1 **Insect temperature-body size trends common to laboratory,**

2 **latitudinal and seasonal gradients are not found across altitudes**

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17 **Author Contributions**

18 CH, AGH and DA designed the study and wrote the paper. CH collected the data and

19 performed the statistical analyses.

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ABSTRACT

1. Body size affects rates of most biological and ecological processes, from individual performance to ecosystem function, and is fundamentally linked to organism fitness. Within species, size at maturity can vary systematically with environmental temperature in the laboratory and across seasons, as well as over latitudinal gradients. Recent meta-analyses have revealed a close match in the magnitude and direction of these size gradients in various arthropod orders, suggesting that these size responses share common drivers.

2. As with increasing latitude, temperature also decreases with increasing altitude. Although the general direction of body size clines along altitudinal gradients has been examined previously, to our knowledge altitude-body size (A-S) clines have never been synthesised quantitatively, nor compared with temperature-size (T-S) responses measured under controlled laboratory conditions.

3. Here we quantitatively examine variation in intraspecific A-S clines among 121 insect species from 50 different global locations, representing 12 taxonomic orders. While some taxa were better represented in the literature than others, our analysis reveals extensive variation in the magnitude and direction of A-S clines. Following the assumption that temperature on average declines by 1°C per 150m increase in altitude, order-specific A-S clines in the field appear to deviate from laboratory T-S responses. Specifically, the magnitude of A-S clines and T-S responses are more closely matched in some taxonomic orders (e.g. Diptera) than others (e.g. Orthoptera). These findings contrast with the strong co-variation observed between latitude-size clines and T-S responses, and between laboratory and seasonal T-S responses.

4. The lack of clear size relationships with elevation, and hence temperature, is likely due to the counteracting effects of other major drivers with altitude, including season length and oxygen partial pressure. Switches in voltinism within species across altitude, and the dispersal of individuals across different elevations, may also obscure trends.

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INTRODUCTION

Body size is fundamentally linked to an organism's fitness, and is correlated with vital physiological rates and life history characteristics (Kleiber 1947; Brown *et al.* 2004; Hirst, Glazier & Atkinson 2014). Further, both within and between species, body size has been found to co-vary with a range of environmental conditions (Bergmann 1847; James 1970; Atkinson 1994). Changes in the body size of organisms can affect not only the fate of individuals and populations (e.g. by influencing survival, fecundity, population production), but also ecosystem functioning via size-dependent processes (e.g. predator-prey dynamics, food web connectivity) (Peters 1983; Honěk 1993). Thus, understanding and ultimately predicting body size variation with environmental conditions presents a significant ecological challenge of global importance.

In ectothermic species, intraspecific variation in body size has been shown to strongly correlate with temperature, both in controlled laboratory conditions (Atkinson 1994; Forster, Hirst & Atkinson 2012) and seasonally across the year (Horne *et al.* 2016; Horne, Hirst & Atkinson 2017). Recurrent patterns in body size also occur across latitudes, and have led to prominent biogeographical 'rules', such as Bergmann's rule (across taxa, larger species are found at higher, colder latitudes) (Bergmann 1847), and James' rule (within species, larger individuals are found at higher, colder latitudes) (James 1970). In arthropods, including insects, close parallels were recently identified between phenotypically plastic size responses to temperature measured in the laboratory, and intraspecific changes in body size observed in the field, both across latitudes and seasonally over an annual cycle. Consistent differences in both the magnitude and direction of body size gradients were found among taxonomic orders, between environments (aquatic vs. terrestrial, suggesting an important role for oxygen availability), and between univoltine and multivoltine terrestrial species (likely an evolutionary adaptation to changing season length) (Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). This co-variation suggests that these widespread body size phenomena may be driven by similar selective pressures, and that temperature is an important correlate of size

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78   responses in the field, despite a number of confounding factors that can also influence body

79   size (e.g. resource availability, mortality risk and competition) (Chown & Gaston 2010).

80

81   As with increasing latitude, temperature commonly declines with increasing altitude. On

82   average, temperature falls by 5.5 to 6.5°C per 1000m increase in elevation (Anslow & Shawn

83   2002), though this of course varies considerably depending on other climatic and topographic

84   influences, including aspect, wind speed and cloud cover amongst others (Hodkinson 2005).

85   We might therefore expect those species that grow to a larger adult size in the cold and with

86   increasing latitude will also exhibit a positive cline in body size with increasing altitude. To our

87   knowledge, most large syntheses of altitude-body size (A-S) clines have predominantly

88   focused on whether body size gradients were negative, positive or non-significant (e.g. Dillon,

89   Frazier & Dudley 2006; Shelomi 2012). Yet, by quantifying and comparing the magnitude of

90   A-S clines between species and among higher taxonomic groups, we can begin to link

91   variation in the strength of these body size gradients with key life history characteristics,

92   habitat and/or functional groups. Furthermore, quantitative data capturing the magnitude of A-

93   S clines allow for a more direct comparison with laboratory temperature-size (T-S) responses.

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95   To our knowledge, A-S clines and laboratory T-S responses have not previously been

96   compared quantitatively. Such a comparison provides an opportunity to explore the extent to

97   which changes in other environmental parameters might be confounding the effects of

98   temperature across altitude in the field. For example, an increase in altitude is also

99   accompanied by a decline in the partial pressure of atmospheric gases, including oxygen,

100   which decreases near linearly with altitude, such that inspired oxygen pressure at 5500m

101   elevation is 50% of that at sea level (Peacock 1998). The length of the season in which

102   individuals are able to grow also typically declines with increasing altitude: these growth

103   conditions encompass not just appropriate temperatures but also extent of resource

104   availability (Hodkinson 2005) (see Fig. 1 for an illustration of the predicted effects of these key

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105 parameters on body size). Other biologically relevant environmental factors that increase with

106 altitude include wind speed, precipitation, and UV radiation (Hodkinson 2005).

