

# Phylogenetic clustering of wingbeat frequency and flight-associated morphometrics across insect orders

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6 Flight strategies between insect orders

7

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17

18 **KEY WORDS:** Comparative morphology, wing loading, body mass, flight strategy, insect  
19 evolution, high-speed filming.

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22

23 **Summary statement**

24 Insect flight strategy varies between orders but is generally well conserved within orders, this  
25 has important evolutionary and ecological implications at high taxonomic levels.

26

27 **ABSTRACT**

28 Wingbeat frequency in insects is an important variable in aerodynamic and energetic  
29 analyses of insect flight and has been studied previously on a family- or species-level basis.  
30 Meta-analyses of these studies have found order-level patterns that suggests flight strategy  
31 is moderately well conserved phylogenetically. Studies incorporated into these meta-  
32 analyses, however, use variable methodologies across different temperatures that may  
33 confound results and phylogenetic patterns. Here, a high-speed camera was used to  
34 measure wingbeat frequency in a wide variety of species ( $n = 102$ ) in controlled conditions to  
35 determine the validity of previous meta-analyses that show phylogenetic clustering of flight  
36 strategy and to identify new evolutionary patterns between wingbeat frequency, body mass,  
37 wing area, wing length, and wing loading at the order level. All flight-associated  
38 morphometrics significantly affected wingbeat frequency. Linear models show that wing area  
39 explained the most amount of variation in wingbeat frequency ( $R^2 = 0.59$ ,  $p = <0.001$ ), whilst  
40 body mass explained the least ( $R^2 = 0.09$ ,  $p = <0.01$ ). A multiple regression model  
41 incorporating both body mass and wing area was the best overall predictor of wingbeat  
42 frequency ( $R^2 = 0.84$ ,  $p = <0.001$ ). Order-level phylogenetic patterns across relationships  
43 were consistent with previous studies. Thus, the present study provides experimental  
44 validation of previous meta-analyses and provides new insights into phylogenetically  
45 conserved flight strategies across insect orders.

46

47

## 48 INTRODUCTION

49 Wingbeat frequency in insects varies with body mass and wing area within and between  
50 species (Byrne et al., 1988; Dudley, 2000), from 5.5 Hz in the helicopter damselfly  
51 *Megalopterus caerulatus* (Rüppell and Fincke, 1989) to over 1000 Hz in a ceratopogonid  
52 *Forcipomyia* sp. midge (Sotavalta, 1953). How frequently an insect beats its wings is an  
53 important variable when considering the biomechanics and physiology of insect flight  
54 (Ellington, 1984a-f; Dudley, 2000; Alexander, 2002; Vogel, 2013). For any given body mass,  
55 variables such as wing length, wing area, wing loading (body mass/wing area), wingbeat  
56 frequency and stroke amplitude can differ substantially and affect the energetics and  
57 biomechanics of insect flight, which is usually linked to evolutionary history (Byrne et al., 1988).  
58 Stroke amplitude, the angle between the points of wing reversal, has been shown to vary  
59 between taxa, from 66° in syrphids (Ellington, 1984c) to 180° in beetles (Atkins, 1960) and  
60 moths (Wilkins, 1991) and may vary significantly during a single flight as shown in dragonflies  
61 (Alexander, 1986), orchid bees (Dudley, 1995; Dillon and Dudley, 2004), and fruit flies  
62 (Lehmann and Dickinson, 1998; Fry et al., 2003). Though undeniably important to  
63 understanding insect flight strategy and aerodynamics, stroke amplitude was not measured in  
64 the current study. This is because although both wingbeat frequency and stroke amplitude  
65 change during a single flight, wingbeat frequency is kept relatively constant because of the  
66 high energetic cost of deviating from the resonant frequency of the flight apparatus (Dudley,  
67 2000). Conversely, stroke amplitude may be altered extremely rapidly to change direction (Fry  
68 et al., 2003) or flight mode e.g. from hovering to forward flight (Dillon and Dudley, 2004).  
69 Because of this variability, stroke amplitude is likely to be a slightly less reliable indicator of  
70 flight strategy than wingbeat frequency.

71 The variables that influence the energetic and biomechanical aspects of flight could be used  
72 to broadly characterize flight strategies between different orders of insects. Typically, higher  
73 wingbeat frequencies are associated with insects of smaller size, to overcome the increasingly  
74 viscous forces of the air present at small spatial scales, represented by low Reynolds numbers

75 Re in the order of 10-100 in the smallest insects (Ellington, 1999; Wang, 2005), and to better  
76 control their direction in a windswept world (Vogel, 2013). Furthermore, frequencies of >100  
77 Hz are facilitated by asynchronous, or myogenic, flight muscle present in endopterygote  
78 (Coleoptera, Diptera, Hymenoptera) and exopterygote (Thysanoptera and Hemiptera) groups  
79 (Dudley, 2000) where one nerve impulse can initiate several wingbeats through stretch-  
80 activation caused by mechanical loading on the wing (Pringle, 1967). Thus, the highest  
81 wingbeat frequencies are found in smaller members of these groups (Byrne et al., 1988).  
82 Members from other orders possess large wings that they beat at lower frequencies relative  
83 to other insects of comparable body mass e.g. Lepidoptera and Neuroptera (Dudley, 2000)  
84 and Orthoptera (Snelling *et al.*, 2012, 2017). Larger wings can produce more force per beat  
85 than smaller wings, and therefore fewer beats are needed per unit time. Moreover, larger  
86 wings afford lower wing loadings for insects of the same body mass, so wingbeat frequency  
87 may be reduced further. It is possible then that flight-associated morphometrics, such as wing  
88 area, can be used to predict wingbeat frequency and characterize flight for different groups of  
89 insect using the same stroke strategy (i.e. conventional wingbeat or clap-fling).

90

91 Flight morphology and wingbeat frequency are dependent on the aerodynamic needs of the  
92 insect according to their ecological niche and oxygen consumption increases with wingbeat  
93 frequency (Bartholomew and Casey, 1978). Species with similar wing loadings may have  
94 different wingbeat frequencies based on the flight velocity required to fulfil their ecological role.  
95 Substantial variation in wingbeat frequency and flight morphology as a product of ecological  
96 needs also exists within orders, such as the differences between Sphingidae and Nymphalidae  
97 (Lepidoptera), where sphingids have small wings, rapid beat frequencies and very fast flight,  
98 whilst nymphalids have much larger wings and lower wingbeat frequencies, usually flying at  
99 overall slower speeds (Dudley, 2000). Such variation could conceal relationships between  
100 flight-associated morphometrics and wingbeat frequency across higher taxonomic levels,  
101 decreasing the overall level of phylogenetic grouping of flight strategy.

