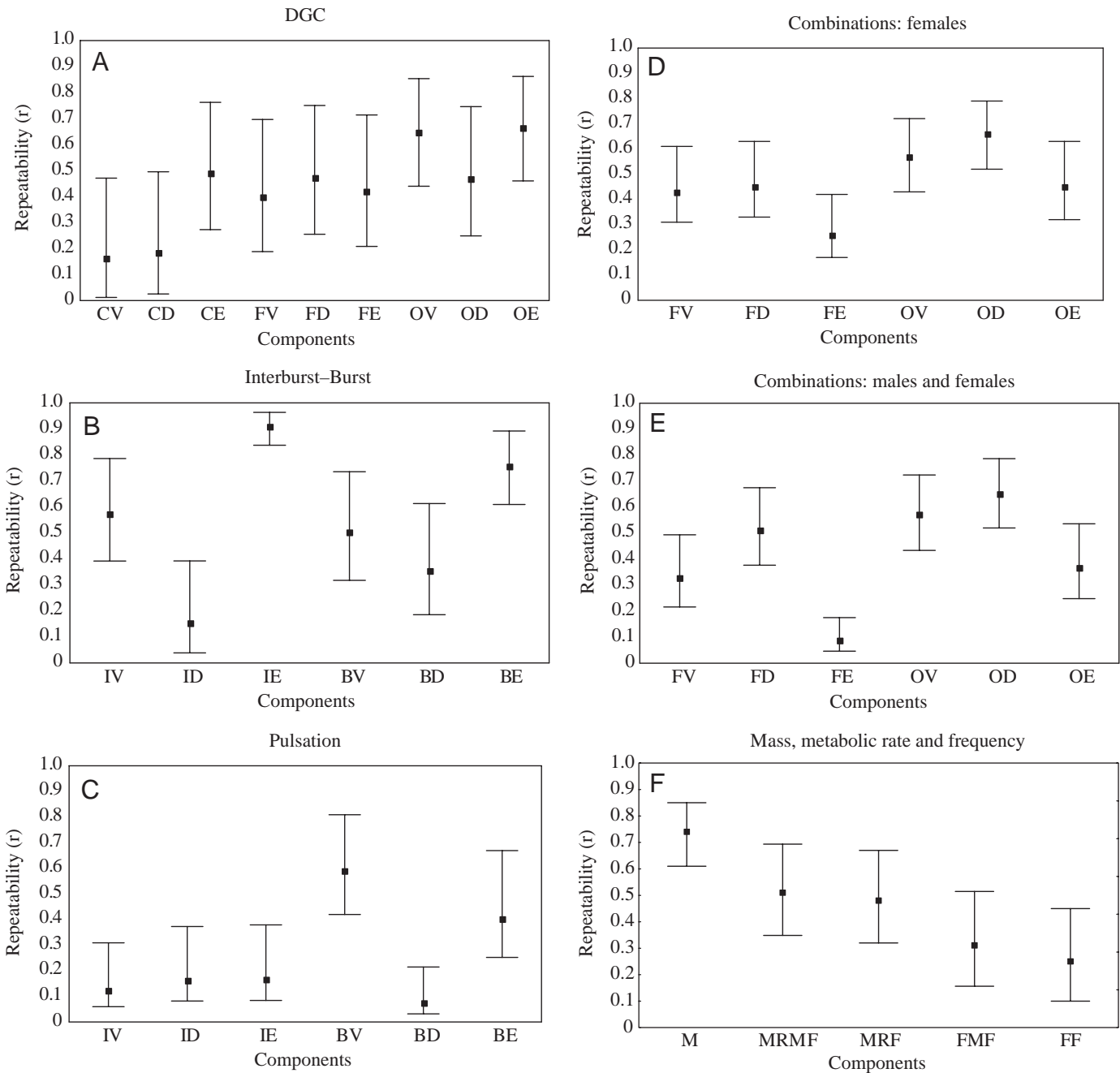


Fig. 2. Repeatability values (r), and their upper and lower 95% confidence limits for all the components of the cyclic patterns, as well as for the components across patterns. (A) Discontinuous gas exchange cycle pattern, (B) Interburst-Burst pattern, (C) Pulsation pattern, (D) the three gas exchange patterns combined for females only, (E) the three gas exchange patterns combined for males and females, (F) mass, metabolic rate and frequency. Mnemonics are as follows: in A–E, O = O-period, C = C-period, F = F-period, I = Interburst period, B = Burst period, E = emission rate, V = volume, D = duration; in F, M = mass, MRMF = metabolic rate for males and females combined, MRF = metabolic rate for females only, FMF = frequency for males and females combined, FF = frequency for females only.