107

108 Variation in the direction of A-S clines among species has often been attributed to seasonality

109 and differences in reproductive strategy (Chown & Klok 2003; Shelomi 2012). Multivoltine

110 species are predicted to grow to a larger adult size at higher colder altitudes, in accordance

111 with the temperature-size rule (Atkinson 1994). In contrast, univoltine species are much more

112 dependent on season length and hence on time available for growth (often positively

113 correlated with temperature) (Chown & Gaston 1999; Blanckenhorn & Demont 2004;

114 Kozłowski, Czarnoleski & Danko 2004; Shelomi 2012). Consequently, univoltine species are

115 predicted to reduce their body size with increasing altitude. The extent to which season length

116 decreases with increasing altitude may also influence the magnitude of A-S clines, particularly

117 in univoltine species for which time available for growth is limiting. Increased time constraints

118 with elevation may be especially important in more thermally seasonal environments, such as

119 at higher latitudes, where the decline in season length along altitudinal gradients is much more

120 pronounced than in the tropics (Körner 2000; Chown & Klok 2003). Of course, voltinism itself

121 can vary with environmental conditions, and any switch in reproductive strategy could also

122 affect the magnitude of any apparent size change (Roff 1980; Mousseau & Roff 1989; Zeuss,

123 Brunzel & Brandl 2016).

124

125 The spatial distance covered by an A-S cline and the dispersal or migratory ability of a species

126 may also affect the magnitude of the A-S cline. Latitudinal-size (L-S) clines are typically

127 measured over larger distances than A-S clines, sometimes spanning thousands of

128 kilometres, whole continents, and entire species' ranges (e.g. Hassall 2013). By contrast,

129 altitudinal clines in the size of species are often studied along localized transects (e.g. Smith

130 *et al.* 2000), or in some cases over tens to hundreds of kilometres at most (e.g. Eweleit &

131 Reinhold 2014). Consequently, populations are more likely to become interconnected by

132 individual movement along relatively short-distance elevation gradients (for example by flight

133 and/or passive transport by wind) than along longer-distance latitudinal transects. Therefore,

134 adults collected at one altitude might have developed at another, where environmental

135 conditions were very different. This shift between environmental conditions during ontogeny

136 is more likely for more mobile species with greater dispersal ability, such as winged species

137 capable of flight (Roff & Fairbairn 2007). As a result, A-S clines in more mobile species may

138 be obscured or appear weaker.

139

140 Here we explore these different potential influences on A-S clines by quantifying and

141 examining variation in intraspecific A-S clines in insects, along with a small number of

142 arachnids and Crustacea. We compare the magnitude and direction of A-S clines based on

143 taxonomy, voltinism, flight capability, and environment type (freshwater vs. terrestrial). We

144 then compare these clines to T-S responses measured under controlled laboratory conditions,

145 to determine whether these major size gradients co-vary, and thus to better understand to

146 what extent changes in other environmental parameters might be confounding the effects of

147 temperature on body size across altitude in the field.

148

149 **MATERIALS AND METHODS**

150 To quantitatively describe species-specific altitudinal-size clines, we searched the literature

151 using the Web of Science database (http://apps.webofknowledge.com/) and Google Scholar

152 for published field records of adult body size measured at different altitudes, covering at least

153 150m in altitudinal range. Most studies were conducted along individual mountain transects,

154 but occasionally sampling locations were more widely distributed within a local area or region;

155 in these instances, we only accepted studies in which the sampling locations were distributed

156 over less than 5º latitude. The primary search term combinations used were: ("altitud*" OR

157 "elevation" OR "mountain") AND "size" AND ("arthropod" OR "insect" OR "[<insert taxonomic

158 order or common name of taxon>]"). We also used reference lists from the papers we found

159 to identify additional key literature. Adult size data were collected as lengths, or dry, wet or

160 carbon masses and subsequently standardised to dry mass (mg) using published intra-

161   specific regressions and conversion factors (see Dataset S1 in Supplementary Information).

162   In cases where species-specific regressions were unavailable, regressions for related

163   species, or more general inter-specific regressions were used. All altitudinal measurements

164   were standardized to metres above sea level.

165

166   We derived species-specific slopes of ordinary least-squares (OLS) regressions between ln-

167   transformed dry mass (mg) and altitude (m). Slopes were calculated for each sex separately

168   where these data were available. We used this exponential function of body mass as it has

169   consistently been found to be the best for modelling body size gradients with both temperature

170   (in the laboratory and seasonally) and across latitude (Forster, Hirst & Atkinson 2012; Horne,

171   Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). Further, this exponential function

172   allowed us to easily compare these different size gradients with one another. Altitudinal-size

173   clines were then transformed into percentage change in dry mass per metre of elevation, using

174   the formula $(exp^{(slope)} -1)*100$ = % change in mass per m (Forster, Hirst & Atkinson 2012).

175   Thus, a positive cline denotes an increase in body size with increasing altitude, whereas a

176   negative cline denotes a decrease in body size with increasing altitude. In general,

177   temperature decreases with increasing altitude at a rate of between 5.5°C and 6.5°C per

178   1000m (Anslow & Shawn 2002), which equates to approximately 1°C per 150m elevation.

179   Thus, we also calculated the percentage change in dry mass per 150m, using the formula

180    $(exp^{(slope \times 150)} -1)*100$ = % change in mass per 150m elevation (or approximately per ºC) . This

181   measure of size change allowed us to more appropriately compare the strength of A-S clines

182   measured in the field with T-S responses (% change in mass per ºC) measured in the

183   laboratory.

184

185   **Statistical analyses**

186   All statistical analyses were conducted in R (version 3.4.1) (R Core Team 2014). Using A-S

187   clines (% change in mass per 150m) as the dependent variable, we compared several

188   candidate models to best predict variation in the magnitude and direction of A-S clines based

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189   on the Akaike's information criterion (AIC). The following were incorporated as fixed variables

190   in a global linear mixed effects model (using package lme4): environment (freshwater vs.