103 Order-level taxonomic relationships to these flight-associated morphometrics have been  
104 studied before (see Byrne et al., 1988; Dudley, 2000) but meta-analyses suffer from  
105 differences in both ambient conditions and methods of measuring wingbeat frequency  
106 between studies that may confuse relationships. For example, acoustic methods,  
107 stroboscopes, and high-speed cameras were used across studies incorporated into Dudley  
108 (2000) and Byrne et al.'s (1988) meta-analyses. Chadwick (1939) suggested stroboscopic  
109 methods are difficult to use effectively to glean kinematic data in insects because of the slight  
110 variations in wingbeat frequency and movements of the specimen during testing, making  
111 visualisation of the wing at the frequency of the strobe light challenging and Unwin and  
112 Ellington (1979) suggested picking up acoustic signals of smaller species difficult even with  
113 highly sensitive microphones. Both stroboscopic (e.g. Chen *et al.*, 2014) and acoustic (e.g.  
114 Raman *et al.*, 2007) methods have, however, been used successfully to measure wingbeat  
115 frequency in insects since advancement in the quality of measurement instruments (i.e. optical  
116 tachometers and microphones). Nevertheless, stroboscopic/optical and acoustic methods are  
117 not absolute measures of wingbeat frequency. High-speed cameras, in contrast, allow the  
118 recording of a temporally magnified visual depiction of the motion of insect wings. The  
119 reliability of the methods used in studies incorporated into important meta-analyses varies  
120 because of the problems faced when the technology was less well developed. Furthermore,  
121 temperatures vary from 7-25°C between studies used in previous meta-analyses. Insect  
122 wingbeat frequency has been shown to increase with higher temperatures (Unwin and Corbet,  
123 1984; Oertli, 1989) and, therefore, meta-analyses of the relationships between measured  
124 characteristics may be confounded. An experimental approach using high-speed cameras in  
125 controlled conditions recording flight in species across several orders has not previously been  
126 done. Using common UK species of insect, relationships between body mass, wing length,  
127 wing area, wing loading and wingbeat frequency were investigated to determine if flight  
128 strategies could be broadly characterized between different orders of insect.

129

## 130 **MATERIALS AND METHODS**

### 131 Study specimens

132 Adult insects were caught using either sweep net (EFE & GB Nets, Totnes, Devon, UK –  
133 handle length = 0.3 m; net diameter = 0.5 m; net depth = 0.7 m), pooter (NHBS, Totnes, Devon,  
134 UK – barrel diameter = 30 mm, length = 55 mm, suction tube diameter = 5mm), or hand  
135 collected into small sampling pots (varying sizes) within a 20 km radius of Harper Adams  
136 University, Shropshire, UK (latitude ~52.772°N, longitude ~2.411°W) over the course of June  
137 and July, 2017. In total, 112 specimens across 102 species in 10 orders were used in the  
138 analysis.

139

### 140 Filming area and conditions

141 Filming took place inside a Fitotron® Standard Growth Room unit (Weiss Technik, Ebbw Vale,  
142 UK) set to a constant 20°C and 60% relative humidity. This temperature was selected to film  
143 flight behaviour of insects in standardised conditions and is unlikely to represent an extreme  
144 for tested species, which were all collected during summer days and therefore active within  
145 ~±5°C of the ambient temperature used. Ambient lighting intensity was 280  $\mu\text{mol m}^{-2} \text{s}^{-1}$  inside  
146 the Fitotron® unit and no other external light source was used. A flight box made of 6  
147 transparent Perspex® panels, measuring 30x30x30 cm once constructed, was used to contain  
148 flights of the specimens whilst filming. Study specimens were introduced to the flight box either  
149 via a 2.5 cm diameter aperture made in the centre of one of the panels by offering up an open  
150 test tube containing a specimen, or, for larger specimens, the entire panel could be removed  
151 and the specimen introduced.

152

### 153 Filming procedure

154 Each specimen was filmed 2-5 times using an FPS1000HD monochromatic high-speed  
155 camera (The Slow Motion Camera Company, London, UK). Specimens were filmed each time  
156 during free flight. For each flight recorded, the camera was handheld in order to track insects  
157 in free flight. This helped increase total length of each video and thus more reliably count  
158 wingbeats. Across videos, insects were filmed from various angles, but this did not affect video  
159 analysis. Sufficient video footage was gathered in <10 minutes for each specimen.

160

#### 161 Morphological measurements

162 Specimens were killed in a killing jar (a jar with a base of plaster of Paris to which ethyl acetate  
163 was intermittently added when needed) after the last video was recorded and immediately  
164 weighed using a precision balance (Cahn C-33 Microbalance, Cerritos, California, USA). The  
165 functional wing (in insects with only one pair of functional wings e.g. Diptera and Coleoptera)  
166 or wing couple on the right side (i.e. the fore- and hindwing on the right side of the insect  
167 viewed dorsally) was removed by dissection under a stereo microscope and forewing length  
168 (henceforth wing length) was measured using a pair of digital calipers (0.01 mm precision),  
169 measured from the base of the forewing to the most distal tip. A photo was taken of the  
170 dissected wing couple using a microscope camera making sure the wings were perpendicular  
171 to the camera lens. Wing area was measured in ImageJ version 1.49 (Schindelin et al., 2012)  
172 by using the photo and following the ImageJ process for measuring leaf area (Reinking, 2007)  
173 as in previous studies on insect wings (e.g. Outomuro *et al.*, 2013); the wing area value was  
174 multiplied by 2 to quantify total wing area assuming symmetry. Wing loading was determined  
175 by dividing body mass by total wing area.

176

#### 177 Video analysis

178 Videos were first converted into a viewable format using ImageJ, where video frames-per-  
179 second (FPS) was then altered to allow individual wingbeats to be clearly visible. A wingbeat

180 was judged to be both a full downstroke and full upstroke, terminating at pronation before the  
181 next wingbeat (Fig. 1), and in all groups except for Odonata, fore- and hindwings beat at the  
182 same time. For odonates, forewing and hindwing pairs were measured separately then the  
183 mean was calculated; the difference between the wing pairs did not exceed 2 beats in any of  
184 the odonate specimens. Sections of videos were carefully selected to represent free-flight,  
185 omitting wingbeats immediately after take-off until a more regular rhythm was observed, which  
186 was usually more rapid. The number of wingbeats  $n_v$  during free-flight was counted for each  
187 video. Equation 1 was used to determine the wingbeat frequency  $n$  (Hz) from each video  
188 where  $t_v$  is the length of the video in seconds, and  $f_m$  is the multiplication factor (the factor that  
189 describes by how much time is magnified in each video), which is calculated by dividing filming  
190 FPS by video playback FPS. All species were filmed at 1000 FPS except for 6 species of  
191 nematoceran Diptera, which were filmed at 2000 FPS.

192

### 193 Statistical Analysis

194 Statistical analysis was conducted using R version 3.4.1. “Single Candle” (R Core Team,  
195 2017) with packages MASS (Venebles and Ripley, 2002), ggplot2 (Wickham, 2009), caret  
196 (Kuhn, 2017), hydroGOF (Zambrano-Bigiarini, 2014), relaimpo (Grömping, 2006), and  
197 gridExtra (Auguie, 2016) used. Both simple and multiple linear regression analyses were  
198 conducted to determine the relationships between morphological variables and wingbeat  
199 frequency. Data were log-transformed to reduce skew and allow analysis by linear regression.  
200 To better measure the level of phylogenetic clustering of flight strategy, a principal component  
201 analysis (PCA) was conducted.

202

## 203 RESULTS

204 Morphometric data

205 Table 1 compiles the range and mean statistics for morphological measurements and  
206 wingbeat frequency in each sampled order. Across all 112 specimens, wingbeat frequency  
207 covered a range between 12.468 to 557.351 Hz ( $\bar{x}$  = 121.588, sd = 92.679, se = 8.767), body  
208 mass a range of 0.0003 to 2.245 g ( $\bar{x}$  = 0.097, sd = 0.256, se = 0.024), wing length a range of  
209 0.172 to 5.214 cm ( $\bar{x}$  = 1.184, sd = 0.919, se = 0.087), wing area a range of 0.022 to 23.362  
210 cm<sup>2</sup> ( $\bar{x}$  = 2.022, sd = 4.088, se = 0.386), and wing loading a range of 0.0028 to 0.245 g/cm<sup>2</sup>  
211 ( $\bar{x}$  = 0.061, sd = 0.059, se = 0.006).