of animals at rest with low metabolic rates. These high repeatabilities were not a consequence of pronounced differences between the genders, with the notable exception of the absence of a Pulsation pattern in males. However, the exclusion of body size variation did tend to result in lower repeatabilities. Although most studies first remove the effects of size variation before examining repeatability, it might also

be argued that this should not be done. This is most readily demonstrated in the context of metabolic rate variation. Several models have demonstrated the importance of metabolic rate for body size evolution (e.g. Kozłowski and Weiner, 1997), and Kozłowski and Gawelczyk (2002) have clearly shown that the major factors influencing optimal size are the size dependence of production rate (which is influenced by metabolic rate; see

Table 2. The distribution of variance of volume, duration and emission rates for periods (DGC, Interburst-Burst and Pulsation), frequency and metabolic rate

Component		Gender	Individual	Time of day	Trial	Error
DGC						
Closed period	log ₁₀ (volume + 2)	10.0	0.1	0	69.8***	20.1
	Duration	0	19.9	0	52.2***	27.9
	Emission rate	41.8*	0	19.1	28.0***	11.1
Flutter period	log ₁₀ volume	4.2	31.0*	0	30.0***	34.8
	Duration	0	17.4	29.1	19.5**	34.0
	Emission rate	31.8*	24.8**	3.4*	0	40.0
Open period	log ₁₀ volume	5.1	46.8	0	34.9***	13.2
	log ₁₀ duration	0	36.4	0	30.6***	33.0
	Emission rate	0	62.2**	0	9.8*	28.0
Interburst-Burst						
Interburst	log ₁₀ volume	26.3	12.0	42.9*	3.0	15.8
	Duration	0	0	39.3	10.0	50.7
	Emission rate	0	89.5***	2.6	0	7.9
Burst	log ₁₀ volume	0	25.3	24.2	22.3**	28.2
	Duration	0	2.1	14.2	37.9**	45.8
	log ₁₀ emission rate	0	66.6**	10.6	9.2**	14.2
Pulsation						
Interburst	log ₁₀ volume	—	0	18.5	9.2***	72.3
	log ₁₀ duration	—	2.8	23.6*	0.1	73.5
	log ₁₀ emission rate	—	13.8*	0	7.9**	78.3
Burst	log ₁₀ volume	—	44.5*	13.0	17.3***	25.2
	Duration	—	4.5	5.1	0.4	90.0
	Emission rate	—	29.0*	0	30.7***	40.3
Flutter period and Interbursts for the three cyclic patterns	Volume	0	18.7	26.5*	27.8***	27.0
	Duration	30.1*	28.1**	11.2	16.6***	13.9
	Emission rate	4.2	7.3*	8.7**	0	79.8
Burst and Open for the three cyclic patterns	log ₁₀ volume	54.2**	14.4*	17.2**	12.5***	1.8
	log ₁₀ duration	54.8**	20.8**	13.3**	9.4***	1.9
	log ₁₀ emission rate	0	32.8**	4.3	28.4***	34.5
Flutter period and Interbursts for the three cyclic patterns, Females	Volume	—	21.7	30.4*	26.6***	21.3
	Duration	—	30.3**	21.1*	17.7***	30.9
	Emission rate	—	18.3**	16.9*	1.5	63.3
Burst and Open for the three cyclic patterns, Females	log ₁₀ volume	—	43.5**	30.2**	23.1***	3.3
	log ₁₀ duration	—	56.0***	23.2**	17.4***	3.5
	log ₁₀ emission rate	—	34.2***	6.4	24.6***	34.8
Frequency		23.7*	17.6*	7.2	—	51.5
Metabolic rate (MR)	log ₁₀ MR	17.1	40.2***	0.9	—	41.8

Trial, between trials; Error, within a given trial.

Tabulated values are percentages of the total variance accounted for at each successive level (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Significance values did not change appreciably following Bonferroni correction.

