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**Insect temperature-body size trends common to laboratory,
latitudinal and seasonal gradients are not found across altitudes**

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Author Contributions

CH, AGH and DA designed the study and wrote the paper. CH collected the data and performed the statistical analyses.

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.

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

responses in the field, despite a number of confounding factors that can also influence body size (e.g. resource availability, mortality risk and competition) (Chown & Gaston 2010).

As with increasing latitude, temperature commonly declines with increasing altitude. On average, temperature falls by 5.5 to 6.5°C per 1000m increase in elevation (Anslow & Shawn 2002), though this of course varies considerably depending on other climatic and topographic influences, including aspect, wind speed and cloud cover amongst others (Hodkinson 2005). We might therefore expect those species that grow to a larger adult size in the cold and with increasing latitude will also exhibit a positive cline in body size with increasing altitude. To our knowledge, most large syntheses of altitude-body size (A-S) clines have predominantly focused on whether body size gradients were negative, positive or non-significant (e.g. Dillon, Frazier & Dudley 2006; Shelomi 2012). Yet, by quantifying and comparing the magnitude of A-S clines between species and among higher taxonomic groups, we can begin to link variation in the strength of these body size gradients with key life history characteristics, habitat and/or functional groups. Furthermore, quantitative data capturing the magnitude of A-S clines allow for a more direct comparison with laboratory temperature-size (T-S) responses.

To our knowledge, A-S clines and laboratory T-S responses have not previously been compared quantitatively. Such a comparison provides an opportunity to explore the extent to which changes in other environmental parameters might be confounding the effects of temperature across altitude in the field. For example, an increase in altitude is also accompanied by a decline in the partial pressure of atmospheric gases, including oxygen, which decreases near linearly with altitude, such that inspired oxygen pressure at 5500m elevation is 50% of that at sea level (Peacock 1998). The length of the season in which individuals are able to grow also typically declines with increasing altitude: these growth conditions encompass not just appropriate temperatures but also extent of resource availability (Hodkinson 2005) (see Fig. 1 for an illustration of the predicted effects of these key

parameters on body size). Other biologically relevant environmental factors that increase with altitude include wind speed, precipitation, and UV radiation (Hodkinson 2005).

Variation in the direction of A-S clines among species has often been attributed to seasonality and differences in reproductive strategy (Chown & Klok 2003; Shelomi 2012). Multivoltine species are predicted to grow to a larger adult size at higher colder altitudes, in accordance with the temperature-size rule (Atkinson 1994). In contrast, univoltine species are much more dependent on season length and hence on time available for growth (often positively correlated with temperature) (Chown & Gaston 1999; Blanckenhorn & Demont 2004; Kozłowski, Czarnoleski & Danko 2004; Shelomi 2012). Consequently, univoltine species are predicted to reduce their body size with increasing altitude. The extent to which season length decreases with increasing altitude may also influence the magnitude of A-S clines, particularly in univoltine species for which time available for growth is limiting. Increased time constraints with elevation may be especially important in more thermally seasonal environments, such as at higher latitudes, where the decline in season length along altitudinal gradients is much more pronounced than in the tropics (Körner 2000; Chown & Klok 2003). Of course, voltinism itself can vary with environmental conditions, and any switch in reproductive strategy could also affect the magnitude of any apparent size change (Roff 1980; Mousseau & Roff 1989; Zeuss, Brunzel & Brandl 2016).

The spatial distance covered by an A-S cline and the dispersal or migratory ability of a species may also affect the magnitude of the A-S cline. Latitudinal-size (L-S) clines are typically measured over larger distances than A-S clines, sometimes spanning thousands of kilometres, whole continents, and entire species' ranges (e.g. Hassall 2013). By contrast, altitudinal clines in the size of species are often studied along localized transects (e.g. Smith *et al.* 2000), or in some cases over tens to hundreds of kilometres at most (e.g. Eweleit & Reinhold 2014). Consequently, populations are more likely to become interconnected by individual movement along relatively short-distance elevation gradients (for example by flight

and/or passive transport by wind) than along longer-distance latitudinal transects. Therefore, adults collected at one altitude might have developed at another, where environmental conditions were very different. This shift between environmental conditions during ontogeny is more likely for more mobile species with greater dispersal ability, such as winged species capable of flight (Roff & Fairbairn 2007). As a result, A-S clines in more mobile species may be obscured or appear weaker.