191   terrestrial), voltinism, categorised here as species that are generally regarded as having ≤1

192   generation per year (includes univoltine) or >1 generation per year (multivoltine), flight

193   capability (flying vs. flightless), and $\log_{10}$-transformed species body mass (calculated at 1500m

194   altitude using species-specific A-S slopes, and representing the approximate mid-point of the

195   altitudinal range across all the studies in our data set). $\log_{10}$-transformed species body mass

196   was included as a covariate to determine if A-S clines were dependent on the body size of the

197   species. A-S clines from multiple studies of the same species were included in our analyses;

198   thus, given that species have shared evolutionary histories and so are not completely

199   independent, we included levels of taxonomic classification (order, family, and species) as

200   nested (hierarchical) random effects on the intercept in all models to help control for

201   phylogeny, and to account for the disproportionate representation of taxonomic orders in our

202   data set. Given that A-S clines did not differ significantly between males and females, and sex

203   was not reported in all studies, we chose to exclude it as a random effect in the models. A-S

204   clines for multiple species were sometimes measured in similar study locations or regions,

205   and so we also included location (categorized using latitude and longitude) as a random effect

206   on the intercept, to help control for potential geographical clumping of the data. Finally, we

207   accounted for variation in information quality by weighting each A-S cline by the inverse of the

208   variance of its slope estimate (using the 'weights' function in R) (Koricheva, Gurevitch &

209   Mengersen 2013). This helped to account for the fact that A-S clines were derived from data

210   that varied in their goodness of fit between studies and species. We compared all possible

211   combinations of the global model terms using the dredge function in the MuMIn package

212   (Barton 2017); this included an intercept-only model, which contained no independent

213   variables and predicted that the best estimate of A-S clines was the intercept. The best model

214   was identified as that with the lowest small-samples corrected AIC (AICc). Where the

215   difference between a model's AICc and the lowest AICc (i.e. ΔAICc) was <2, a set of best fit

216   models, rather than a single best model, was assumed. Model averaging was then used to

identify the best predictor variables across the top candidate models, and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of F tests were used to verify the significance ($p < 0.05$) of each parameter's effect on the strength of the seasonal T-S gradient.

To compare A-S clines with laboratory controlled T-S responses, we used the data compilation of Horne *et al.* (2015) . For each data set, we first generated single species-specific body size gradients by combining size gradients from multiple studies of the same species into a simple mean. We then averaged these species-specific gradients for each taxonomic order, separated by voltinism, and plotted the resulting order-specific A-S clines against order-specific laboratory T-S responses. We then assessed the extent to which both of these body size gradients co-varied (i.e. whether a 1% increase in body size per 150m altitude = 1% decrease in body size per °C in the laboratory).

We acknowledge that the use of interspecific length-mass conversions may increase the likelihood of inaccuracy when determining body size gradients, particularly as small deviations in the equation's power term can result in substantial over- or under-estimation of the percentage change in body size. Wherever possible, therefore, we used species-specific length-mass regressions, but sometimes had to rely on family- and order-specific conversions, and authors often employed a variety of equation forms. To reduce risks that uncertainties in these length-to-mass conversions would bias our conclusions, we therefore repeated our analysis using length in place of dry mass to generate a second set of A-S clines (% change in length $150m^{-1}$). To do this we used either the original length measurements reported, or calculated the cube-root of mass when this was given.

RESULTS

We derived a total of 229 altitude-size clines representing 121 species from 12 taxonomic orders. These size clines were recorded at 50 different global locations ranging from 53° South to 66° North, with most locations falling around mid-latitude regions (Fig. 2). The data set contained a near even distribution of negative and positive A-S clines, with 49% of clines showing a decrease in adult body size with increasing altitude. We found that neither of the sexes within species showed a consistently stronger cline in body size with altitude than the other ($F_{1,227}$=0.0004, p=0.98; also see Fig. 3), which parallels the lack of sex differences in laboratory T-S responses within arthropod species (Hirst, Horne & Atkinson 2015). Across species, the magnitude of A-S clines varied considerably, with some of the strongest size clines observed in the Coleoptera, Orthoptera and Hymenoptera (see Fig. 3). Approximately 69% of the A-S clines in our data set were based on measurements of body length, width or direct measurements of mass, whereas the remaining clines were derived from limb measurements, including femur length and wing length, as well as head width (all of which had been converted to mass). Whether A-S clines were originally derived from whole- or part-body measurements had no significant effect on the magnitude of A-S clines in our data set, ($F_{1,227}$=1.99, p=0.16), and this was also the case when A-S clines were calculated using length in place of dry mass ($F_{1,227}$=1.88, p=0.17).

None of the fixed variables included in our global model (environment, voltinism, flight capability and log_{10}-transformed species body mass) could significantly explain variation in A-S clines. Although the best supported model contained environment type (freshwater vs. terrestrial), voltinism, and log_{10}-transformed species body mass as independent variables (R^2=0.26) (see Table S1 in Supplementary Information for AIC output), 6 alternative models had a ΔAICc less than 2; this included an intercept-only model, which contained no independent variables and predicted that the best estimate of A-S clines was the intercept, which did not differ significantly from zero (3.9% change in body mass 150m^{-1}; ±7.1 95% CI).

270   Coleoptera (-1.5% body mass 150m^{-1}; ±4.5 95% CI) and Orthoptera (-0.7% body mass 150m^{-1}

271   ^{1}; ±2.3 95% CI) were among those taxonomic orders to show on average a decrease in body

272   size with increasing altitude, whereas orders such as Diptera (1.2% body mass 150m^{-1}; ±2.3

273   95% CI), Hymenoptera (1.8% body mass 150m^{-1} ±5.2 95% CI) and Lepidoptera (1.1% body

274   mass 150m^{-1}; ±1.2 95% CI) tended to show the opposite pattern (Fig. 4). Yet, these patterns

275   were not strong enough for a significant effect of taxonomic order on the magnitude of the A-

276   S cline to be detected ($F_{11,217}=0.80$, $p=0.64$).

277

278   When we categorized species into those which commonly have one generation or fewer per

279   year, or species that have multiple generations per year, the former showed on average a

280   reduction in their body size with increasing altitude (-0.4% body mass 150m^{-1}; ±1.4 95% CI),

281   whereas the latter showed a mean increase in body size with increasing altitude (1.0% body

282   mass 150m^{-1}; ±2.0 95% CI) (Fig. 5a). However, again these patterns were not strong enough

283   to detect a significant effect of voltinism on the magnitude of the A-S cline ($F_{1,227}=0.66$, $p=0.42$).