Sibly and Calow, 1986) and the size dependence of mortality rate (which could be influenced by metabolic rate; see Chown and Gaston, 1999). Thus, it seems much more likely that

selection will act on the metabolic rate of an animal of a given size than on the residual variation of that trait once size has been taken into account. McNab (1999) arrived at a similar

conclusion, pointing out “...that total units of metabolism are the ecologically and evolutionary relevant units”. This argument can readily be applied to all of the other traits we examined, and indeed, in our view, to most other physiological and life history traits.

Therefore, we can conclude that for most of the characteristics we examined variation among individuals was typically significant, and often considerable. These results provide strong evidence that one of the conditions for considering natural selection an important process in the evolution both of gas exchange traits and standard metabolic rate has been met (Endler, 1986; Bech et al., 1999). They also provide a line of evidence, independent of that of comparative analyses, suggesting that variation in these traits among species and populations might well be adaptive. The only exceptions appeared to be the characteristics of the Closed period (in DGC) and Interburst period (in the other cyclic patterns), where repeatability was generally low. Thus, of the gas exchange characteristics examined, those associated with the Closed and Interburst periods are least likely to be the subject of selection. This finding is in keeping with evidence demonstrating that among species with discontinuous gas exchange cycles it is most often the F- and O-periods that vary in a way consistent with adaptive change (Lighton, 1988; Lighton et al., 1993a; Bosch et al., 2000; Duncan and Byrne, 2000; Duncan, 2003; Chown and Davis, 2003).

To date, no other studies have convincingly demonstrated consistent among-individual variation in standard metabolic rate and gas exchange characteristics in insects. Prior to this investigation, repeatability in one or more of these traits had only been examined on two occasions. Buck and Keister (1955) reported, but did not provide the statistics for, analyses of variance, which apparently revealed that among-individual variation in O-period volume in diapausing moth pupae was larger than that within individuals, but that several other characteristics of the DGC showed “about as much variation between different cycles of a single pupa as between pupae”. Much later, Chappell and Rogowitz (2000) reported repeatability of standard metabolic rate and DGC characteristics for two species of longicorn beetles (see also Rogowitz and Chappell, 2000), but included both species in their analysis without distinguishing them, factored out body size before the analyses, and considered their non-significant results a consequence of small sample size. Our work takes these initial, useful analyses a step further and demonstrates that, in general, both standard metabolic rate and gas exchange characteristics are significantly repeatable, so meeting one of the major requirements for selection.

Although repeatability estimates for physiological traits in insects and other arthropods are comparatively rare, our data are in keeping with the work that has been undertaken to date. For example, Chappell and Rogowitz (2000) found r values in the range 0.26–0.57 for DGC characteristics in the longicorn beetles they examined. Our values for DGC characteristics not only span a broader range, but unlike theirs were also all significant. This difference is particularly important in the

context of metabolic rate. Their analysis indicated a low and non-significant repeatability (0.38), whilst ours suggested that repeatability of metabolic rate was both higher (0.48–0.51) and significant. Nonetheless, it should be kept in mind that the inclusion of body mass as a covariate makes a considerable difference to the value of r in our analysis, but not to its significance (repeatability declined from 0.51 to 0.21 when the effects of mass were controlled for). Considering other physiological traits, in *Melanoplus* grasshoppers, repeatability of tethered flight duration varies between 0.6 and 0.7 (Kent and Rankin, 2001), whilst in *Rhizoglyphus* mites, repeatability of sperm competitive ability is much lower (0.22) (Radwan, 1998).

Our repeatability estimates for metabolic rate in *Perisphaeria* sp. were also well within the range of values typically found in vertebrates. For example, repeatability estimates ranged from 0.35 to 0.52 in breeding female kittiwakes measured over an interval of one year (Bech et al., 1999) and, in a variety of small mammals and birds, varied between 0.261 in meadow voles measured over an interval of 42 days (Berteaux et al., 1996) and 0.64 in kittiwakes measured over the course of a single day (Fyhn et al., 2001).

In conclusion, we have provided evidence that at least one of the prerequisites for natural selection for metabolic rate and gas exchange characteristics in insects is satisfied, and therefore that variation in these traits might be considered adaptive. Whilst our work does not provide conclusive evidence for adaptation in these traits, when considered in conjunction with selection experiments (reviewed in Gibbs, 1999), and comparative analyses (reviewed in Chown and Gaston, 1999), it does make the argument for adaptive variation more compelling than it has been. In the past, investigations of variation in gas exchange characteristics in particular have suffered from an unduly Panglossian approach.

