



Environmental drivers of voltinism and body size in insect assemblages across Europe

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ABSTRACT

Aim General geographical patterns of insect body size are still a matter of considerable debate, mainly because the annual number of generations (voltinism) and its relationship with body size have largely been ignored. We present the first analyses of voltinism and body size of insect assemblages at a continental scale using lepidopteran and odonate species. We hypothesize that voltinism is strongly driven by environmental conditions and constrains body size on macroecological scales.

Location Europe.

Methods We compiled the distribution, voltinism and body size of 943 lepidopteran and odonate species within a 50 km × 50 km grid system, thereby presenting a novel method for estimating the body volume of species from digital images. Regressions and structural equation modelling were applied to distinguish the effects of temperature, productivity and season length on mean voltinism and body size within grid cells. We accounted for spatial autocorrelation with autoregressive models and analysed the possible effect of species richness and intraspecific variability.

Results Voltinism consistently decreased with latitude for both lepidopterans ($r^2 = 0.76$) and odonates ($r^2 = 0.86$), with species having on average fewer generations per year in northern Europe and more generations per year in southern Europe. The effects of temperature, productivity and season length on body size contrasted in sign between lepidopterans and odonates, leading to opposing geographical patterns across Europe.

Main conclusions Voltinism in insect assemblages is strongly driven by environmental temperature, and trade-offs between voltinism and body size influence the occurrence of species at macroecological scales. Insects with the ability to extend their generation time over multiple years can overcome this constraint, allowing for a relatively large body size in cold areas. Our results furthermore support the idea that body sizes of terrestrial and aquatic insects form contrasting geographical patterns because they are differently affected by temperature and resource constraints.

Keywords

Bergmann's rule, ectotherm, insect, intraspecific variability, Lepidoptera, Odonata, productivity, season length, temperature–size rule.

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INTRODUCTION

The most important trait influencing the physiological and ecological processes of an animal is its body size (LaBarbera, 1989). Body size affects almost all physiological rates (e.g. the rate of oxygen consumption; Woods, 1999; Atkinson *et al.*, 2006), which subsequently determine or constrain fertility, mortality and ecological processes such as competitive interactions between individuals or species. In this way, body size is ultimately linked to the spatio-temporal distribution and abundance of animals (Blackburn & Gaston, 2001) and has important implications for the impact of climate warming – from biomass production by single species to the structure and dynamics of communities (Sheridan & Bickford, 2011; Forster *et al.*, 2012). This link between physiology and distribution was first recognized by Carl Bergmann (1848), who noted that body size increases with increasing latitude within and/or between closely related species of endothermic animals. Bergmann proposed that thermoregulatory advantages explain this pattern because of the low surface-to-volume ratio of large organisms and consequent improved heat retention in cold geographical areas. Since the publication of Bergmann's seminal work, considerable debates have flourished, especially with regard to whether Bergmann's rule constitutes a pattern, a process or both, whether it applies to intraspecific, interspecific or assemblage levels, and whether it also applies to ectothermic animals (e.g. Watt *et al.*, 2010).

The body size of endotherms appears to increase consistently with decreasing environmental temperature (e.g. Ashton *et al.*, 2000; Meiri & Dayan, 2003; Olson *et al.*, 2009), although it is clear that thermoregulation is not the only factor that influences the evolution of body size, given the many processes in which it is involved (see also Geist, 1987). However, for ectotherms, especially insects, general patterns in the geographical variation of body size are still debated. The number of intraspecific and interspecific studies that show an increase in insect body size with latitude or elevation is nearly equal to those that show a decrease (Shelomi, 2012). It is important to keep in mind that the physiological processes influencing geographical patterns of body size of ectotherms may fundamentally differ from those of endotherms. Three major hypotheses relating the body size of ectotherms to environmental conditions have been proposed, all of which operate at the individual level. However, if these hypotheses are also important during speciation, then their effects should be mirrored by the convergence of species traits in a particular environment (e.g. Gaston *et al.*, 2008).

The first hypothesis, the *temperature–size rule* (TSR; Atkinson, 1994), is based on the widely observed phenomenon that development and growth rates respond differently to variations in environmental temperature, specifically that increasing temperature accelerates the development rate more than the growth rate; hence, the adult stage is reached at a smaller body size with increasing temperature. This holds true for both unicellular and multicellular organisms despite differences in reproductive methods and ontogenetic timing,

which suggests that there are different ultimate causes for the TSR (Forster *et al.*, 2011). For example, it has been proposed that oxygen supply is an important driver of the TSR in animals, especially in aquatic environments where oxygen uptake is more costly than in terrestrial environments (e.g. Forster *et al.*, 2012; Horne *et al.*, 2015; but see also Angilletta *et al.*, 2004; Walters & Hassall, 2006; Kingsolver & Huey, 2008). From a geographical perspective, the TSR predicts a decrease in body size with increasing environmental temperature.

The second hypothesis deals with the *availability of resources*, which is essential for growth and thus for an animal's body size. Animals adjust their body size to the potential food supply of the environment in which they occur (Atkinson & Sibly, 1997). Therefore, animals can grow larger in a resource-rich environment but must stay small when resources are limited. Productivity and latitude are usually negatively correlated; hence, this resource effect should, in contrast to the expectation from the TSR, lead to a decrease in body size with increasing latitude.