284   Similarly, we found no significant effect of flight capability ($F_{1,227}=2.25$, $p=0.14$) (Fig. 5b) or

285    $\log_{10}$ transformed species body mass, when calculated either at 1500m for all species

286   ($F_{1,227}=1.11$, $p=0.29$), or at the mid-altitude of each individual study ($F_{1,227}=0.64$, $p=0.42$). Akin

287   to patterns observed in temperature-size (laboratory and seasonal) and latitude-size

288   gradients, on average freshwater species exhibited stronger positive A-S clines (4.8% body

289   mass 150m^{-1}; ±4.0 95% CI) relative to terrestrial species (-0.1% body mass 150m^{-1}; ±1.2 95%

290   CI), though again this effect was not significant ($F_{1,227}=2.36$, $p=0.13$) (Fig. 5c).

291

292   We tested the robustness of our conclusion that none of the fixed parameters significantly

293   affected the strength of the A-S cline. First, we responded to the more variable strengths of A-

294   S clines observed over relatively small altitudinal ranges by re-running analyses only for A-S

295   clines measured over ≥500m altitudinal range (determined by the point at which variation in

296   the magnitude of A-S clines appeared to stabilize; see Figure S1). Again, none of the fixed

297   variables included in our global model could significantly explain variation in A-S clines.

11

298   Second, re-analysis using A-S clines calculated from original length measurements, in place

299   of dry mass, also revealed no significant effects of any of the fixed variables. The findings from

300   this approach are summarised in more detail in the Supplementary Information.

301

302   Finally, we examined whether A-S clines were similar in direction and magnitude to

303   temperature-size responses measured under controlled laboratory conditions (at the level of

304   taxonomic order, given that A-S and T-S data sets largely contained different species). The

305   plot of order-specific A-S clines (% change in dry mass per 150m) against laboratory T-S

306   responses (% change in dry mass per °C), taken from the comprehensive data set of Horne

307   *et al.* (2015), is shown in Figure 6. Given the low correlation coefficient (r^2=0.24) between A-

308   S clines measured in the field and T-S responses measured in the laboratory, following Smith

309   (2009), we chose not to fit an RMA regression through the data. However, from visual

310   inspection we might ascertain from Figure 6 that temperature is a more important driver of

311   body size change in some taxa compared to others. These comparisons largely test the

312   degree to which A-S clines and laboratory T-S responses are influenced by temperature

313   gradients. However, even when comparing order-specific A-S clines with latitudinal-size

314   clines, which capture variation both in season length and temperature, we did not observe a

315   significant correlation (RMA slope=0.50±0.50 95% CI, r^2=0.01). This leads us to suggest that

316   the confounding factors affecting the direction and magnitude of body size clines across

317   altitudes are much stronger than those across latitudes, particularly given the strong co-

318   variation between L-S clines and laboratory T-S responses (Horne, Hirst & Atkinson 2015).

319

320   **DISCUSSION**

321   Our synthesis of A-S clines in insects reveals widespread variation not just in the direction of

322   these body size gradients, but also in their magnitude. The lack of statistical support for the

323   effects of our model parameters [environment (aquatic vs. terrestrial), voltinism, flight

324   capability and species body mass], and the large amount of unexplained variation in A-S

325   clines, suggests that other environmental factors or life history traits not captured by our model

326   are influencing altitudinal clines in body size.

327

328   We suggest that genotype mixing is more likely along short-distance elevation gradients

329   (compared to longer-distance latitudinal gradients), and thus A-S clines may result primarily

330   from phenotypic plasticity, as is observed in laboratory T-S responses, rather than genetic

331   variation (Keller *et al.* 2013). Thus, if temperature is a major correlate of body size variation

332   with altitude, we might expect a particularly close match between A-S clines and plastic

333   laboratory T-S responses. However, although A-S clines observed at the level of taxonomic

334   order were generally similar in direction to temperature-body size responses measured under

335   controlled laboratory conditions (falling within the non-shaded areas in Fig. 6), they did not

336   significantly correlate in magnitude.

337

338   The lack of correlation between A-S clines and T-S responses is in contrast to the strong

339   correlations observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015),

340   and also between laboratory and seasonal T-S responses (Horne, Hirst & Atkinson 2017),

341   which did not differ significantly from a 1:1 relationship on average. We note however, that the

342   A-S and T-S data sets largely contain different species, with some taxonomic orders better

343   represented than others in our data set. We also re-emphasize that the extent to which these

344   two body size gradients co-vary is based upon the assumption that temperature on average

345   declines by 1°C per 150m increase in altitude, which does not capture the variation between

346   study locations (Hodkinson 2005).

347

348   Another counter argument to our conclusion that the environmental parameters confounding

349   the effects of temperature are stronger across altitudes than across latitudes, is that the strong

350   correlation observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015) is

351   due to the inclusion of additional taxonomic orders, particularly aquatic crustaceans. Yet, even

352   when those orders not present in the A-S data set are excluded from the data of Horne, Hirst

353   and Atkinson (2015), we still observe a significant negative correlation (inferred from 95% CIs)

354   between order-specific laboratory T-S responses and L-S clines (RMA slope=-0.55±0.27 95%

355   CI) which explains much of the variability in the data (r^2=0.88). Therefore, we suggest that the

356   mismatch in the magnitude of A-S clines and laboratory T-S responses arises from other

357   environmental parameters confounding the effects of temperature on the magnitude of A-S

358   clines.

359

360   In previous studies (Chown & Klok 2003; Shelomi 2012), differences in the direction of A-S

361   clines were observed between taxa with different reproductive strategies (i.e. voltinism), which

362   corresponds with intraspecific body size patterns observed across latitudes, and also with T-

363   S responses measured in the laboratory (Chown & Gaston 1999; Blanckenhorn & Demont

364   2004; Horne, Hirst & Atkinson 2015). Season length generally declines at higher elevations,

365   and unlike species with multiple generations per year, adult sizes of univoltine species are

366   likely to be much more sensitive to changes in seasonality: as season length decreases, so

367   does time available for growth and reproduction, which imposes limits on size at maturity; thus,

368   the maximisation of fitness may come from utilising as much of the available season length as

369   possible. In contrast, generation time in multivoltine species is relatively short and

370   consequently adult size in each generation is less sensitive to changes in season length;

371   instead, size at maturity in multivoltine species is predicted to be more dependent on changes

372   in temperature (Chown & Gaston 1999; Kozłowski, Czarnoleski & Danko 2004). Yet here, we

373   found no significant effect of voltinism on A-S clines.