212

213 These values show that some orders were better sampled than others and in some cases this  
214 is reflected in the ranges of different variables recorded. Average values, however, are  
215 generally in agreement with expected values for UK insects. Synchronous fliers  
216 (Ephemeroptera, Lepidoptera, Mecoptera, Neuroptera, Odonata, Trichoptera) were overall  
217 less well sampled than asynchronous fliers (Coleoptera, Diptera, Hemiptera, Hymenoptera)  
218 and should be similarly taken into account when considering ranges of variables.

219

220 Relationships between morphometrics and wingbeat frequency

221 Figure 2 shows the relevant linear relationships between the log<sub>10</sub> transformed morphometric  
222 data. Of these, wing area (cm<sup>2</sup>) was the best predictor of wingbeat frequency ( $R^2$  = 0.59, p =  
223 <0.001). The strongest overall linear relationship between all morphometric measurements  
224 was between wing length (cm) and wing area ( $R^2$  = 0.93, p = <0.001). Body mass explained  
225 only 9% of the variation in wingbeat frequency across specimens ( $R^2$  = 0.09, p = <0.01) and  
226 represented the poorest predictor of wingbeat frequency across the measured morphometrics.  
227 Taxonomic distribution on the graphs (Fig. 2, especially A-D) sees a diffuse but identifiable  
228 clustering of the orders most intensively sampled, suggesting that orders may broadly adhere  
229 to a specific strategy and some new phylogenetic clustering between wingbeat frequency,  
230 wing area, wing length, and wing loading have been revealed where previous meta-analyses

231 focussed solely on taxonomic grouping in relation to wingbeat frequency and body mass. For  
232 example, looking at Figure 2D, Hymenoptera are quite closely clustered at the higher end of  
233 the wing loading range and the upper-middle range of wingbeat frequency, denoting that most  
234 hymenopterans sampled have small wings relative to their body mass, which they beat at  
235 above average frequencies compared to other orders.

236

237 A multiple regression model using  $\log_{10}$  values of wing area ( $\beta = -0.034$ ,  $p = <0.001$ ) and body  
238 mass ( $\beta = 0.001$ ,  $p = <0.001$ ), with a fit of  $R^2 = 0.84$  was the best overall model predicting  
239 wingbeat frequency in insects: wingbeat frequency = (wing area \* -0.77) + (body mass \* 0.37)  
240 + 5.56.

241

242 A dominance analysis (Azen and Budescu, 2003; Grömping, 2006) was conducted to  
243 determine the relative importance of the explanatory variables to the response variable in the  
244 model and showed that body mass and wing area explained 17.3% and 67.2% of the change  
245 in wingbeat frequency, respectively.

246

247 Phylogenetic clustering of flight strategy

248 Wingbeat frequency and morphometric variables for all specimens were reduced to a dataset  
249 summarising the variance and covariance between each using a Principle Component  
250 Analysis (PCA). Initial eigenvalues indicated the first two principle components explained  
251 64.039% and 33.291% of the data, respectively (97.331% cumulatively). Dimension 1 is  
252 mainly loaded towards wing area (30.417%), wing length (30.073%), body mass (22.882%),  
253 and wing loading (16.388%), whereas Dimension 2 is mainly loaded towards wing loading  
254 (58.325%), wingbeat frequency (24.979%), and body mass (15.821%). Having determined the  
255 loadings, a PCA biplot was produced to view the relationship between variables and whether

256 insect orders were clustered on the graph. Figure 3 reveals that most insect specimens are in  
257 close proximity to their associated centroid (the mean value of the x and y coordinates for  
258 each order), shown by the ellipses, which represent one standard deviation along each axis  
259 and is rotated toward the direction of maximum spread of the point cloud. This strongly  
260 suggests that flight strategy is well conserved at the order level.

261

## 262 **DISCUSSION**

263 Phylogenetic clustering apparent in this study broadly agrees with results from previous meta-  
264 analyses (Byrne et al., 1988; Dudley, 2000). Past research looking at differences in wingbeat  
265 frequency and flight-associated morphometrics are, therefore, experimentally validated by the  
266 present study through the use of high-speed filming. However, although all measured  
267 characteristics significantly affected wingbeat frequency, body mass did not show as clear a  
268 relationship to it as in previous meta-analysis (Dudley, 2000). This is likely because of the lack  
269 of specimen variation in the present study, compared to the very high number of different  
270 specimens across a much broader body mass range in the meta-analysis (Dudley, 2000).  
271 Indeed, previous meta-analyses included species from a much wider geographical range,  
272 incorporating studies from many different countries and ecosystems, including those from  
273 tropical forests.

274

275 Wing length and wing area are both able to predict wingbeat frequency moderately accurately,  
276 explaining 42% and 59% of its variation, respectively. Wing length may affect wingbeat  
277 frequency as a product of increasing body mass, where larger insects have slightly longer  
278 wings to offset the lower wingbeat frequency and maintain good advance ratios (Vogel, 2013),  
279 though this is also connected to wing area (Fig. 2E). Area of the wing generally increases with  
280 body mass to accommodate the greater level of lift generation required and longer wings tend  
281 to have a greater area than shorter ones. Thus, an increased area means fewer beats are

282 necessary per unit time to generate the same amount of lift. This is supported by the positive  
283 relationship between wing loading and wingbeat frequency, where heavily loaded wings are  
284 generally beaten more rapidly to generate enough lift. Relatively heavily loaded wings must  
285 keep a weight aloft with a reduced area and are associated with larger insects (Fig. 2F)  
286 because wing area, proportional to the square of body length, cannot keep pace with body  
287 mass, proportional to the cube of body length, as insect size increases (Bartholomew and  
288 Heinrich, 1973; Byrne et al., 1988; Ennos, 1989; Dudley, 2000; Vogel, 2013). Despite this,  
289 heavier insects tended to also have lower wingbeat frequencies (Fig. 2C). Whilst initially  
290 paradoxical that heavier insects with greater wing loading beat their wings relatively less  
291 frequently, this is because smaller insects must overcome the increasingly viscous forces of  
292 air at small scales, greater relative drag, and the greater effect of the wind on their direction  
293 by beating their wings comparatively faster (Dudley, 2000; Alexander, 2002; Vogel, 2013) and  
294 because the oscillatory frequency of the thorax is inversely dependent on its size, which  
295 directly influences wingbeat frequency in asynchronous fliers (Pringle, 1949, 1967; Dickinson  
296 and Tu, 1997; Dudley, 2000).

297

298 The best overall model explaining the variation in wingbeat frequency incorporated body mass  
299 and wing area, the relative importances of which were 17.3% and 67.2%, respectively. This  
300 suggests that despite the weak linear relationship between body mass and wingbeat  
301 frequency, together with wing area the variables can explain 84% of the variation in wingbeat  
302 frequency. These findings support previous agreement (Jensen, 1956; Ellington, 1984b-c,  
303 1999; Dudley, 1990, 2000; Alexander, 2002) that wingbeat frequency is in large part  
304 dependent on wing area and body mass.