The third hypothesis concerns the *length of the season*, which affects the time available for growth and development. A longer season means a longer growing period and hence a larger final body size (Mousseau, 1997; Chown & Gaston, 2010). At high latitudes, the growing season is short, which, like the expectation from considerations of resource availability, leads to the prediction that body size should decrease with increasing latitude.

These three hypotheses, however, are influenced by one important factor that might constrain body size, namely volinism, i.e. the number of generations per year. In a given area, a species with multiple generations per year has less time per generation available for growth than a species with only one generation per year. Since total growing time and body size are positively correlated, multivoltine species or populations should be smaller than univoltine species or populations (Roff, 1980; but see Kivelä *et al.*, 2011), as already suggested by Masaki (1967) and shown, for example, for the striped ground cricket, *Allonemobius fascia* (Mousseau & Roff, 1989). In a recent intraspecific study, Horne *et al.* (2015) found that volinism significantly affects strength and direction of latitude–size clines across different orders of terrestrial arthropods. Furthermore, an increase in temperature allows an increase in the number of generations per year, because it accelerates development rates so that more than one generation can be completed during the growing season (Gillooly, 2001; Altermatt, 2010). Finally, the maximum number of generations per year is also constrained by resource availability and season length (e.g. Mousseau & Roff, 1989). These possible indirect effects on body size via the number of generations per year stand in contrast to the possible direct effects of resource availability and season length on insect body size. Whether organisms in a particular environment invest in a large adult size or in several small generations is a matter of resource allocation, and of course differs within and between species (Kozłowski *et al.*, 2004).

Here, we analyse direct and indirect effects of temperature, productivity and season length on geographical patterns of voltinism and body size of assemblages of lepidopterans and odonates within grid cells across Europe. So far it is largely unknown how environmental filters influence the large-scale geographical distribution of voltinism and body size of insect species, which might lead to the convergence of traits within local assemblages. In contrast to previous studies (e.g. Altermatt, 2010; Forster *et al.*, 2012), our analyses hence focus on assemblages on a large spatial scale. For example, Horne *et al.* (2015), in an intraspecific study, found that body size of aquatic arthropod species generally decreases with warming and decreasing latitude in contrast to body size of terrestrial arthropod species, which shows reduced and often opposite clines. A calculation of averages across the species co-occurring within an assemblage may reveal subtle trends in the variation of traits across environmental gradients, despite considerable variation within and between species. We chose lepidopterans and odonates as study groups because reliable data on their large-scale distributions are available. Furthermore, the two groups differ in important biological aspects: butterflies and moths are terrestrial herbivores, whereas dragonflies and damselflies are carnivores with aquatic larvae. Lepidopterans have to cope with the phenology and low nitrogen content of many plants, whereas odonates continuously feed on a comparatively nitrogen-rich diet.

In particular, we: (1) analyse the relationships of voltinism and body size within assemblages to temperature, productivity and season length and (2) test the hypothesis that voltinism constrains the body size of lepidopterans as well as odonates. We predict that the body size of terrestrial lepidopterans decreases with latitude, whereas the body size of semi-aquatic odonates increases with latitude. We furthermore hypothesize that voltinism is strongly determined by environmental conditions and constrains insect body size at macro-ecological scales.

MATERIALS AND METHODS

Body size data

Linear measurements, such as wing length or head width, are commonly used as a proxy for the body size of lepidopterans and odonates (e.g. Hawkins & Lawton, 1995; Bried, 2009; but see Hassall *et al.*, 2008). Instead of these linear measures, we used the body volume (caput, thorax and abdomen) as a proxy for body size. The advantages of this three-dimensional measure are that it also captures differences in shape that influence body mass and that it is not confounded by variations in wing morphology, which co-varies with habitat or landscape structure (Van Dyck & Matthysen, 1999). We scanned illustrations of adult species (imagos) from existing literature (see Appendix S1 in the Supporting Information) with an Epson Perfection 4490 photo scanner and manually clipped background, wings and legs with GIMP 2.6 tools. We then calculated the total body volume as the sum

of the volumes of each row of the clipped image ($\pi \times r^2 \times \text{edge length pixel}$) using the resolution of the image to obtain metric values in mm^3 . This procedure assumes that the body shapes are axially symmetric. Given the bilateral symmetry of higher organisms, this assumption seems to be acceptable. The images that we analysed were all of the same scale, and we obtained real size values by applying the scale factors given in the data sources. The obtained values are a surrogate for the average adult body size of species (ignoring intraspecific variability). However, there might be intraspecific variability in body size due to, for example, sexual dimorphism, time of year, number of specimens used to estimate the body size of species and differences in body size within the distributional range of species, and such variability could bias the results. Therefore, we accounted for the possible effect of intraspecific variability on the results by running simulation tests (see the section 'Statistical analyses'). This seemed to be the most appropriate solution to this problem as sufficient spatially and temporally explicit data on within-species body size variation are not available for the species studied here.

We tested the comparability of our method of estimating the body size of species by analysing species body size values obtained from different books and found that body size values of odonates were highly correlated between two important sources ($r^2 = 0.95$, $P < 0.001$; Appendix S2). We used images of female lepidopterans and male odonates because of their availability, and excluded all subspecies and families with only one species, which led to a dataset of 943 species. Body sizes of males and females were highly positively correlated (lepidopterans, $r^2 = 0.86$, $P < 0.001$; odonates, $r^2 = 0.96$, $P < 0.001$; Appendix S3). Therefore, we conclude that our decision to compare female lepidopterans and male odonates does not bias the comparison between the two orders. We averaged body size values if more than one individual of a species was available, with a total of 1867 processed images and an average number of specimens per species of 1.98. The body size data used in this study are provided in Table S1. We would like to note that our method can also be applied to many other species to obtain metric body size values.