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Supplemental Data

Appendix 1A. ANOVA table used to calculate the repeatability values and their upper and lower 95% confidence limits for each of the components of the cyclic patterns, as well as comparable components across all three of the cyclic patterns, metabolic rate, frequency and body mass

Components	Source of variation	d.f.	SS	MS	F-ratio	P	r	Lower confidence limit	Upper confidence limit
DGC volume									
C-period [log ₁₀ (+2)]	Among groups	8	1.01×10 ⁻⁷	1.26×10 ⁻⁸	2.29	0.03	0.16	0.01	0.47
	Within groups	53	2.92×10 ⁻⁷	5.51×10 ⁻⁹					
	Total	61	3.93×10 ⁻⁷						
F-period (log ₁₀)	Among groups	8	4.32	0.54	5.37	5.78×10 ⁻⁵	0.40	0.19	0.70
	Within groups	53	5.33	0.10					
	Total	61	9.65						
O-period (log ₁₀)	Among groups	8	1.76	0.22	13.13	3.2×10 ⁻¹⁰	0.65	0.44	0.85
	Within groups	53	0.89	0.02					
	Total	61	2.65						
DGC duration									
C-period	Among groups	8	1.58×10 ⁶	1.97×10 ⁵	2.49	0.02	0.18	0.03	0.50
	Within groups	53	4.21×10 ⁶	7.94×10 ⁴					
	Total	61	5.79×10 ⁶						
F-period	Among groups	8	2.19×10 ⁶	2.74×10 ⁵	6.92	3.34×10 ⁻⁶	0.47	0.26	0.75
	Within groups	53	2.09×10 ⁶	3.95×10 ⁴					
	Total	61	4.28×10 ⁶						
O-period (log ₁₀)	Among groups	8	0.94	0.12	6.77	4.41×10 ⁻⁶	0.47	0.25	0.75
	Within groups	53	0.92	0.02					
	Total	61	1.86						
DGC emission rate									
C-period	Among groups	8	1.42×10 ⁻⁸	1.78×10 ⁻⁹	7.38	1.52×10 ⁻⁶	0.49	0.27	0.76
	Within groups	53	1.28×10 ⁻⁸	2.41×10 ⁻¹⁰					
	Total	61	2.70×10 ⁻⁸						
F-period (log ₁₀)	Among groups	8	1.13	0.14	5.81	2.55×10 ⁻⁵	0.42	0.21	0.71
	Within groups	53	1.29	0.02					
	Total	61	2.42						
O-period	Among groups	8	3.38×10 ⁻⁶	4.32×10 ⁻⁷	14.07	9.93×10 ⁻¹¹	0.66	0.46	0.86
	Within groups	53	1.59×10 ⁻⁶	3.01×10 ⁻⁸					
	Total	61	4.97×10 ⁻⁶						
Interburst–Burst volume									
Burst (log ₁₀)	Among groups	10	5.23	0.52	10.09	2.76×10 ⁻¹¹	0.50	0.32	0.74
	Within groups	93	4.82	0.05					
	Total	103	10.05						
Interburst (log ₁₀)	Among groups	10	21.99	2.20	13.22	4.14×10 ⁻¹⁴	0.57	0.39	0.79
	Within groups	93	15.46	0.17					
	Total	103	37.45						
Interburst–Burst duration									
Burst	Among groups	10	3.39×10 ⁶	3.39×10 ⁵	5.93	6.92×10 ⁻⁷	0.35	0.19	0.61
	Within groups	93	5.31×10 ⁶	5.71×10 ⁴					
	Total	103	8.70×10 ⁶						
Interburst	Among groups	10	6.74×10 ⁶	6.74×10 ⁵	2.64	7.00×10 ⁻³	0.15	0.04	0.39
	Within groups	93	2.37×10 ⁷	2.55×10 ⁵					
	Total	103	3.05×10 ⁷						
Interburst–Burst emission rate									
Burst (log ₁₀)	Among groups	10	5.30	0.53	29.28	1.51×10 ⁻²⁴	0.76	0.61	0.89
	Within groups	93	1.68	0.02					
	Total	103	6.98						
Interburst	Among groups	10	4.26×10 ⁻⁶	4.26×10 ⁻⁷	92.14	0	0.91	0.84	0.96
	Within groups	93	4.30×10 ⁻⁷	4.62×10 ⁻⁹					
	Total	103							