Here we explore these different potential influences on A-S clines by quantifying and examining variation in intraspecific A-S clines in insects, along with a small number of arachnids and Crustacea. We compare the magnitude and direction of A-S clines based on taxonomy, voltinism, flight capability, and environment type (freshwater vs. terrestrial). We then compare these clines to T-S responses measured under controlled laboratory conditions, to determine whether these major size gradients co-vary, and thus to better understand to what extent changes in other environmental parameters might be confounding the effects of temperature on body size across altitude in the field.

MATERIALS AND METHODS

To quantitatively describe species-specific altitudinal-size clines, we searched the literature using the Web of Science database (<http://apps.webofknowledge.com/>) and Google Scholar for published field records of adult body size measured at different altitudes, covering at least 150m in altitudinal range. Most studies were conducted along individual mountain transects, but occasionally sampling locations were more widely distributed within a local area or region; in these instances, we only accepted studies in which the sampling locations were distributed over less than 5° latitude. The primary search term combinations used were: (“altitud*” OR “elevation” OR “mountain”) AND “size” AND (“arthropod” OR “insect” OR “[<insert taxonomic order or common name of taxon>]”). We also used reference lists from the papers we found to identify additional key literature. Adult size data were collected as lengths, or dry, wet or carbon masses and subsequently standardised to dry mass (mg) using published intra-

specific regressions and conversion factors (see Dataset S1 in Supplementary Information). In cases where species-specific regressions were unavailable, regressions for related species, or more general inter-specific regressions were used. All altitudinal measurements were standardized to metres above sea level.

We derived species-specific slopes of ordinary least-squares (OLS) regressions between ln-transformed dry mass (mg) and altitude (m). Slopes were calculated for each sex separately where these data were available. We used this exponential function of body mass as it has consistently been found to be the best for modelling body size gradients with both temperature (in the laboratory and seasonally) and across latitude (Forster, Hirst & Atkinson 2012; Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). Further, this exponential function allowed us to easily compare these different size gradients with one another. Altitudinal-size clines were then transformed into percentage change in dry mass per metre of elevation, using the formula $(\exp^{(\text{slope})} - 1) * 100 = \% \text{ change in mass per m}$ (Forster, Hirst & Atkinson 2012). Thus, a positive cline denotes an increase in body size with increasing altitude, whereas a negative cline denotes a decrease in body size with increasing altitude. In general, temperature decreases with increasing altitude at a rate of between 5.5°C and 6.5°C per 1000m (Anslow & Shawn 2002), which equates to approximately 1°C per 150m elevation. Thus, we also calculated the percentage change in dry mass per 150m, using the formula $(\exp^{(\text{slope} \times 150)} - 1) * 100 = \% \text{ change in mass per 150m elevation (or approximately per } ^\circ\text{C)}$. This measure of size change allowed us to more appropriately compare the strength of A-S clines measured in the field with T-S responses (% change in mass per °C) measured in the laboratory.

Statistical analyses

All statistical analyses were conducted in R (version 3.4.1) (R Core Team 2014). Using A-S clines (% change in mass per 150m) as the dependent variable, we compared several candidate models to best predict variation in the magnitude and direction of A-S clines based

on the Akaike's information criterion (AIC). The following were incorporated as fixed variables in a global linear mixed effects model (using package lme4): environment (freshwater vs. terrestrial), voltinism, categorised here as species that are generally regarded as having ≤ 1 generation per year (includes univoltine) or >1 generation per year (multivoltine), flight capability (flying vs. flightless), and $\log_{10}$ -transformed species body mass (calculated at 1500m altitude using species-specific A-S slopes, and representing the approximate mid-point of the altitudinal range across all the studies in our data set). $\log_{10}$ -transformed species body mass was included as a covariate to determine if A-S clines were dependent on the body size of the species. A-S clines from multiple studies of the same species were included in our analyses; thus, given that species have shared evolutionary histories and so are not completely independent, we included levels of taxonomic classification (order, family, and species) as nested (hierarchical) random effects on the intercept in all models to help control for phylogeny, and to account for the disproportionate representation of taxonomic orders in our data set. Given that A-S clines did not differ significantly between males and females, and sex was not reported in all studies, we chose to exclude it as a random effect in the models. A-S clines for multiple species were sometimes measured in similar study locations or regions, and so we also included location (categorized using latitude and longitude) as a random effect on the intercept, to help control for potential geographical clumping of the data. Finally, we accounted for variation in information quality by weighting each A-S cline by the inverse of the variance of its slope estimate (using the 'weights' function in R) (Koricheva, Gurevitch & Mengersen 2013). This helped to account for the fact that A-S clines were derived from data that varied in their goodness of fit between studies and species. We compared all possible combinations of the global model terms using the dredge function in the MuMIn package (Barton 2017); this included an intercept-only model, which contained no independent variables and predicted that the best estimate of A-S clines was the intercept. The best model was identified as that with the lowest small-samples corrected AIC (AICc). Where the difference between a model's AICc and the lowest AICc (i.e. $\Delta AICc$) was <2 , a set of best fit 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 $\Delta AICc$ 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).