Voltinism data

We assembled data on voltinism of European species of lepidopterans and odonates from existing literature (Appendix S1). In particular, we used the number of generations per year and coded different levels of voltinism into numerical values. We scored uni-, bi- and trivoltinism at values of 1, 2 and 3, respectively. Multi- and polyvoltinism scored 3. Some species of odonates need more than 1 year to complete one generation. Consequently, parti- and semivoltinism were scored at values of 0.33 and 0.5, respectively. If more than one value was available for a single species (228 of 943 species), we averaged the scores. We are aware that this averaging ignores intraspecific variability in the number of generations per year across the range of a species. For example, a species that completes one generation per year in its

northern distributional range and two generations per year in its southern distributional range entered our analysis with 1.5 generations per year throughout its distributional range. However, according to the literature, only 18 of 943 species show an intraspecific increase in voltinism with latitude in single parts of their distributional range. We therefore did not run simulation tests for intraspecific variability in voltinism. We conclude that our averaging flattens north–south clines in the number of generations per year and therefore real geographical clines in the number of generations per year might be even steeper than the results of our study suggest, due to intraspecific variability. We provide all data on the number of generations per year in Table S1.

Distributional data

Our study covers Europe as defined in Kudrna *et al.* (2011), excluding Belarus, Ukraine, Moldova and Turkey, for which distributional data are not reliable. We divided the study area into 50 km × 50 km grid cells of a polygon mask layer (Common European Chorological Grid Reference System). We excluded all islands except Great Britain and Ireland and grids cells with land coverage < 50% (coastal regions), yielding 1937 grid cells covering 4,833,693 km². We then digitized distribution maps of lepidopterans and odonates from existing literature (Appendix S1) with the software *WORLDMAP* to estimate presence and absence of species within the grid system.

Data on the body size, number of generations per year and distribution were available for 845 species of lepidopterans and 98 species of odonates (Table S1). We used the ln-transformed body size measures of each species. All assemblage-level analyses of body size were conducted using the arithmetic mean of ln-transformed body size values of all species in each grid cell, unless otherwise stated. All assemblage-level analyses of voltinism were conducted using the arithmetic mean of the number of generations per year of all species in each grid cell.

Environmental data

Besides latitude, three distinct environmental variables were selected to analyse the geographical patterns in the number of generations per year and body size of lepidopteran and odonate assemblages: *annual mean temperature* (AMT; °C) was chosen because of its direct relationship to the TSR (data from Hijmans *et al.*, 2005; available at <http://www.worldclim.org/current>); *net primary productivity* (NPP; g dry matter m⁻² year⁻¹) was chosen as an indicator of resource availability (data from FAO, available at <http://www.fao.org/geonetwork/srv/en/metadata.show?id=36915>, period 1976–2000); *growing degree days* (GDD; base 5°C), represents the length of the season and hence the time available for growth in a certain area (data from New *et al.*, 1999; available at <http://www.sage.wisc.edu/atlas/maps.php?datasetid=31&includerelatedlinks=1&dataset=31>). We calculated mean values of these variables for each 50 km × 50 km grid cell using GRASS GIS 6.4.3.

Statistical analyses

Regression analyses

In the first step, we used quantile regression to test for a link between the number of generations per year and body size at the species level (R package ‘quantreg’). Quantile regression is a suitable tool for analysing ecological relationships when the relationships are expected to be influenced by multiple variables (Cade & Noon, 2003) and when the variance is not homogeneous across the range of the predictors, as was the case here (Levene’s test, $P < 0.05$ for both lepidopterans and odonates). We then used ordinary least-squares regressions to explain the geographical variation in the average number of generations per year and body size of lepidopteran and odonate assemblages with AMT, NPP and GDD as predictors. We built single and multiple regression models and also included species richness, which we calculated from the distribution data for each grid cell, as a weight in this analysis. We accounted for spatial autocorrelation with autoregressive models (R package ‘spdep’), in which the error term is predefined from a spatial neighbourhood matrix of a certain distance. This distance was identified using a spatial correlogram (R package ‘ncf’) and was set to 2000 km for both groups of analysed insects (Appendix S4).

Structural equation modelling

We used structural equation modelling (SEM) to investigate direct and indirect effects of AMT, NPP and GDD on the average number of generations per year and body size within assemblages (R package ‘lavaan’). SEM allows the calculation of direct and indirect effects of predictor variables in a hierarchical linear regression setting of interconnected predictor and response variables. This setting can be mapped by a path model that represents the assumed causal relationships among the variables (Rosseel, 2012). We specified our models based on the hypothesized relationships presented in the Introduction, with the intercorrelated dependent variables generations per year and body size and the intercorrelated explanatory variables AMT, NPP and GDD (Appendix S5).