Components	Source of variation	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i>	r	Lower confidence limit	Upper confidence limit
Pulsation volume									
Burst (log ₁₀)	Among groups	8	13.75	1.72	83.15	0	0.59	0.42	0.81
	Within groups	541	11.19	0.02					
	Total	549	24.94						
Interburst (log ₁₀)	Among groups	8	18.80	2.35	9.09	9.0×10 ⁻¹²	0.12	0.06	0.31
	Within groups	541	1.39×10 ²	0.26					
	Total	549	1.58×10 ²						
Pulsation duration									
Burst	Among groups	8	4.33×10 ³	5.41×10 ²	5.68	6.0×10 ⁻⁷	0.08	0.03	0.21
	Within groups	541	5.16×10 ⁴	95.38					
	Total	549	5.59×10 ⁴						
Interburst (log ₁₀)	Among groups	8	42.19	5.27	11.95	9.0×10 ⁻¹⁶	0.16	0.08	0.37
	Within groups	541	2.39×10 ²	0.44					
	Total	549	2.81×10 ²						
Pulsation emission rate									
Burst (log ₁₀)	Among groups	8	5.51	0.69	39.79	0	0.40	0.25	0.67
	Within groups	541	9.37	0.02					
	Total	549	14.88						
Interburst (log ₁₀)	Among groups	8	13.42	1.68	12.27	3.0×10 ⁻¹⁶	0.16	0.08	0.38
	Within groups	541	73.66	0.14					
	Total	549	87.08						
Metabolic rate									
(males and females) (log ₁₀)	Among groups	19	3.47	0.18	6.25	2.01×10 ⁻⁹	0.51	0.35	0.69
	Within groups	80	2.33	0.03					
	Total	99	5.80						
(females) (log ₁₀)	Among groups	16	2.57	0.16	5.67	1.57×10 ⁻⁷	0.48	0.32	0.67
	Within groups	68	1.93	0.03					
	Total	84	4.50						
Frequency (males and females)	Among groups	19	1.98×10 ⁴	1.04×10 ³	3.37	5.9×10 ⁻⁵	0.31	0.15	0.52
	Within groups	87	2.69×10 ⁴	3.10×10 ²					
	Total	106	4.68×10 ⁴						
(females)	Among groups	16	1.58×10 ⁴	9.87×10 ²	2.78	0.001	0.25	0.10	0.45
	Within groups	76	2.69×10 ⁴	3.55×10 ²					
	Total	92	4.27×10 ⁴						
Mass (males and females)	Among groups	19	0.64	0.03	15.12	3.93×10 ⁻¹⁹	0.74	0.61	0.85
	Within groups	80	0.18	2.0×10 ⁻³					
	Total	99	0.82						
Flutter period and interbursts for the three cyclic patterns (males and females)									
Volume	Among groups	19	2.54×10 ⁻⁴	1.34×10 ⁻⁵	24.13	0	0.33	0.22	0.49
	Within groups	754	4.17×10 ⁻⁴	5.53×10 ⁻⁷					
	Total	773	6.71×10 ⁻⁴						
Duration	Among groups	19	5.87×10 ⁷	3.09×10 ⁶	50.82	0	0.51	0.38	0.68
	Within groups	754	4.59×10 ⁷	6.08×10 ⁴					
	Total	773	10.46×10 ⁷						
Emission rate	Among groups	19	6.64×10 ⁻⁶	3.49×10 ⁻⁷	5.65	2.88×10 ⁻¹³	0.09	0.05	0.18
	Within groups	754	4.66×10 ⁻⁵	6.18×10 ⁻⁸					
	Total	773	5.32×10 ⁻⁵						