374

375   Voltinism itself can vary with environmental conditions within a species (Zeuss, Brunzel &

376   Brandl 2016), and this has the potential to obscure body size gradients in the field. It is

377   plausible that species with longer generation times may switch between a bivoltine and

378   univoltine life cycle at higher altitudes, for example, allowing more time for growth despite a

379   decrease in season length, thus weakening any negative cline in body size. Such a switch in

380   voltinism is predicted to result in a 'saw-tooth' body size cline (Roff 1980), and consequently

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381 the slope of size change across the entire altitudinal gradient would appear shallower.

382 Similarly, although multivoltine species with very short generation times are predicted to

383 increase in size at higher colder altitudes, in species with just two generations per year,

384 variation in size at maturity may be more strongly dependent on season length than

385 temperature. Thus, these species may be more likely to exhibit a negative A-S cline. Of

386 course, the extent to which season length decreases with altitude can vary between study

387 locations, influencing the magnitude of A-S clines; for example, Chown and Klok (2003) found

388 opposing A-S clines in weevil species sampled from two regions that differed in their

389 seasonality. Although altitudinal variation in seasonality is often more pronounced at higher

390 latitudes (Körner 2000), latitude is only a proxy for seasonality, corresponding with relatively

391 broad changes in environmental conditions. We lack the high-resolution climate data,

392 including changes in season length and resource availability with altitude, that would provide

393 a much more robust test of this hypothesis. This lack of high-resolution data is particularly

394 important for mountainous regions, as fine-scale variation in environmental conditions due to

395 local topography can result in microclimates that differ from surrounding regions (Suggitt *et al.*

396 2011).

397

398 We also hypothesised that a reduction in oxygen partial pressure (i.e. a reduction in oxygen

399 availability per unit volume of air) with altitude might limit energy available for growth if species

400 cannot increase their air intake, confounding the effects of temperature on body size (Peacock

401 1998). Indeed, under experimental conditions, lower proportional oxygen concentrations have

402 been shown to lead to a reduction in size-at-stage (Frazier, Woods & Harrison 2001; Peck &

403 Maddrell 2005; Atkinson, Morley & Hughes 2006; Walczyńska *et al.* 2015). This might act to

404 weaken any potential increase in adult body size at higher colder altitudes, or even exacerbate

405 body size reduction in those species that already grow to a smaller size with decreasing

406 season length. In aquatic species, a greater increase in oxygen demand than supply with

407 warming has also been proposed as an important driver of body size reduction (Atkinson,

408 Morley & Hughes 2006), with species developing in water exhibiting particularly strong

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409   reductions in size with warming and towards the equator compared to those on land (Forster,

410   Hirst & Atkinson 2012; Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). These

411   patterns would correspond with an increase in size at higher colder altitudes, and although not

412   significant, on average aquatic species did show stronger positive A-S clines relative to

413   terrestrial species. Obtaining data for freshwater species proved extremely difficult, with many

414   studies having explored inter- as opposed to intra-specific patterns in body size.

415   Consequently, species from aquatic environments contribute only a very small proportion of

416   the data analysed here. A-S clines for aquatic species represent a significant gap in the

417   literature, and we would strongly encourage field studies to incorporate such measurements

418   in the future. We caution, however, that many insects lay their eggs upstream, which can then

419   drift downstream, making it difficult to know exactly where they developed.

420

421   Of the taxonomic orders better represented in our data set, Orthopterans exhibited a

422   particularly weak A-S cline relative to their T-S response. Although overall we found no

423   significant effect of flight ability on the magnitude of the A-S cline, Orthoptera are

424   hemimetabolous, producing nymphs that resemble the adult phase (Daly, Doyen & Ehrlich

425   1978). Although nymphs cannot fly, the increased mobility of individuals during ontogeny (c.f.

426   relatively sedentary larvae in holometabolous taxa, such as Diptera) could potentially obscure

427   A-S clines observed in this taxon. In a study of the bush cricket *Pholidoptera griseoaptera*,

428   juveniles and imagos exhibited equally good dispersal ability (Diekötter *et al.* 2005), whilst

429   Alexander (1964) reported collecting many species of montane grasshopper as much as

430   several thousand feet above their normal breeding range. Consequently, altitude at time of

431   collection may not necessarily resemble altitude during ontogeny in these more mobile

432   species, increasing the likelihood of dispersal and/or migration between sampling locations.

433   Even when stricter screening criteria excluded clines covering less than 500m in altitude,

434   potentially removing some of the noise in the data caused by the movement of individuals

435   between nearby sampling locations, the estimated average A-S cline for univoltine Orthoptera

436   was still half that of their laboratory T-S response. One alternative way to examine the effect

16

437 of dispersal on A-S clines would be to measure body size in individuals grown in field
438 enclosures at different elevations, and which hence would not be able to disperse across
439 altitudes. Such an approach helps exclude not just the effect of dispersive individuals, but also
440 other confounding biotic factors, such as certain types of predation and competition (e.g. Joern
441 & Klucas 1993; Blanckenhorn 1998; Darr *et al.* 2016). However, lack of such data prevented
442 a quantitative investigation of species with highly mobile juveniles reared inside enclosures
443 throughout their ontogeny at natural rather than manipulated densities, and which would allow
444 body size to be compared within versus outside enclosures across a suitable altitudinal range.
445
446 Given that altitudinal environmental changes can differ substantially between study locations,
447 currently it seems that only broad predictions can be made about the direction of A-S clines.
448 Although altitude correlates with average temperature, it also correlates with many other
449 environmental parameters, and disentangling their effects is challenging. For example, a
450 reduction in the partial pressure of respiratory gases with altitude not only reduces oxygen
451 availability, but also serves to reduce overall air density (Hodkinson 2005), which can be
452 particularly problematic for active fliers. Selection in these species may favour larger wings
453 with greater surface area to maintain flight performance at higher altitudes, or alternatively,
454 smaller body mass to reduce wing loading (Hodkinson 2005). A recent study also showed that,
455 on average, predation risk for insects increases with decreasing latitude and elevation (Roslin
456 *et al.* 2017), and greater predation risk at lower altitudes may select for earlier maturation at a
457 smaller adult size. To more accurately predict the magnitude of intraspecific body size clines,
458 a detailed understanding of the life history of the species or taxonomic group in question,
459 coupled with fine scale information on local environmental conditions and topography, should
460 be provided on a case by case basis. Nevertheless, by comparing A-S clines with laboratory
461 T-S responses, in which many confounding variables are controlled, we can begin to postulate
462 which environmental factors and/or life history traits are important in driving variation in A-S
463 clines between different taxonomic groups. Although we observe a relatively close match in
464 the magnitude of A-S clines and laboratory T-S responses in some taxa (e.g. Diptera,

17

465   Coleoptera, Hymenoptera), suggesting a particularly important role for temperature,

466   deviations away from a 1:1 relationship in other taxa (e.g. Orthoptera) may indicate that other

467   environmental variables confound the effects of temperature on body size along elevation

468   gradients.