305

306 Palaeopterous insects using direct flight muscles and neopterous insects using synchronous  
307 flight muscles show generally lower wingbeat frequencies than insects with asynchronous

308 flight muscles (Figure 2) and these two groups are further clustered in Figure 3 (Neuroptera,  
309 Lepidoptera, Odonata – bottom right; asynchronous fliers – middle/top left). The weak  
310 relationship between wingbeat frequency and body mass in the present study as well as past  
311 meta-analyses may arise because of the differences in scaling between these groups. Insects  
312 with indirect synchronous flight muscles conduct wingbeats by single nerve impulses to the  
313 tergosternal (wing depressor) and dorsal-longitudinal (wing elevator) muscles. Thus, the  
314 wingbeat frequency of insects with synchronous musculature is determined by the frequency  
315 of nervous stimulation to the muscles. In contrast, insects that possess asynchronous muscles  
316 have essentially random nervous stimulation relative to the wingbeat frequency (Dickinson  
317 and Tu, 1997). Wingbeat frequency in asynchronous fliers is determined primarily by the  
318 resonant features of the pterothoracic apparatus to maximise efficiency of energy expenditure  
319 (Pringle, 1949; Dickinson and Tu, 1997), as well as behavioural changes during rapid  
320 manoeuvring (Nachtigall and Wilson, 1967). Asynchronous muscles are stretch-activated  
321 (Pringle, 1949, 1967) by their antagonistic pair within the pterothorax and are therefore  
322 dependent on mechanical loading. The inertial load of the whole thorax-wing system must  
323 increase with body mass and wingbeat frequency has been shown to vary inversely with wing  
324 inertia (Sotavalta, 1952). For asynchronous fliers, scaling of the resonant flight apparatus is  
325 therefore especially important, as the oscillatory frequency of the pterothorax is inversely  
326 dependent on its size, which directly influences wingbeat frequency (Pringle, 1949, 1967;  
327 Dickinson and Tu, 1997; Dudley, 2000). In synchronous fliers, wing amputation experiments  
328 to lower wing inertia results in only a relatively small increase in wingbeat frequency in  
329 *Periplaneta* cockroaches and *Agrotia* moths compared to asynchronous fliers (Roeder,  
330 1951), suggesting wingbeat frequency in synchronous fliers is independent of mechanical  
331 load. Thus, asynchronous fliers are more likely to show a stronger scaling relationship  
332 between wingbeat frequency and body mass than other insects. No strong inferences relating  
333 to scaling differences between synchronous and asynchronous fliers can be made in the  
334 present study because Lepidoptera encompassed the only well sampled synchronous fliers.

335

336 Orders are shown to be clustered when wingbeat frequency is viewed as a function of one of  
337 the other measured morphometrics (Fig. 2A-C), supporting the idea that flight strategy can be  
338 generally characterized based on evolutionary history. This may be because of a combination  
339 of several factors: 1) species inherit a flight apparatus that can only be changed to a certain  
340 extent in a given time to fit a new role/niche e.g. Coleoptera inherit heavy elytra, one pair of  
341 functional wings, asynchronous flight muscles, and low flight muscle mass ratio relative to  
342 body mass (Marden, 1987; Dudley, 2000) making it unlikely for them to be able to fill the role  
343 of an aerial predator but well adapted to infrequent spells of sustained flight; 2) species may  
344 need to fly in the same way even though they have different ecological niches, which may  
345 increase the level of intra-order clustering because the existing flight apparatus can be used  
346 to fulfil the same aerodynamic needs despite interacting with different organisms e.g.  
347 Syrphidae and Tabanidae need to fly in similar ways – visiting flowers vs. visiting vertebrate  
348 hosts (female tabanids), ability to hover above resources, ability to change direction rapidly to  
349 regularly escape predators or swatting etc.; and 3) a specific goal may be achieved in more  
350 than one way e.g. Diptera: Asilidae and Odonata are both aerial predators with a high  
351 proportion of relative flight muscle mass (Marden, 1987), but likely utilise completely different  
352 flight strategies because of their very different inherited flight apparatuses. Combined, these  
353 factors suggest that although an inherited flight apparatus is predisposed to certain flight  
354 strategies and precludes others, it can be somewhat modified in some instances to fit new  
355 ecological niches or maintained if aerodynamic needs do not change with differing ecological  
356 interactions. Ultimately, this may improve levels of flight strategy conservation at the order  
357 level.

358

359 Order-level flight strategies may have interesting energetic, ecological, and evolutionary  
360 implications though intra-order exceptions exist where some groups fly in unconventional  
361 ways. For example, flies are very light to medium weight with high wingbeat frequencies,

362 medium to low wing area and wing length, and medium to high wing loading (Fig. 2A-D). These  
363 attributes afford flies the ability to fly quickly, perform complex aerobatic manoeuvres and to  
364 hover, conferring obvious ecological advantages to certain groups. Mosquitos and  
365 chironomids, however, possess wingbeat frequencies that are unusually high, and wing  
366 loadings that are unusually low relative to other Diptera (Table S1, Supplementary Information)  
367 that likely increases energetic costs of flight substantially, and may be used for acoustic  
368 communication during swarming and mating (Neems et al., 1992; Takken et al., 2006;  
369 Bompfrey et al., 2017). One potential explanation of this presumably highly energetically  
370 expensive trait uncharacteristic of most other members of the order may be related to sexual  
371 selection, where males and females “duet” by reaching a common harmonic tone based on  
372 their usually different wingbeat frequencies (Cator et al., 2009; Robert, 2009; Bompfrey et al.,  
373 2017).

374

375 The variation between different clades within orders suggests broad categorization is possible,  
376 with infrequent exceptions. For most orders, however, relationships between wingbeat  
377 frequency and flight-associated morphometrics show moderately well conserved patterns  
378 across the graphs. These align with previous meta-analyses (Byrne et al., 1988; Dudley, 2000)  
379 looking at wingbeat frequency in relation to body mass, with the same orders covering the  
380 same areas on the graphs (see Fig. 3.3B in Dudley, 2000). The present study therefore  
381 provides strong experimental evidence that flight strategy is broadly conserved at the order  
382 level, as specimens are generally clustered phylogenetically, and this validates previous meta-  
383 analyses investigating wingbeat frequency and flight-associated morphometrics, although  
384 there is evidence that some flight strategies show similarity between certain groups. The PCA  
385 analysis could though be improved by incorporating other variables, such as relative flight  
386 muscle mass, which is shown to be important when considering the ecology of different orders  
387 (Marden, 1987; Dudley, 2000).

388

389 Energetic and ecological costs and benefits of differing flight behaviours are still poorly known  
390 in most insect groups, though some have received attention e.g. Hymenoptera: Apidae:  
391 Euglossini (see Casey et al., 1985; Dudley, 1995; Dillon and Dudley, 2004), Lepidoptera:  
392 Sphingidae and Saturniidae (Bartholomew and Casey, 1978), Orthoptera: Acrididae (Snelling  
393 et al., 2012), and Hymenoptera: Apidae: Bombini (Ellington et al., 1990). Elucidation of the  
394 ecological pressures leading to adaptation of specific flight strategies and the energetic costs  
395 associated may help illuminate evolutionary trade-offs. These trade-offs are likely to explain  
396 the phylogenetic clustering found across flight-associated morphometrics and wingbeat  
397 frequency in the present study. Studies that combine quantitative evaluation of insect flight  
398 energetics with additional qualitative comparisons between orders can go some way in  
399 revealing why different groups utilise different flight strategies (e.g. between bees, moths, and  
400 locusts in Snelling *et al.*, 2012). Further work to reveal ecological pressures and energetic  
401 costs of broad flight strategies in different orders is therefore required to infer why insect  
402 groups fly the way they do.