Components	Source of variation	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i>	r	Lower confidence limit	Upper confidence limit
Open period and bursts for the three cyclic patterns (males and females)									
Volume (log ₁₀)	Among groups	19	1.87×10 ²	9.86	63.99	0	0.57	0.43	0.72
	Within groups	754	1.16×10 ²	0.15					
	Total	773	3.04×10 ²						
Duration (log ₁₀)	Among groups	19	2.10×10 ²	11.0	89.83	0	0.65	0.52	0.79
	Within groups	754	92.7	0.12					
	Total	773	3.03×10 ²						
Emission rate (log ₁₀)	Among groups	19	10.34	0.55	28.52	0	0.37	0.25	0.54
	Within groups	754	14.47	0.02					
	Total	773	24.81						
Flutter period and interbursts for the three cyclic patterns (females)									
Volume	Among groups	16	2.56×10 ²	16.01	31.52	0	0.43	0.31	0.61
	Within groups	700	3.55×10 ²	0.51					
	Total	716	6.11×10 ²						
Duration	Among groups	16	3.09×10 ²	19.32	33.99	0	0.45	0.33	0.63
	Within groups	700	3.98×10 ²	0.57					
	Total	716	7.07×10 ²						
Emission rate	Among groups	16	31.43	1.96	15.12	0	0.26	0.17	0.42
	Within groups	700	90.88	0.13					
	Total	716	1.22×10 ²						
Open period and bursts for the three cyclic patterns (females)									
Volume (log ₁₀)	Among groups	16	1.39×10 ²	8.71	52.87	0	0.57	0.43	0.72
	Within groups	700	1.15×10 ²	0.17					
	Total	716	2.54×10 ²						
Duration (log ₁₀)	Among groups	16	1.60×10 ²	10.01	77.30	0	0.66	0.53	0.79
	Within groups	700	90.64	0.13					
	Total	716	2.50×10 ²						
Emission rate (log ₁₀)	Among groups	16	10.46	0.65	33.45	0	0.45	0.32	0.63
	Within groups	700	13.68	0.02					
	Total	716	24.14						
Data were log ₁₀ transformed in some cases to normalize the distributions. Repeatabilities were calculated for females unless indicated otherwise. Sample sizes for individuals used for each pattern are given in Table 1.									

Appendix 1B. ANOVA table used to calculate the repeatability values and their upper and lower 95% confidence limits for each of the components of the cyclic patterns, as well as comparable components across all three of the cyclic patterns, metabolic rate, frequency and body mass, with body mass included as a covariate

Components	Source of variation	d.f.	SS	MS	F-ratio	P	r	Lower confidence limit	Upper confidence limit
DGC volume									
F-period	Among groups	8	2.05	0.26	3.03	0.007	0.23	0.06	0.55
	Within groups	52	4.40	0.08					
	Total	60	6.45						
DGC duration									
C-period	Among groups	8	2.02×10 ⁶	2.53×10 ⁵	3.59	0.002	0.28	0.10	0.60
	Within groups	52	3.66×10 ⁶	7.03×10 ⁴					
	Total	60	5.68×10 ⁶						
F-period	Among groups	8	1.50×10 ⁶	1.87×10 ⁵	5.05	1.14×10 ⁻⁴	0.38	0.17	0.68
	Within groups	52	1.93×10 ⁶	3.71×10 ⁴					
	Total	60	3.43×10 ⁶						
Interburst–Burst volume									
Burst (log ₁₀)	Among groups	10	1.50	0.15	3.63	4.18×10 ⁻⁴	0.22	0.09	0.48
	Within groups	92	3.80	0.04					
	Total	102	5.30						
Interburst–Burst duration									
Burst	Among groups	10	3.86×10 ⁶	3.86×10 ⁵	7.34	1.9×10 ⁻⁸	0.41	0.24	0.67
	Within groups	92	4.84×10 ⁶	5.26×10 ⁴					
	Total	102	8.70×10 ⁶						
Interburst–Burst emission rate									
Interburst	Among groups	10	1.85×10 ⁻⁶	1.85×10 ⁻⁷	42.73	2.85×10 ⁻³⁰	0.82	0.70	0.92
	Within groups	92	3.99×10 ⁻⁷	4.34×10 ⁻⁹					
	Total	102	1.27×10 ²						
Pulsation volume									
Burst (log ₁₀)	Among groups	8	14.45	1.8	93.68	0	0.62	0.42	0.81
	Within groups	540	10.41	0.02					
	Total	548	24.86						
Interburst (log ₁₀)	Among groups	8	20.86	2.61	10.27	2.08×10 ⁻¹³	0.14	0.07	0.34
	Within groups	540	1.37×10 ²	0.25					
	Total	548	1.58×10 ²						
Pulsation duration									
Burst	Among groups	8	4.14×10 ³	5.18×10 ²	5.49	1.12×10 ⁻⁶	0.07	0.03	0.21
	Within groups	541	5.10×10 ⁴	94.38					
	Total	549	5.52×10 ⁴						
Interburst (log ₁₀)	Among groups	8	47.40	5.93	13.99	1.48×10 ⁻¹⁸	0.19	0.08	0.37
	Within groups	540	2.30×10 ²	0.42					
	Total	548	2.77×10 ²						
Emission rate									
Interburst (log ₁₀)	Among groups	8	13.37	1.67	12.59	1.23×10 ⁻¹⁶	0.17	0.10	0.41
	Within groups	540	71.41	0.13					
	Total	548	84.78						
Metabolic rate									
(males and females) (log ₁₀)	Among groups	19	1.97	0.05	2.39	0.004	0.22	0.07	0.42
	Within groups	79	1.69	0.02					
	Total	98	2.66						
(females) (log ₁₀)	Among groups	16	0.92	0.06	3.05	7.14×10 ⁻⁴	0.29	0.12	0.52
	Within groups	67	1.27	0.02					
	Total	83	2.19						