Coleoptera (-1.5% body mass 150m^{-1} ; ± 4.5 95% CI) and Orthoptera (-0.7% body mass 150m^{-1} ; ± 2.3 95% CI) were among those taxonomic orders to show on average a decrease in body size with increasing altitude, whereas orders such as Diptera (1.2% body mass 150m^{-1} ; ± 2.3 95% CI), Hymenoptera (1.8% body mass 150m^{-1} ± 5.2 95% CI) and Lepidoptera (1.1% body mass 150m^{-1} ; ± 1.2 95% CI) tended to show the opposite pattern (Fig. 4). Yet, these patterns were not strong enough for a significant effect of taxonomic order on the magnitude of the A-S cline to be detected ($F_{11,217}=0.80$, $p=0.64$).

When we categorized species into those which commonly have one generation or fewer per year, or species that have multiple generations per year, the former showed on average a reduction in their body size with increasing altitude (-0.4% body mass 150m^{-1} ; ± 1.4 95% CI), whereas the latter showed a mean increase in body size with increasing altitude (1.0% body mass 150m^{-1} ; ± 2.0 95% CI) (Fig. 5a). However, again these patterns were not strong enough to detect a significant effect of voltinism on the magnitude of the A-S cline ($F_{1,227}=0.66$, $p=0.42$). Similarly, we found no significant effect of flight capability ($F_{1,227}=2.25$, $p=0.14$) (Fig. 5b) or $\log_{10}$ transformed species body mass, when calculated either at 1500m for all species ($F_{1,227}=1.11$, $p=0.29$), or at the mid-altitude of each individual study ($F_{1,227}=0.64$, $p=0.42$). Akin to patterns observed in temperature-size (laboratory and seasonal) and latitude-size gradients, on average freshwater species exhibited stronger positive A-S clines (4.8% body mass 150m^{-1} ; ± 4.0 95% CI) relative to terrestrial species (-0.1% body mass 150m^{-1} ; ± 1.2 95% CI), though again this effect was not significant ($F_{1,227}=2.36$, $p=0.13$) (Fig. 5c).

We tested the robustness of our conclusion that none of the fixed parameters significantly affected the strength of the A-S cline. First, we responded to the more variable strengths of A-S clines observed over relatively small altitudinal ranges by re-running analyses only for A-S clines measured over $\geq 500\text{m}$ altitudinal range (determined by the point at which variation in the magnitude of A-S clines appeared to stabilize; see Figure S1). Again, none of the fixed variables included in our global model could significantly explain variation in A-S clines.

Second, re-analysis using A-S clines calculated from original length measurements, in place of dry mass, also revealed no significant effects of any of the fixed variables. The findings from this approach are summarised in more detail in the Supplementary Information.