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#### 410 **COMPETING INTERESTS**

411 The authors declare no competing or financial interests.

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553 **Figure 1. Images a-k show a complete wingbeat in the beetle *Rutpela maculata***  
554 **(Coleoptera: Cerambycidae);  $t$  is time in milliseconds from the start of the wingbeat. a. the**  
555 **end of pronation; b-e. downstroke translation; e-g. supination; h-j. upstroke translation; j-k.**  
556 **pronation.**

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574 **Figure 2. Relationships between  $\log_{10}$  transformed morphometric variables.** **a**, wingbeat  
575 frequency (Hz) as a function of wing length (cm): wingbeat frequency =  $-0.764 * \text{wing length} +$   
576  $4.479$ ,  $R^2 = 0.42$ ,  $p = <0.001$ ; **b**, wingbeat frequency as a function of wing area ( $\text{cm}^2$ ): wingbeat  
577 frequency =  $-0.413 * \text{wing area} + 4.345$ ,  $R^2 = 0.59$ ,  $p = <0.001$ ; **c**, wingbeat frequency as a  
578 function of body mass (g): wingbeat frequency =  $-0.129 * \text{body mass} + 4.04$ ,  $R^2 = 0.09$ ,  $p =$   
579  $<0.01$ ; **d**, wingbeat frequency as a function of wing loading ( $\text{g}/\text{cm}^2$ ): wingbeat frequency =  
580  $0.385 * \text{wing loading} + 5.799$ ,  $R^2 = 0.29$ ,  $p = <0.001$ ; **e**, wing area as a function of wing length:  
581 wing area =  $2.105 * \text{wing length} - 0.307$ ,  $R^2 = 0.93$ ,  $p = <0.001$ ; **f**, wing loading as a function  
582 of body mass: wing loading =  $0.356 * \text{body mass} - 1.977$ ,  $R^2 = 0.34$ ,  $p = <0.001$ ; **g**, wing area  
583 as a function of body mass: wing area =  $0.644 * \text{body mass} + 1.977$ ,  $R^2 = 0.63$ ,  $p = <0.001$ .

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596 **Figure 3. Principle component data for Dimensions 1 and 2, categorised into different**  
597 **insect orders by symbol shape and colour.** Small translucent symbols represent  
598 specimens and large opaque symbols represent the centroids for each order. The ellipses  
599 around each centroid represent one standard deviation along each axis of the associated  
600 order and are rotated in the direction of maximum spread. Trichoptera, Ephemeroptera, and  
601 Mecoptera lack ellipses because of an insufficient sample size. The Dimension scores show  
602 a moderate-high level of clustering of orders in relation to measured variables, as specimens  
603 are generally in close proximity to their associated centroid. The black point in the top-right  
604 quarter of the graph is the mean direction of the arrows and suggests the variables are on  
605 average positively correlated with dimensions 1 and 2.

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619 **Table 1.** Range and mean of wingbeat frequency and associated morphological  
620 measurements in each sampled order. Number of species are denoted in parentheses beside  
621 sample size in the right-most column.

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640 **Supplementary Information**

641 **Table S1.** Wingbeat frequency and morphological measurements of all specimens. Lists  
642 specimens by body mass in ascending order. Cells with a “-“ denote the specimen failed to  
643 be identified to the associated taxonomic rank.

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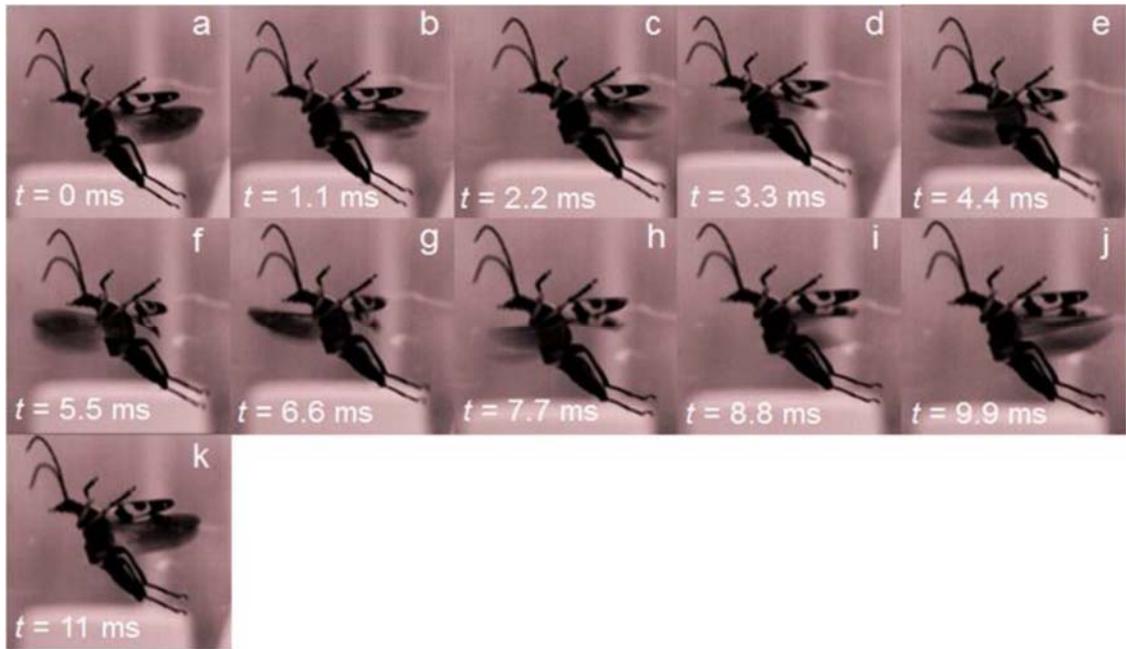
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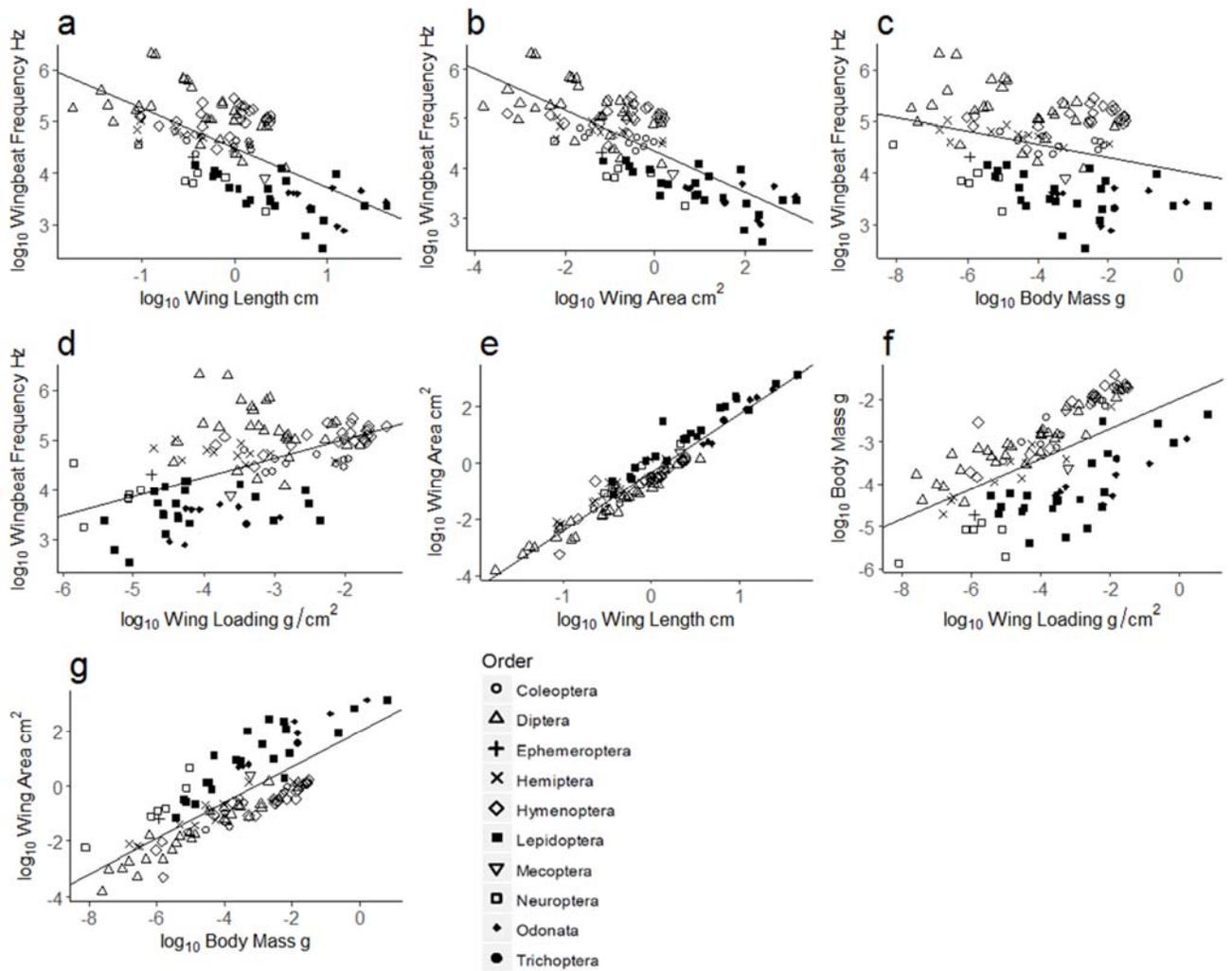
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662 **Figure 1.**