Components	Source of variation	d.f.	SS	MS	<i>F</i> -ratio	<i>P</i>	r	Lower confidence limit	Upper confidence limit
Frequency									
(males and females)	Among groups	19	2.11×10 ⁴	1.11×10 ³	3.76	1.25×10 ⁻⁵	0.35	0.19	0.55
	Within groups	86	2.55×10 ⁴	2.96×10 ²					
	Total	105	4.66×10 ⁴						
(females)	Among groups	16	1.71×10 ⁴	1.07×10 ³	3.16	3.96×10 ⁻⁴	0.29	0.13	0.51
	Within groups	75	2.54×10 ⁴	3.39×10 ²					
	Total	91	4.25×10 ⁴						
Flutter period and interbursts for the three cyclic patterns (males and females)									
Volume	Among groups	19	2.54×10 ⁻³	1.34×10 ⁻⁴	42.45	0	0.47	0.35	0.63
	Within groups	753	2.4×10 ⁻³	3.19×10 ⁻⁶					
	Total	772	4.94×10 ⁻³						
Duration	Among groups	19	6.12×10 ⁷	3.22×10 ⁶	54.35	0	0.53	0.41	0.68
	Within groups	753	4.47×10 ⁷	5.90×10 ⁴					
	Total	772	10.59×10 ⁷						
Emission rate	Among groups	19	2.63×10 ⁻⁵	1.38×10 ⁻⁶	15.60	0	0.24	0.16	0.38
	Within groups	753	6.67×10 ⁻⁵	8.86×10 ⁻⁸					
	Total	772	9.30×10 ⁻⁵						
Open period and bursts for the three cyclic patterns (males and females)									
Volume (log ₁₀)	Among groups	19	1.29×10 ²	6.81	69.04	0	0.59	0.47	0.73
	Within groups	753	74.24	0.10					
	Total	772	2.03×10 ²						
Duration (log ₁₀)	Among groups	19	2.20×10 ²	11.6	138.12	0	0.74	0.64	0.85
	Within groups	753	63.22	0.08					
	Total	772	2.83×10 ²						
Flutter period and interbursts for the three cyclic patterns (females)									
Volume	Among groups	16	3.95×10 ²	24.68	43.14	0	0.51	0.39	0.68
	Within groups	700	3.99×10 ²	0.57					
	Total	716	6.11×10 ²						
Duration	Among groups	16	3.45×10 ²	21.54	38.29	0	0.48	0.36	0.66
	Within groups	700	3.93×10 ²	0.56					
	Total	716	7.07×10 ²						
Emission rate	Among groups	16	34.94	2.18	14.23	0	0.25	0.16	0.41
	Within groups	700	90.88	0.15					
	Total	716	1.07×10 ²						
Open period and bursts for the three cyclic patterns (females)									
Volume (log ₁₀)	Among groups	16	1.10×10 ²	6.94	74.25	0	0.65	0.52	0.79
	Within groups	700	65.39	0.09					
	Total	716	1.75×10 ²						
Duration (log ₁₀)	Among groups	16	1.67×10 ²	10.47	121.93	0	0.75	0.65	0.86
	Within groups	700	60.13	0.09					
	Total	716	2.27×10 ²						
Data were log ₁₀ transformed in some cases to normalize the distributions.									
Repeatabilities were calculated for females unless indicated otherwise.									
Sample sizes for individuals used for each pattern are given in Table 1.									