Finally, we examined whether A-S clines were similar in direction and magnitude to temperature-size responses measured under controlled laboratory conditions (at the level of taxonomic order, given that A-S and T-S data sets largely contained different species). The plot of order-specific A-S clines (% change in dry mass per 150m) against laboratory T-S responses (% change in dry mass per °C), taken from the comprehensive data set of Horne *et al.* (2015), is shown in Figure 6. Given the low correlation coefficient ($r^2=0.24$) between A-S clines measured in the field and T-S responses measured in the laboratory, following Smith (2009), we chose not to fit an RMA regression through the data. However, from visual inspection we might ascertain from Figure 6 that temperature is a more important driver of body size change in some taxa compared to others. These comparisons largely test the degree to which A-S clines and laboratory T-S responses are influenced by temperature gradients. However, even when comparing order-specific A-S clines with latitudinal-size clines, which capture variation both in season length and temperature, we did not observe a significant correlation (RMA slope= 0.50 ± 0.50 95% CI, $r^2=0.01$). This leads us to suggest that the confounding factors affecting the direction and magnitude of body size clines across altitudes are much stronger than those across latitudes, particularly given the strong co-variation between L-S clines and laboratory T-S responses (Horne, Hirst & Atkinson 2015).

DISCUSSION

Our synthesis of A-S clines in insects reveals widespread variation not just in the direction of these body size gradients, but also in their magnitude. The lack of statistical support for the effects of our model parameters [environment (aquatic vs. terrestrial), voltinism, flight capability and species body mass], and the large amount of unexplained variation in A-S

clines, suggests that other environmental factors or life history traits not captured by our model are influencing altitudinal clines in body size.

We suggest that genotype mixing is more likely along short-distance elevation gradients (compared to longer-distance latitudinal gradients), and thus A-S clines may result primarily from phenotypic plasticity, as is observed in laboratory T-S responses, rather than genetic variation (Keller *et al.* 2013). Thus, if temperature is a major correlate of body size variation with altitude, we might expect a particularly close match between A-S clines and plastic laboratory T-S responses. However, although A-S clines observed at the level of taxonomic order were generally similar in direction to temperature-body size responses measured under controlled laboratory conditions (falling within the non-shaded areas in Fig. 6), they did not significantly correlate in magnitude.

The lack of correlation between A-S clines and T-S responses is in contrast to the strong correlations observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015), and also between laboratory and seasonal T-S responses (Horne, Hirst & Atkinson 2017), which did not differ significantly from a 1:1 relationship on average. We note however, that the A-S and T-S data sets largely contain different species, with some taxonomic orders better represented than others in our data set. We also re-emphasize that the extent to which these two body size gradients co-vary is based upon the assumption that temperature on average declines by 1°C per 150m increase in altitude, which does not capture the variation between study locations (Hodkinson 2005).

Another counter argument to our conclusion that the environmental parameters confounding the effects of temperature are stronger across altitudes than across latitudes, is that the strong correlation observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015) is due to the inclusion of additional taxonomic orders, particularly aquatic crustaceans. Yet, even when those orders not present in the A-S data set are excluded from the data of Horne, Hirst

and Atkinson (2015), we still observe a significant negative correlation (inferred from 95% CIs) between order-specific laboratory T-S responses and L-S clines (RMA slope= -0.55 ± 0.27 95% CI) which explains much of the variability in the data ($r^2=0.88$). Therefore, we suggest that the mismatch in the magnitude of A-S clines and laboratory T-S responses arises from other environmental parameters confounding the effects of temperature on the magnitude of A-S clines.

In previous studies (Chown & Klok 2003; Shelomi 2012), differences in the direction of A-S clines were observed between taxa with different reproductive strategies (i.e. voltinism), which corresponds with intraspecific body size patterns observed across latitudes, and also with T-S responses measured in the laboratory (Chown & Gaston 1999; Blanckenhorn & Demont 2004; Horne, Hirst & Atkinson 2015). Season length generally declines at higher elevations, and unlike species with multiple generations per year, adult sizes of univoltine species are likely to be much more sensitive to changes in seasonality: as season length decreases, so does time available for growth and reproduction, which imposes limits on size at maturity; thus, the maximisation of fitness may come from utilising as much of the available season length as possible. In contrast, generation time in multivoltine species is relatively short and consequently adult size in each generation is less sensitive to changes in season length; instead, size at maturity in multivoltine species is predicted to be more dependent on changes in temperature (Chown & Gaston 1999; Kozłowski, Czarnoleski & Danko 2004). Yet here, we found no significant effect of voltinism on A-S clines.