Randomization tests

We also analysed the possible effect of species richness on the average number of generations per year and the average body size within grid cells with two different randomization tests. First, we created 1000 datasets for lepidopterans and odonates by randomly sampling five species from each grid cell with at least five species ($n = 1937$ grid cells). We then calculated the average number of generations per year and the average ln-transformed body size within each grid cell and regressed these values against latitude for each dataset. Second, we randomized the assignment of voltinism and ln-transformed body size to lepidopteran and odonate species and calculated the average number of generations per year and the average body size within grid cells for the randomized data sets. We then calculated the standardized effect size as the observed average number of generations per year and body size within grid cells minus

the expected values from the randomizations, divided by the standard deviation of the expectation. We repeated this procedure 1000 times and calculated the average effect size for each grid cell. The average effect size within grid cells was then regressed against AMT, NPP and GDD for lepidopterans and odonates (Appendix S6).

Simulation of intraspecific variability of body size

To test for the possible influence of intraspecific variability on the general results of our study we set up two scenarios of body size variations within species across their distributional range. In each scenario, we first assumed that all species show intraspecific clines in body size and iteratively increased the relative level of intraspecific variability (in %). We then determined the percentage of intraspecific variability up to which our main results are robust and compared these obtained thresholds with known levels of intraspecific variability given in the original data sources. In both scenarios, we simulated intraspecific variability by calculating the intraspecific body size range for each species as $\ln[\text{body size} \pm (\text{body size} \times \text{variability} \times 0.5)]$. Subsequently, we divided the intraspecific body size range of each species into quantiles. These quantiles were calculated for each species by the number of grid cells in the north–south direction of the distributional range. Each grid cell in the distributional range was then assigned the corresponding value of the body size quantiles according to latitude – as either increasing or decreasing with latitude. In the first scenario (*random clines*), we randomly chose the geographical direction of body size clines within each species (increasing or decreasing with latitude). This random selection is reasonable because intraspecific clines in body size are not consistent in direction for either lepidopterans or odonates (see the Discussion). We then calculated simulated datasets for intraspecific variability between 1% and 100% in steps of 1%. Each step was repeated 100 times and the results were averaged per grid cell for each step. In the second scenario (*fixed clines*), we set the geographical direction of the body size clines within each species as increasing with latitude for lepidopterans and decreasing with latitude for odonates. These fixed intraspecific clines are contrary to the observed macroecological trends and the full range of intraspecific variability runs exactly counter to the observed macroecological trends; hence, this scenario is very conservative. We then calculated simulated datasets for intraspecific variability between 1% and 50% in steps of 1%. For both scenarios, we analysed the slopes of the regressions between \ln body size and latitude of each level of intraspecific variability.

All calculations were performed in R version 3.2.3.

RESULTS

At the species level, lepidopterans had an average of 1.38 ± 0.59 (SD, $n = 845$) generations per year and an average body size of $43 \pm 47 \text{ mm}^3$, ranging between 1.4 mm^3 (*Cleta perpusillaria*) and 447 mm^3 (*Papilio alexanor*).

Odonates had an average of 0.98 ± 0.63 (SD, $n = 98$) generations per year and an average body size of $604 \pm 571 \text{ mm}^3$ (SD, $n = 98$), ranging between 27 mm^3 (*Nehalennia speciosa*) and 2507 mm^3 (*Anax imperator*; Appendix S7). Lepidopterans had on average more generations per year and a smaller body size than odonates (t -test, $P < 0.001$). Body size decreased with increasing number of generations per year for both groups of insect species (all slopes were negative for quantiles between 0.02 and 0.98 in steps of 0.01; Fig. 1). However, for a given number of generations within one year, odonates are able to develop to a body volume about ten times larger than lepidopterans.

The average number of generations per year within assemblages decreased clearly with latitude from southern to northern Europe, for both lepidopterans ($r^2 = 0.76$, $P < 0.001$) and odonates ($r^2 = 0.86$, $P < 0.001$; Fig. 2). In contrast, body size trends differed between lepidopterans and odonates: the average body size in assemblages of lepidopterans decreased with latitude ($r^2 = 0.30$, $P < 0.001$), whereas the average body size in assemblages of odonates increased with latitude ($r^2 = 0.35$, $P < 0.001$; Fig. 2).

Consistently throughout all models (linear, linear weighted, spatial), AMT, NPP and GDD were positively correlated with the average number of generations per year in lepidopteran and odonate assemblages (Table 1). Body size was positively correlated with AMT, NPP and GDD in all models of lepidopteran assemblages, and negatively correlated with AMT, NPP and GDD in all models of odonate assemblages. AMT explained the largest amount of variance in most of the models. However, GDD explained a slightly higher amount of variance than AMT in the linear and spatial models for the average number of generations per year in lepidopteran assemblages, and NPP explained a slightly higher amount of variance than AMT in the spatial model for the average body size in lepidopteran assemblages (Table 1). Note that for the average body size within assemblages, the amount of variance explained by all variables together was lower than for the number of generations per year (multiple linear models; Lepidoptera, $0.15 \leq R^2 \leq 0.22$; Odonata, $0.29 \leq R^2 \leq 0.41$; Table 1) but was still higher than what would be expected in interspecific studies of body size variation of insects along environmental gradients (mean = 0.17, median = 0.06, $n = 50$ studies; calculated with data from Shelomi, 2012).

Our structural equation models also showed a strong positive influence of AMT on the average number of generations per year within lepidopteran and odonate assemblages (Fig. 3). The average number of generations per year and body size co-varied positively in lepidopteran assemblages and negatively in odonate assemblages. Hence, AMT had an indirect positive effect on the body size of lepidopterans and an indirect negative effect on the body size of odonates via the average number of generations per year. In addition to this indirect effect of AMT on body size, AMT also had a direct positive effect on body size in lepidopteran assemblages and a direct negative effect on body size in odonate assemblages.