676 **Figure 2.**



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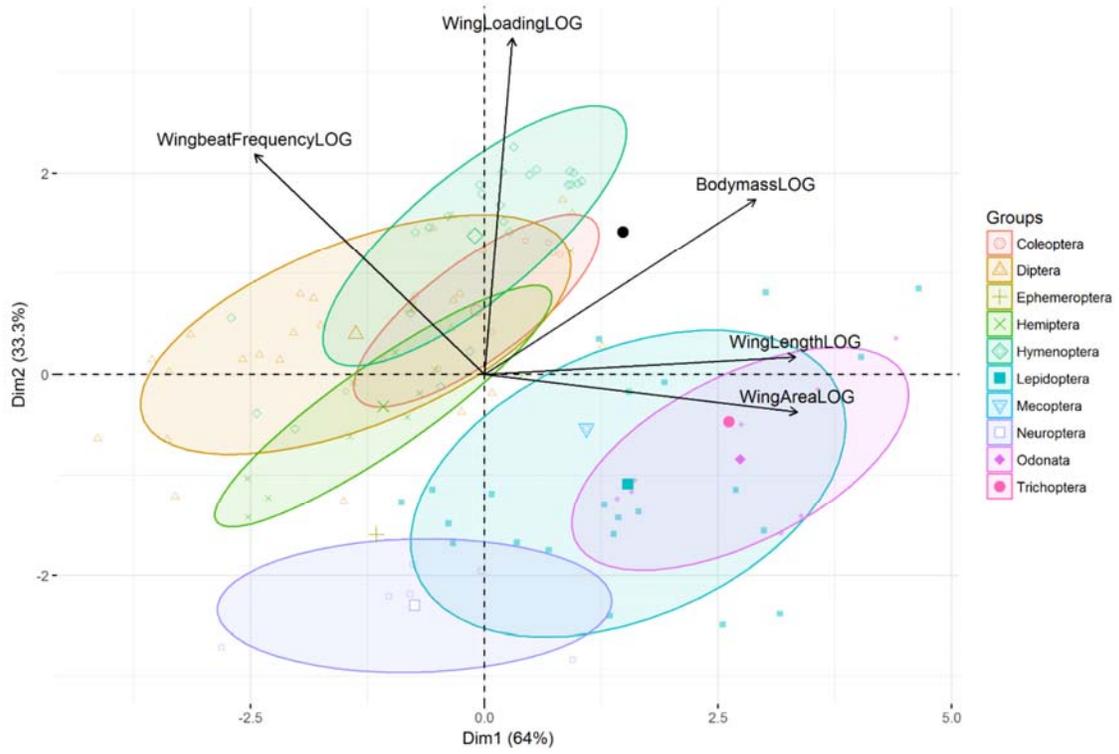
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697 **Table 1.**

	Wingbeat frequency (mean Hz)		Bodymass (g)		Wing length (cm)		Wing area (cm <sup>2</sup> )		Wing loading (g/cm <sup>2</sup> )		Number of specimens
	range	mean	range	mean	range	mean	range	mean	range	mean	
Coleoptera	79 - 123.396	97.512	0.0061 - 0.117	0.0539	0.521 - 1.188	0.898	0.19 - 0.982	0.545	0.0321 - 0.141	0.085	10(10)
Diptera	59.567 - 557.351	208.244	0.0005 - 0.162	0.0268	0.172 - 1.739	0.729	0.022 - 1.17	0.327	0.0119 - 0.168	0.0554	28(28)
Ephemeroptera	n/a	75.0454	n/a	0.0027	n/a	0.634	n/a	0.306	n/a	0.00882	1(1)
Hemiptera	90.222 - 152.247	116.39	0.0011 - 0.14	0.0226	0.345 - 1.185	0.624	0.112 - 1.186	0.445	0.009 - 0.118	0.034	11(11)
Hymenoptera	87.129 - 230.987	163.89	0.0024 - 0.223	0.103	0.356 - 1.48	1.006	0.038 - 1.234	0.64	0.022 - 0.245	0.136	24(15)
Lepidoptera	12.468 - 64.566	39.606	0.0044 - 2.24	0.203	0.646 - 5.214	1.792	0.318 - 23.362	5.031	0.004 - 0.096	0.025	22(22)
Mecoptera	n/a	48.885	n/a	0.0398	n/a	1.387	n/a	1.492	n/a	0.027	1(1)
Neuroptera	25-923 - 94.413	52.801	0.0003 - 0.0065	0.0035	0.352 - 1.393	0.757	0.106 - 1.972	0.701	0.003 - 0.007	0.005	6(6)
Odonata	17.847 - 40.665	32.331	0.0278 - 1.23	0.27	1.795 - 5.158	3.002	1.964 - 22.784	8.768	0.0112 - 0.054	0.022	8(6)
Trichoptera	n/a	27.515	n/a	0.159	n/a	2.267	n/a	4.738	n/a	0.0336	1(1)

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711 **Supplementary Table.**