Voltinism itself can vary with environmental conditions within a species (Zeuss, Brunzel & Brandl 2016), and this has the potential to obscure body size gradients in the field. It is plausible that species with longer generation times may switch between a bivoltine and univoltine life cycle at higher altitudes, for example, allowing more time for growth despite a decrease in season length, thus weakening any negative cline in body size. Such a switch in voltinism is predicted to result in a 'saw-tooth' body size cline (Roff 1980), and consequently

the slope of size change across the entire altitudinal gradient would appear shallower. Similarly, although multivoltine species with very short generation times are predicted to increase in size at higher colder altitudes, in species with just two generations per year, variation in size at maturity may be more strongly dependent on season length than temperature. Thus, these species may be more likely to exhibit a negative A-S cline. Of course, the extent to which season length decreases with altitude can vary between study locations, influencing the magnitude of A-S clines; for example, Chown and Klok (2003) found opposing A-S clines in weevil species sampled from two regions that differed in their seasonality. Although altitudinal variation in seasonality is often more pronounced at higher latitudes (Körner 2000), latitude is only a proxy for seasonality, corresponding with relatively broad changes in environmental conditions. We lack the high-resolution climate data, including changes in season length and resource availability with altitude, that would provide a much more robust test of this hypothesis. This lack of high-resolution data is particularly important for mountainous regions, as fine-scale variation in environmental conditions due to local topography can result in microclimates that differ from surrounding regions (Suggitt *et al.* 2011).

We also hypothesised that a reduction in oxygen partial pressure (i.e. a reduction in oxygen availability per unit volume of air) with altitude might limit energy available for growth if species cannot increase their air intake, confounding the effects of temperature on body size (Peacock 1998). Indeed, under experimental conditions, lower proportional oxygen concentrations have been shown to lead to a reduction in size-at-stage (Frazier, Woods & Harrison 2001; Peck & Maddrell 2005; Atkinson, Morley & Hughes 2006; Walczyńska *et al.* 2015). This might act to weaken any potential increase in adult body size at higher colder altitudes, or even exacerbate body size reduction in those species that already grow to a smaller size with decreasing season length. In aquatic species, a greater increase in oxygen demand than supply with warming has also been proposed as an important driver of body size reduction (Atkinson, Morley & Hughes 2006), with species developing in water exhibiting particularly strong

reductions in size with warming and towards the equator compared to those on land (Forster, Hirst & Atkinson 2012; Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). These patterns would correspond with an increase in size at higher colder altitudes, and although not significant, on average aquatic species did show stronger positive A-S clines relative to terrestrial species. Obtaining data for freshwater species proved extremely difficult, with many studies having explored inter- as opposed to intra-specific patterns in body size. Consequently, species from aquatic environments contribute only a very small proportion of the data analysed here. A-S clines for aquatic species represent a significant gap in the literature, and we would strongly encourage field studies to incorporate such measurements in the future. We caution, however, that many insects lay their eggs upstream, which can then drift downstream, making it difficult to know exactly where they developed.

Of the taxonomic orders better represented in our data set, Orthopterans exhibited a particularly weak A-S cline relative to their T-S response. Although overall we found no significant effect of flight ability on the magnitude of the A-S cline, Orthoptera are hemimetabolous, producing nymphs that resemble the adult phase (Daly, Doyen & Ehrlich 1978). Although nymphs cannot fly, the increased mobility of individuals during ontogeny (c.f. relatively sedentary larvae in holometabolous taxa, such as Diptera) could potentially obscure A-S clines observed in this taxon. In a study of the bush cricket *Pholidoptera griseoaptera*, juveniles and imagos exhibited equally good dispersal ability (Diekötter *et al.* 2005), whilst Alexander (1964) reported collecting many species of montane grasshopper as much as several thousand feet above their normal breeding range. Consequently, altitude at time of collection may not necessarily resemble altitude during ontogeny in these more mobile species, increasing the likelihood of dispersal and/or migration between sampling locations. Even when stricter screening criteria excluded clines covering less than 500m in altitude, potentially removing some of the noise in the data caused by the movement of individuals between nearby sampling locations, the estimated average A-S cline for univoltine Orthoptera was still half that of their laboratory T-S response. One alternative way to examine the effect

of dispersal on A-S clines would be to measure body size in individuals grown in field enclosures at different elevations, and which hence would not be able to disperse across altitudes. Such an approach helps exclude not just the effect of dispersive individuals, but also other confounding biotic factors, such as certain types of predation and competition (e.g. Joern & Klucas 1993; Blanckenhorn 1998; Darr *et al.* 2016). However, lack of such data prevented a quantitative investigation of species with highly mobile juveniles reared inside enclosures throughout their ontogeny at natural rather than manipulated densities, and which would allow body size to be compared within versus outside enclosures across a suitable altitudinal range.