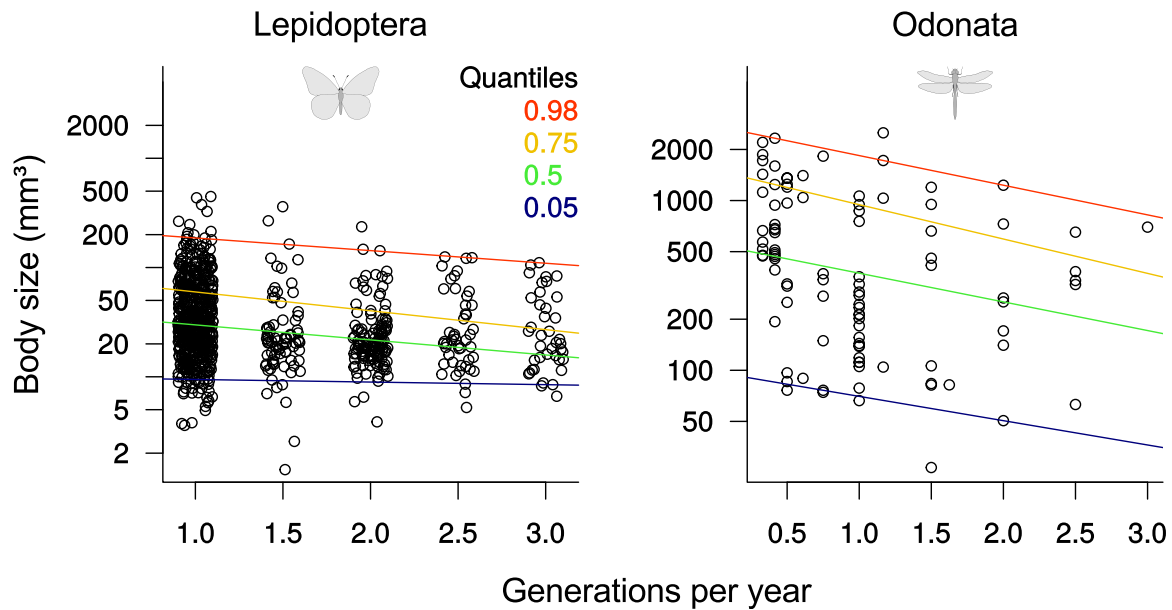


Figure 1 Relationship between body size and number of generations per year (voltinism) for lepidopteran ($n = 845$) and odonate species ($n = 98$) in Europe. Each dot represents one species. Jitter was added to the generations per year for lepidopterans to better distinguish single species. Note that body size decreases with increasing number of generations in both groups (quantile regressions, all slopes negative for quantiles between 0.02 and 0.98 in steps of 0.01). Note also that species with few generations per year can be both small and large, whereas species with more generations per year must be small.

NPP had a direct positive effect on body size in lepidopteran assemblages, and GDD had a direct positive effect on the number of generations per year in lepidopteran assemblages (Fig. 3).

An increase in body size with latitude was also found in assemblages of odonates when only species with voltinism < 1 (more than one year per generation) were considered ($r^2 = 0.24$, $P < 0.001$; Fig. 4). However, the average body size in assemblages decreased clearly with latitude for species with voltinism > 1 (more than one generation per year) for both lepidopterans ($r^2 = 0.42$, $P < 0.001$) and odonates ($r^2 = 0.70$, $P < 0.001$; Fig. 4) and AMT explained the largest amount of variance in almost all models (Appendix S8).

Randomization tests showed that the observed latitudinal trends in voltinism and body size are also consistent for a random subset of species from each grid cell. Additionally, the effect sizes calculated with randomized data revealed trends highly similar to those obtained with the original data (Appendix S6).

Simulation of intraspecific variability showed that the latitudinal trends in body size were robust ($P < 0.001$; no change in the direction of the relationship) up to 100% intraspecific variability according to the random clines scenario and up to 21% for lepidopterans and 30% for odonates according to the fixed clines scenario (Fig. 5). The average intraspecific variability in body size obtained from the literature was 26% for lepidopterans (variability in wingspan, $n = 516$ species; Table S1) and 14% for odonates (variability in body length, $n = 98$ species; Table S1).

DISCUSSION

First, we found that voltinism constrains the body size of lepidopteran and odonate species (Fig. 1). This suggests that there is an upper limit for body size given a particular number of generations per year for species of both lepidopterans and odonates, even though odonates are much larger than lepidopterans. The most important aspect of this finding, however, is that not all species use the limits of the available time: many species produce one generation within a year but nevertheless grow only to a small body size.

We furthermore showed that the average number of generations per year in insect assemblages increases strongly with increasing ambient temperature: species living at high latitudes or in cool climates have a lower number of generations per year than species found at low latitudes or in warm climates. This result was consistent for both insect orders studied. However, we found contrasting geographical patterns of body size for lepidopteran and odonate assemblages across Europe: assemblages in northern Europe are dominated by relatively small lepidopteran species and relatively large odonate species. In addition, direct effects of temperature, productivity and season length on body size contrasted in sign between lepidopterans (positive) and odonates (negative), with temperature having the strongest effect in both groups. Hence, our results support the idea that body sizes of terrestrial and aquatic insects show contrasting geographical patterns because they are differently affected by temperature, resources and time constraints.