469

470   These observations may be particularly informative for predicting effects on body size of

471   terrestrial ectotherms that are shifting their altitudinal distribution with climate warming (Chen

472   *et al.* 2011). These range shifts may enable species to track favourable thermal environments,

473   thereby negating any potential effects of warming on body size; yet, warming of high-altitude

474   environments will not be accompanied by similar changes in other environmental parameters

475   (e.g. oxygen partial pressure, photoperiod). Therefore, in those species whose adult size is

476   influenced more by these other variables, altitudinal range shifts may still be accompanied by

477   significant changes in the size of species.

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613

614 **SUPPORTING INFORMATION**

615 Additional supporting information may be found in the online version of this article.

616

617 **Data Set S1.xlsx:** Raw data used in this study.

618 **Supplementary Information.pdf:** Table S1, Table S2, Figure S1 and additional length-

619 based analyses.

620

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FIGURE LEGENDS

629

630 **Figure 1.** The predicted effects of i) decreasing temperature, ii) decreasing oxygen partial

631 pressure and iii) decreasing season length with increasing altitude on size at maturity in

632 insects. Body size is predicted to increase with decreasing temperature, following the

633 temperature-size rule, particularly in multivoltine species. However, a decrease in the partial

634 pressure of oxygen at higher altitudes may reduce the available energy for growth, acting in

635 the opposite direction to constrain body size. Similarly, shorter season length is predicted to

636 decrease body size at higher altitude in univoltine species, as resource availability and time

637 available for growth is reduced.

638

639 **Figure 2.** World map indicating the location of studies (n=50) from which altitudinal-size clines

640 were recorded.

641

642 **Figure 3.** Altitudinal-size clines (% change in body mass per 150m) of individual species,

643 including both males and females, categorized by taxonomic order (Amphipoda (A), Araneae

644 (Ar), Coleoptera (Col), Diptera (Dip), Hemiptera (He), Hymenoptera (Hym), Isopoda (I),

645 Lepidoptera (Lep), Orthoptera (Orth), Plecoptera (Plec), Trichoptera (T) and Trombidiformes

646 (Tr)). Species within each order are also categorized by voltinism: one generation or less per

647 year (unshaded background), multiple generations per year (shaded background). Dashed

648 horizontal line indicates no change in body size with altitude. Dashed vertical lines divide

649 taxonomic orders.

650

651 **Figure 4.** A-S clines (±95% CI), averaged by taxonomic order and by voltinism (one generation

652 or less per year (≤1), multiple generations per year (>1)). The number of species within each

653 order is given in brackets. Dashed horizontal line indicates no change in body size with altitude

654 There is no significant effect of taxonomic order on the strength of the A-S cline.

655

656 **Figure 5.** Mean A-S clines (±95% CI) in (A) species with one generation or less per year vs.

657 those with multiple generations per year, (B) flightless vs. flying species, and (C) freshwater

658 vs. terrestrial species. The number of A-S clines are given in brackets. Dashed horizontal line

659 indicates no change in body size with altitude. Shared letters above data points indicate no

660 significant difference.

661

662 **Figure 6.** A comparison of order-specific altitudinal-size clines (% change in body mass per

663 150m ±SE) with laboratory temperature-size responses (% change in body mass per °C ±SE)

664 for species with one generation or less per year (≤1; open symbols) and multiple generations

665 per year (>1; shaded symbols). Dashed line indicates a 1:1 relationship. Taxonomic orders