Species	Genus	Family	Order	Wingbeat frequency	Bodymass	Wing length	Wing area	Wing loading
				(mean Hz)	(g)	(cm)	(cm <sup>2</sup> )	(g/cm <sup>2</sup> )
-	<i>Micromus</i>	Hemerobiidae	Neuroptera	94.413	0.0003	0.352	0.106	0.003
-	<i>Syrphus</i>	Syrphidae	Diptera	190.860	0.0005	0.172	0.022	0.023
-	-	Psychodidae	Diptera	144.611	0.0006	0.267	0.048	0.013
-	-	-	Diptera	204.355	0.0009	0.252	0.050	0.018
-	-	Chironomidae	Diptera	557.351	0.0011	0.406	0.064	0.017
-	-	Miridae	Hemiptera	127.872	0.0011	0.345	0.122	0.009
<i>Thaumatomyia notata</i>	<i>Thaumatomyia</i>	Chloropidae	Diptera	269.741	0.0014	0.234	0.038	0.037
-	-	-	Hemiptera	152.247	0.0014	0.358	0.114	0.012
<i>Uroleucon cirsii</i>	<i>Uroleucon</i>	Aphididae	Hemiptera	99.603	0.0015	0.353	0.112	0.013
-	-	Chironomidae	Diptera	544.494	0.0018	0.423	0.070	0.026
-	-	Tipulidae	Diptera	94.606	0.0020	0.687	0.168	0.012
<i>Hemerobius humulinus</i>	<i>Hemerobius</i>	Hemerobiidae	Neuroptera	46.583	0.0021	0.586	0.336	0.006
-	<i>Torymus</i>	Torymidae	Hymenoptera	160.011	0.0024	0.360	0.098	0.024
<i>Wesmaelius subnebulosis</i>	<i>Wesmaelius</i>	Hemerobiidae	Neuroptera	45.304	0.0026	0.640	0.416	0.006
<i>Centroptilum luteolum</i>	<i>Centroptilum</i>	Baetidae	Ephemeroptera	75.045	0.0027	0.634	0.306	0.009
-	-	Braconidae	Hymenoptera	136.261	0.0029	0.438	0.134	0.022
-	-	Chloropidae	Diptera	180.050	0.0030	0.342	0.070	0.043
-	-	Braconidae	Hymenoptera	164.443	0.0030	0.356	0.038	0.079
-	<i>Wesmaelius</i>	Hemerobiidae	Neuroptera	54.583	0.0033	0.666	0.444	0.007
-	-	-	Diptera	195.996	0.0039	0.354	0.098	0.040

-	-	Syrphidae	Diptera	198.890	0.0044	0.403	0.124	0.035
<i>Pseudargyrotoza conwagana</i>	<i>Pseudargyrotoza</i>	Tortricidae	Lepidoptera	64.246	0.0044	0.653	0.318	0.014
-	-	Miridae	Hemiptera	120.832	0.0048	0.530	0.250	0.019
<i>Culex pipiens</i>	<i>Culex</i>	Culicidae	Diptera	334.037	0.0049	0.578	0.158	0.031
-	-	Tortricidae	Lepidoptera	52.214	0.0055	0.785	0.612	0.009
-	-	Crambidae	Lepidoptera	57.948	0.0059	0.799	0.562	0.010
<i>Micromus angulatus</i>	<i>Micromus</i>	Hemerobiidae	Neuroptera	50.000	0.0059	0.904	0.932	0.006
<i>Oulema melanopus</i>	<i>Oulema</i>	Chrysomelidae	Coleoptera	123.398	0.0061	0.521	0.190	0.032
<i>Chrysoperla carnea</i>	<i>Chrysoperla</i>	Chrysopidae	Neuroptera	25.923	0.0065	1.393	1.972	0.003
<i>Aedes cantans</i>	<i>Aedes</i>	Culicidae	Diptera	286.949	0.0066	0.627	0.182	0.036
<i>Culiseta annulata</i>	<i>Culiseta</i>	Culicidae	Diptera	344.160	0.0070	0.572	0.150	0.047
<i>Macrolophus sp.</i>	<i>Macrolophus</i>	Miridae	Hemiptera	139.717	0.0076	0.515	0.244	0.031
<i>Lobesia abscisana</i>	<i>Lobesia</i>	Tortricidae	Lepidoptera	64.566	0.0076	0.646	0.526	0.014
<i>Culiseta annulata</i>	<i>Culiseta</i>	Culicidae	Diptera	331.157	0.0077	0.602	0.174	0.044
-	-	-	Hemiptera	116.865	0.0104	0.619	0.490	0.021
<i>Propylea 14-punctata</i>	<i>Propylea</i>	Coccinellidae	Coleoptera	102.427	0.0105	0.589	0.210	0.050
<i>Pasiphila rectangulata</i>	<i>Pasiphila</i>	Geometridae	Lepidoptera	41.358	0.0107	0.938	1.132	0.009
<i>Pterophorus pentadactyla</i>	<i>Pterophorus</i>	Pterophoridae	Lepidoptera	32.333	0.0114	1.192	1.120	0.010
<i>Nephrotoma flavescens</i>	<i>Nephrotoma</i>	Tipulidae	Diptera	79.470	0.0118	1.015	0.402	0.029
-	-	Miridae	Hemiptera	108.171	0.0119	0.710	0.402	0.030
<i>Pandemis cerasana</i>	<i>Pandemis</i>	Tortricidae	Lepidoptera	54.184	0.0124	0.835	0.890	0.014
<i>Athalia scutellariae</i>	<i>Athalia</i>	Tenthredinidae	Hymenoptera	87.129	0.0132	0.826	0.352	0.038
<i>Xanthorhoe montanata</i>	<i>Xanthorhoe</i>	Geometridae	Lepidoptera	29.243	0.0133	1.561	2.980	0.004
<i>Lygus rugulipennis</i>	<i>Lygus</i>	Miridae	Hemiptera	115.183	0.0140	0.574	0.298	0.047

<i>Nephrotoma quadrifaria</i>	<i>Nephrotoma</i>	Tipulidae	Diptera	67.360	0.0181	1.084	0.464	0.039
<i>Rhagonycha fulva</i>	<i>Rhagonycha</i>	Catharidae	Coleoptera	79.712	0.0183	0.653	0.380	0.048
<i>Chloromyia formosa</i>	<i>Chloromyia</i>	Stratiomyidae	Diptera	156.043	0.0183	0.736	0.312	0.059
<i>Haematopota pluvialis</i>	<i>Haematopota</i>	Tabanidae	Diptera	151.568	0.0183	0.779	0.302	0.061
-	-	-	Hemiptera	112.917	0.0184	0.682	0.518	0.036
-	-	Vespidae: Eumeninae	Hymenoptera	135.597	0.0184	0.703	0.292	0.063
-	-	Empididae	Diptera	151.321	0.0193	0.759	0.288	0.067
<i>Oedemera nobilis</i>	<i>Oedemera</i>	Oedemeridae	Coleoptera	112.656	0.0210	0.698	0.232	0.091
<i>Scathophaga stercoraria</i>	<i>Scathophaga</i>	Scathophagidae	Diptera	104.015	0.0224	0.854	0.366	0.061
-	-	Ichneumonidae	Hymenoptera	110.116	0.0233	0.942	0.546	0.043
<i>Manulea lurideola</i>	<i>Manulea</i>	Erebidae	Lepidoptera	33.095	0.0258	1.470	2.542	0.010
<i>Syrphus ribesii</i>	<i>Syrphus</i>	Syrphidae	Diptera	177.908	0.0273	0.994	0.512	0.053
<i>Coenagrion puella</i>	<i>Coenagrion</i>	Coenagrionidae	Odonata	37.495	0.0277	1.795	1.964	0.014
<i>Harmonia axyridis</i>	<i>Harmonia</i>	Coccinellidae	Coleoptera	79.000	0.0283	0.993	0.644	0.044
<i>Anania hortulata</i>	<i>Anania</i>	Crambidae	Lepidoptera	40.996	0.0293	1.448	2.410	0.012
<i>Episyrphus balteatus</i>	<i>Episyrphus</i>	Syrphidae	Diptera	166.057	0.0294	1.025	0.488	0.060
<i>Idaea aversata</i>	<i>Idaea</i>	Geometridae	Lepidoptera	32.088	0.0303	1.471	2.420	0.013
<i>Coenagrion puella</i>	<i>Coenagrion</i>	Coenagrionidae	Odonata	36.691	0.0316	1.984	2.072	0.015
-	<i>Aphodius</i>	Scarabaeidae	Coleoptera	93.054	0.0327	0.987	0.560	0.058
<i>Aphantopus hyperantus</i>	<i>Aphantopus</i>	Nymphalidae	Lepidoptera	16.014	0.0373	2.168	7.262	0.005
<i>Coenagrion puella</i>	<i>Coenagrion</i>	Coenagrionidae	Odonata	36.839	0.0374	1.902	2.190	0.017
-	<i>Andrena</i>	Apidae	Hymenoptera	213.815	0.0376	0.703	0.352	0.107
-	-	-	Hemiptera	90.222	0.0383	0.989	1.164	0.033
-	-	Syrphidae	Diptera	208.540	0.0385	0.872	0.340	0.113