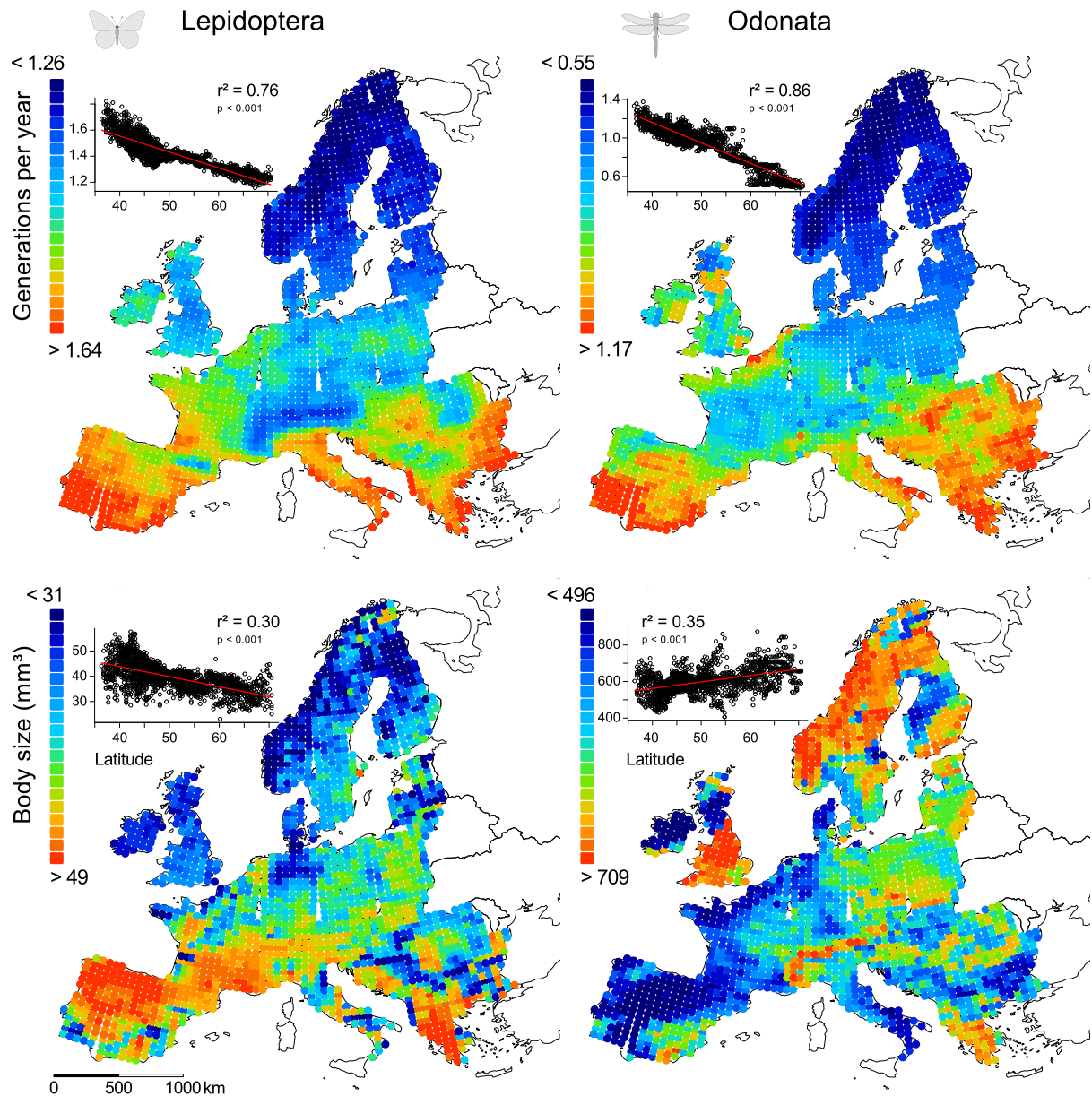


Figure 2 Average number of generations per year (voltinism) and body size within lepidopteran and odonate assemblages across Europe. The colour code was set according to an equal-frequency classification; red indicates assemblages with more species having many generations per year or large body size and blue indicates assemblages with more species having few generations per year or small body size ($n = 1937$ grid cells; EPSG 3575; Lambert azimuthal equal area projection). Note that the average number of generations per year decreases clearly from south to north in both groups. Note also that the average body size within lepidopteran assemblages decreases with increasing latitude, whereas the average body size in odonate assemblages increases with increasing latitude. Values of r^2 and P are from ordinary least-squares regressions with ln-transformed body size data.

Our simulations of intraspecific variability showed that our main findings for body size are robust to within-species variability up to at least 100% in the more likely random clines scenario (Fig. 5). Even in the most unlikely scenario (fixed clines), i.e. when all intraspecific variability of all species was modelled to be exactly opposite to the observed macroecological trends, our main results for body size were robust up to 21% intraspecific variability for lepidopterans and up to 30% intraspecific variability for odonates. Known

levels of intraspecific variability taken from the literature are 26% for lepidopterans (wingspan) and 14% for odonates (body length); this variability also includes sexual dimorphism for both groups and variation in wing morphology for lepidopterans (see Van Dyck & Matthysen, 1999). The results of our simulations showed that our main findings for body size are to some extent sensitive to intraspecific variability, but we conclude that this extent is too low to seriously bias our results and conclusions.