666 which fall within the shaded quadrants exhibit a mismatch in the direction of their mean A-S
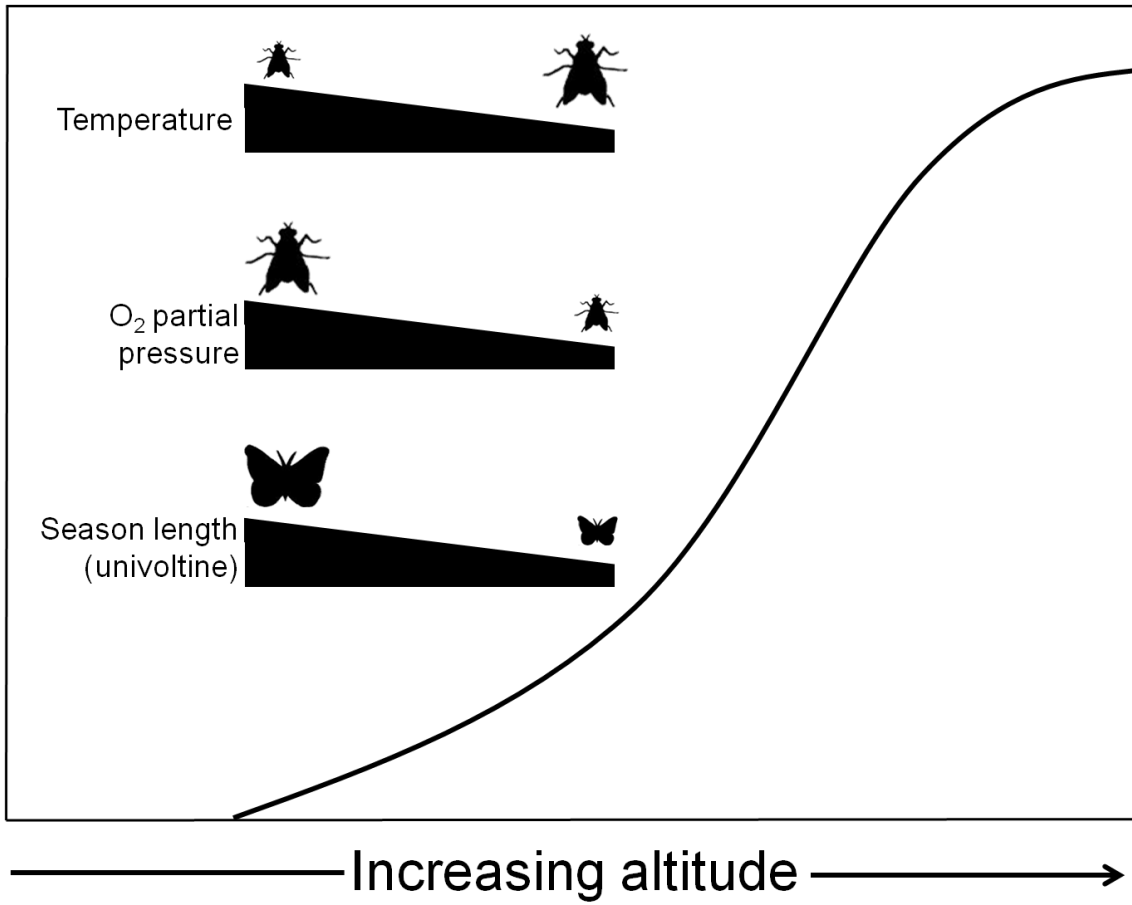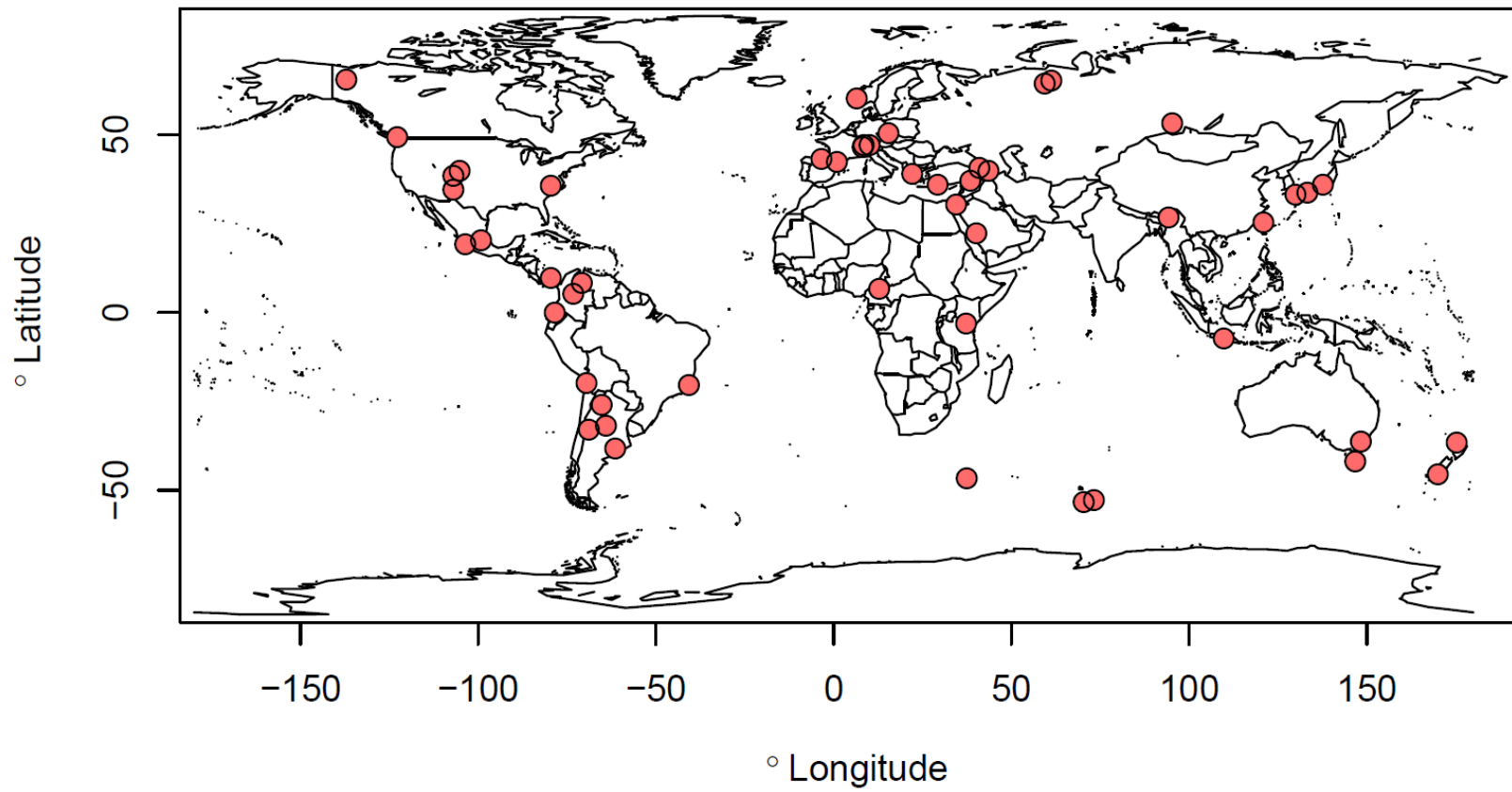
667 cline and T-S gradient.