<i>Panorpa communis</i>	<i>Panorpa</i>	Panorpidae	Mecoptera	48.885	0.0398	1.387	1.492	0.027
-	<i>Andrena</i>	Apidae	Hymenoptera	172.581	0.0453	0.690	0.346	0.131
-	<i>Sarcophaga</i>	Sarcophagidae	Diptera	149.643	0.0540	0.995	0.526	0.103
<i>Calliphora vomitoria</i>	<i>Calliphora</i>	Calliforidae	Diptera	214.835	0.0549	0.874	0.460	0.119
<i>Hypena proboscidalis</i>	<i>Hypena</i>	Noctuidae	Lepidoptera	30.587	0.0565	1.137	4.496	0.013
-	<i>Tipula</i>	Tipulidae	Diptera	59.567	0.0676	1.739	1.170	0.058
<i>Pieris brassicae</i>	<i>Pieris</i>	Pieridae	Lepidoptera	12.468	0.0691	2.593	10.992	0.006
<i>Vespula germanica</i>	<i>Vespula</i>	Vespidae	Hymenoptera	145.156	0.0769	1.126	0.628	0.122
<i>Ectemnius cavifrons</i>	<i>Ectemnius</i>	Crabronidae	Hymenoptera	210.688	0.0800	1.037	0.542	0.148
-	<i>Zygaena</i>	Zygaenidae	Lepidoptera	60.595	0.0804	1.669	2.640	0.030
<i>Vespula germanica</i>	<i>Vespula</i>	Vespidae	Hymenoptera	152.006	0.0818	1.061	0.610	0.134
<i>Vespula germanica</i>	<i>Vespula</i>	Vespidae	Hymenoptera	146.908	0.0833	0.530	0.536	0.155
<i>Vespula vulgaris</i>	<i>Vespula</i>	Vespidae	Hymenoptera	173.277	0.0874	1.081	0.598	0.146
<i>Apis mellifera</i>	<i>Apis</i>	Apidae	Hymenoptera	230.987	0.0886	0.995	0.588	0.151
-	<i>Aphodius</i>	Scarabaeidae	Coleoptera	103.159	0.0929	1.018	0.658	0.141
<i>Rutpela maculata</i>	<i>Rutpela</i>	Cerambycidae	Coleoptera	86.840	0.1026	1.188	0.768	0.134
<i>Geometra papilionaria</i>	<i>Geometra</i>	Geometridae	Lepidoptera	22.023	0.1071	2.632	10.194	0.011
<i>Chrysoteuchia culmella</i>	<i>Chrysoteuchia</i>	Crambidae	Lepidoptera	40.626	0.1090	1.041	1.330	0.082
<i>Calopteryx splendens</i>	<i>Calopteryx</i>	Calopterygidae	Odonata	19.318	0.1092	3.054	9.760	0.011
-	<i>Aphodius</i>	Scarabaeidae	Coleoptera	101.111	0.1095	1.161	0.822	0.133
<i>Polygonia c-album</i>	<i>Polygonia</i>	Nymphalidae	Lepidoptera	27.501	0.1145	2.298	7.696	0.015
<i>Bombus pascuorum</i>	<i>Bombus</i>	Apidae	Hymenoptera	198.274	0.1166	1.074	0.614	0.190
<i>Leptura quadrifasciata</i>	<i>Leptura</i>	Cerambycidae	Coleoptera	93.768	0.1173	1.173	0.982	0.119
<i>Orthosia gothica</i>	<i>Orthosia</i>	Noctuidae	Lepidoptera	47.053	0.1253	1.753	3.260	0.038

<i>Pentatoma rufipes</i>	<i>Pentatoma</i>	Pentatomidae	Hemiptera	96.667	0.1397	1.185	1.186	0.118
<i>Calopteryx virgo</i>	<i>Calopteryx</i>	Calopterygidae	Odonata	17.847	0.1457	3.287	10.466	0.014
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	183.029	0.1504	1.171	0.818	0.184
<i>Bombus lapidarius</i>	<i>Bombus</i>	Apidae	Hymenoptera	199.547	0.1536	1.114	0.626	0.245
<i>Phryganea grandis</i>	<i>Phryganea</i>	Phryganeidae	Trichoptera	27.515	0.1590	2.267	4.738	0.034
<i>Sympetrum striolatum</i>	<i>Sympetrum</i>	Libellulidae	Odonata	40.665	0.1595	2.894	6.944	0.023
<i>Volucella pellucens</i>	<i>Volucella</i>	Syrphidae	Diptera	134.179	0.1613	1.418	1.152	0.140
<i>Volucella bombylans</i>	<i>Volucella</i>	Syrphidae	Diptera	133.078	0.1624	1.337	0.966	0.168
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	186.521	0.1641	1.229	0.864	0.190
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	152.625	0.1854	1.436	1.048	0.177
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	153.182	0.1972	1.422	1.128	0.175
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	144.712	0.2081	1.454	1.134	0.184
<i>Bombus terrestris</i> ♀	<i>Bombus</i>	Apidae	Hymenoptera	161.815	0.2125	1.425	1.086	0.196
<i>Bombus terrestris</i> ♂	<i>Bombus</i>	Apidae	Hymenoptera	165.085	0.2154	1.480	1.152	0.187
<i>Bombus terrestris</i> ♂	<i>Bombus</i>	Apidae	Hymenoptera	149.597	0.2227	1.480	1.234	0.180
<i>Orthetrum cancellatum</i>	<i>Orthetrum</i>	Libellulidae	Odonata	38.577	0.4176	3.944	13.960	0.030
<i>Deilephila elpenor</i>	<i>Deilephila</i>	Sphingidae	Lepidoptera	53.715	0.5281	3.026	6.792	0.078
<i>Laothoe populi</i>	<i>Laothoe</i>	Sphingidae	Lepidoptera	29.330	0.8449	4.085	17.152	0.049
<i>Aeshna grandis</i>	<i>Aeshna</i>	Aeshnidae	Odonata	31.214	1.2296	5.158	22.784	0.054
<i>Acherontia atropos</i>	<i>Acherontia</i>	Sphingidae	Lepidoptera	29.160	2.2403	5.214	23.362	0.096

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715 **Equation 1.**

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Equation 1  $n = n_v \frac{1}{(t_v/f_m)}$

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