Table 1 Models explaining geographical variation in the average number of generations per year (voltinism) and body size of lepidopteran and odonate assemblages in Europe.

Assemblage	Variable	Model	r^2			R^2
			AMT	NPP	GDD	
Lepidoptera	Voltinism	Linear	(+) 0.82*	(+) 0.15*	(+) 0.84*	0.87*
		Linear weighted	(+) 0.78*	(+) 0.06*	(+) 0.78*	0.83*
		Spatial [†]	(+) 0.82*	(+) 0.46***	(+) 0.85*	0.88*
	Body size	Linear	(+) 0.17*	(+) 0.16*	(+) 0.12*	0.21*
		Linear weighted	(+) 0.11*	(+) 0.12*	(+) 0.06*	0.15*
		Spatial [†]	(+) 0.18*	(+) 0.23*	(+) 0.20**	0.22*
Odonata	Voltinism	Linear	(+) 0.78*	(+) 0.29*	(+) 0.62*	0.78*
		Linear weighted	(+) 0.67*	(+) 0.11*	(+) 0.54*	0.67*
		Spatial [†]	(+) 0.80*	(+) 0.58*	(+) 0.72*	0.80*
	Body size	Linear	(-) 0.37*	(-) 0.21*	(-) 0.28*	0.38*
		Linear weighted	(-) 0.28*	(-) 0.10*	(-) 0.21*	0.29*
		Spatial [†]	(-) 0.40*	(-) 0.34*	(-) 0.35*	0.41*

AMT, annual mean temperature; NPP, net primary productivity; GDD, growing degree days.

Statistics were calculated with linear regressions (linear), regressions weighted with the number of species in each assemblage (linear weighted) and models to account for spatial autocorrelation (spatial). The explained variance is given for single variables (r^2 , highest value of each model in bold, calculated with ln-transformed body size data) and for the full model (R^2). +/–, positive/negative relationship.

Lepidoptera: $n = 845$ species across 1937 assemblages. Odonata: $n = 98$ species across 1937 assemblages.

Note that directions of the relationships are consistent for voltinism but contrast for body size between lepidopterans and odonates.

* $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$. [†]Nagelkerke R^2 .

A possible explanation for the contrasting geographical patterns of body size of lepidopterans and odonates may lie in the different characteristics of their habitats, especially of their larvae, and associated life histories. Larvae of butterflies

and moths are terrestrial herbivores that rely on plant tissue for growth, whereas larvae of dragonflies and damselflies are carnivores that feed on a comparatively nitrogen-rich diet. In addition, odonates often need several years to reach the adult

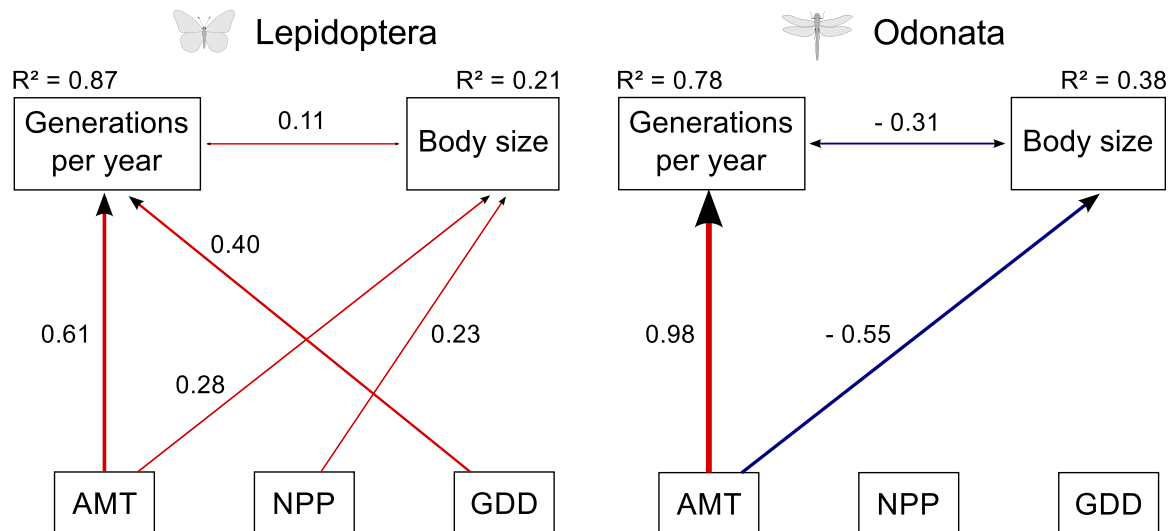


Figure 3 Structural equation models of the relationships between average generations per year (voltinism) and body size of European lepidopteran and odonate assemblages and three environmental variables: AMT, annual mean temperature; NPP, net primary productivity; GDD, growing degree days. Arrows indicate the direction and strength of the effects, and standardized coefficient estimates are given for each path. For convenience, only paths with $P < 0.001$ and predictor estimates > 0.15 are shown. Full model R^2 values for generations per year and body size are given for each group. Note that AMT strongly influences voltinism, which in turn positively covaries with lepidopteran body size and negatively covaries with odonate body size. Note also that the direct effects of AMT on body size of lepidopterans and odonates contrast in sign.