Temperature

O$_2$ partial pressure
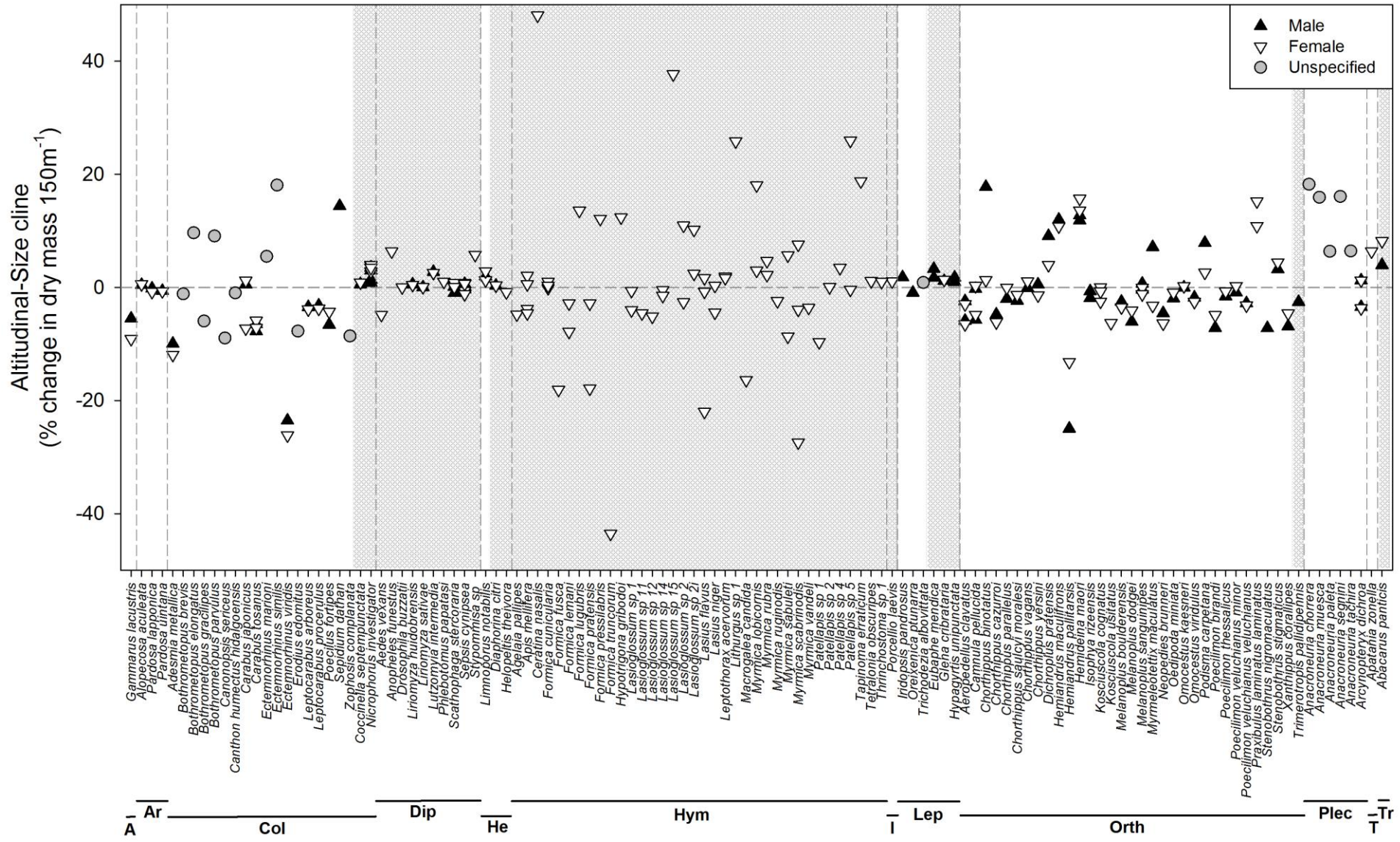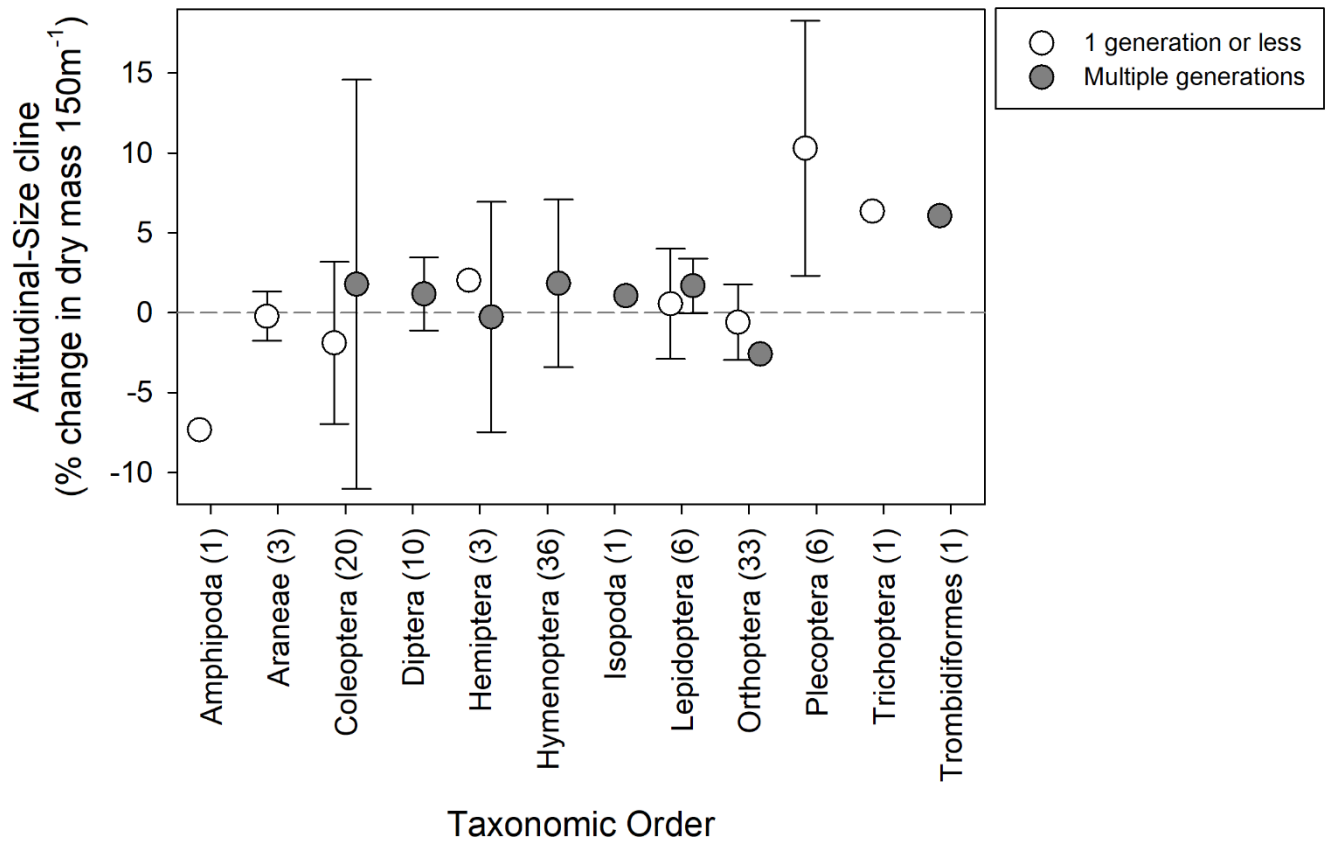
Season length
(univoltine)

Increasing altitude

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6