Given that altitudinal environmental changes can differ substantially between study locations, currently it seems that only broad predictions can be made about the direction of A-S clines. Although altitude correlates with average temperature, it also correlates with many other environmental parameters, and disentangling their effects is challenging. For example, a reduction in the partial pressure of respiratory gases with altitude not only reduces oxygen availability, but also serves to reduce overall air density (Hodkinson 2005), which can be particularly problematic for active fliers. Selection in these species may favour larger wings with greater surface area to maintain flight performance at higher altitudes, or alternatively, smaller body mass to reduce wing loading (Hodkinson 2005). A recent study also showed that, on average, predation risk for insects increases with decreasing latitude and elevation (Roslin *et al.* 2017), and greater predation risk at lower altitudes may select for earlier maturation at a smaller adult size. To more accurately predict the magnitude of intraspecific body size clines, a detailed understanding of the life history of the species or taxonomic group in question, coupled with fine scale information on local environmental conditions and topography, should be provided on a case by case basis. Nevertheless, by comparing A-S clines with laboratory T-S responses, in which many confounding variables are controlled, we can begin to postulate which environmental factors and/or life history traits are important in driving variation in A-S clines between different taxonomic groups. Although we observe a relatively close match in the magnitude of A-S clines and laboratory T-S responses in some taxa (e.g. Diptera,

Coleoptera, Hymenoptera), suggesting a particularly important role for temperature, deviations away from a 1:1 relationship in other taxa (e.g. Orthoptera) may indicate that other environmental variables confound the effects of temperature on body size along elevation gradients.

These observations may be particularly informative for predicting effects on body size of terrestrial ectotherms that are shifting their altitudinal distribution with climate warming (Chen *et al.* 2011). These range shifts may enable species to track favourable thermal environments, thereby negating any potential effects of warming on body size; yet, warming of high-altitude environments will not be accompanied by similar changes in other environmental parameters (e.g. oxygen partial pressure, photoperiod). Therefore, in those species whose adult size is influenced more by these other variables, altitudinal range shifts may still be accompanied by significant changes in the size of species.

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SUPPORTING INFORMATION

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

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

Supplementary Information.pdf: Table S1, Table S2, Figure S1 and additional length-based analyses.

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FIGURE LEGENDS

Figure 1. The predicted effects of i) decreasing temperature, ii) decreasing oxygen partial pressure and iii) decreasing season length with increasing altitude on size at maturity in insects. Body size is predicted to increase with decreasing temperature, following the temperature-size rule, particularly in multivoltine species. However, a decrease in the partial pressure of oxygen at higher altitudes may reduce the available energy for growth, acting in the opposite direction to constrain body size. Similarly, shorter season length is predicted to decrease body size at higher altitude in univoltine species, as resource availability and time available for growth is reduced.

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

Figure 3. Altitudinal-size clines (% change in body mass per 150m) of individual species, including both males and females, categorized by taxonomic order (Amphipoda (A), Araneae (Ar), Coleoptera (Col), Diptera (Dip), Hemiptera (He), Hymenoptera (Hym), Isopoda (I), Lepidoptera (Lep), Orthoptera (Orth), Plecoptera (Plec), Trichoptera (T) and Trombidiformes (Tr)). Species within each order are also categorized by voltinism: one generation or less per year (unshaded background), multiple generations per year (shaded background). Dashed horizontal line indicates no change in body size with altitude. Dashed vertical lines divide taxonomic orders.

Figure 4. A-S clines ($\pm 95\%$ CI), averaged by taxonomic order and by voltinism (one generation or less per year (≤ 1), multiple generations per year (> 1)). The number of species within each order is given in brackets. Dashed horizontal line indicates no change in body size with altitude. There is no significant effect of taxonomic order on the strength of the A-S cline.

655

656 **Figure 5.** Mean A-S clines ($\pm 95\%$ 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 $\pm$ SE) with laboratory temperature-size responses (% change in body mass per $^{\circ}\text{C} \pm$ 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.

Figure 1

Figure 2

Figure 4

Figure 5

Figure 6