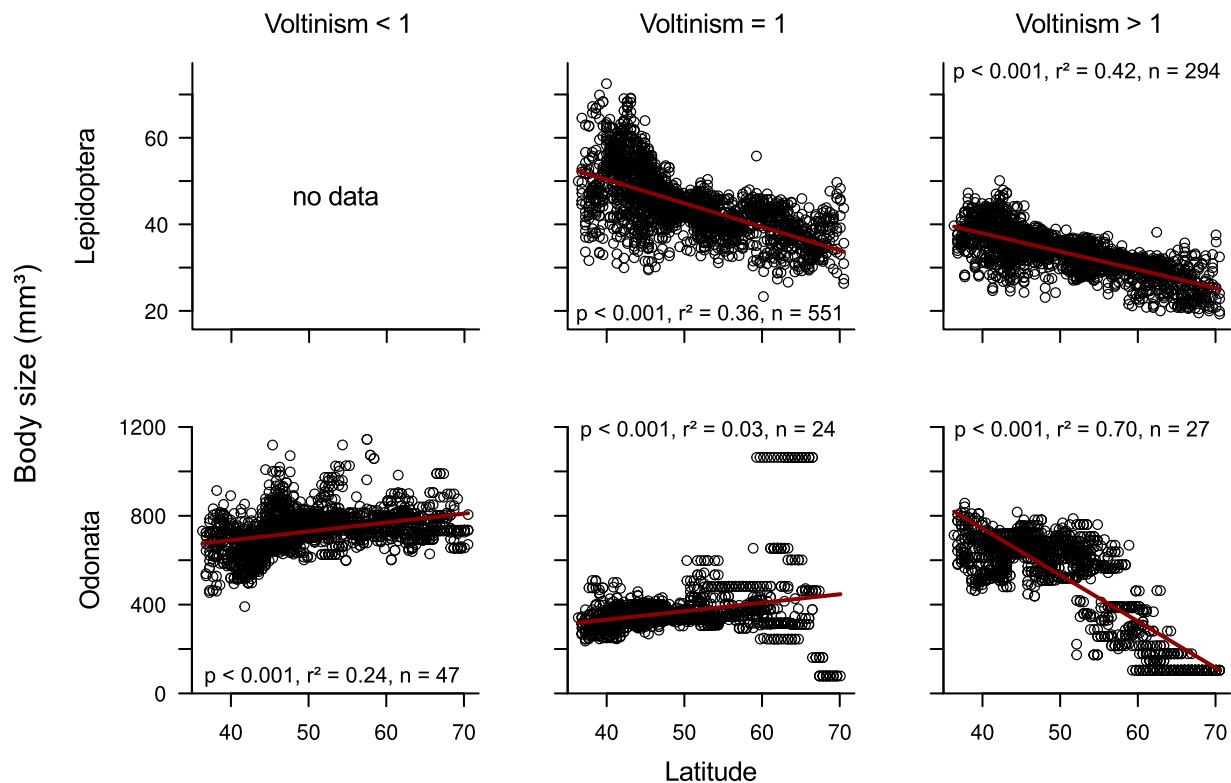


Figure 4 Scatterplots between latitude and average body size in assemblages of lepidopterans and odonates categorized by different levels of voltinism. Voltinism < 1, only assemblages of species which need more than one year per generation; voltinism = 1, only assemblages of species with one generation per year; voltinism > 1, only assemblages of species with more than one generation per year. Note that the dataset of this study does not contain lepidopteran species with voltinism < 1. Values of r^2 and P are from ordinary least-squares regressions with ln-transformed body size data; n refers to the number of species in the analyses. Note also that the average body size in assemblages of both lepidopteran and odonate species with voltinism > 1 decreases with latitude.

stage, whereas lepidopterans often complete several generations within a single year. Hence, odonates on average dedicate a larger proportion of their life cycle to growth. Following this line of argument, the body size of odonates is less affected by resource and time constraints than the body size of lepidopterans.

We conclude that the average body size of co-occurring species of lepidopterans as well as odonates is influenced by temperature, productivity and season length, but the effects differ in strength and direction. For lepidopterans, we suggest that the temperature effect (TSR) is overwritten by resource and time limitations, especially in northern areas. Furthermore, the life history of lepidopterans is dependent on the phenology of their host plants and plant defence mechanisms (Cizek *et al.*, 2006; Altermatt, 2010), which might in part explain the stronger relationship between season length and the number of generations per year for lepidopterans compared with odonates and the high number of small univoltine species of lepidopterans. For odonates, we suppose that the temperature effect is strong because their larvae are more dependent on ambient temperature in the aquatic part of their life history due to the physical properties of water, where heat conduction is high and oxygen consumption is strongly determined by temperature (Verberk *et al.*, 2011). However, a

lot of unexplained variance in body size remained in this correlative study. As body size is involved in or constrained by many ecological processes, the low explanatory power of environmental variables for predicting the average body size of assemblages is not surprising. We also did not consider physiological traits relevant for the energetic budget of insects, for example their coloration (e.g. Zeuss *et al.*, 2014). Given our finding that temperature is both a direct and indirect driver of body size, more detailed energetic considerations might be a promising future research direction for a more holistic understanding of variation in insect body size.

Our results of the average body size in lepidopteran assemblages support the findings of García-Barros (2000), who found a positive correlation between body size and temperature in a global interspecific analysis of 496 species of the superfamilies Papilionoidea and Hesperioidea. In addition, Barlow (1994), who analysed 1058 Australian and 2605 African species of the superfamily Papilionoidea, found a decrease in wingspan with increasing latitude, similar to the findings of Hawkins & Lawton (1995). These latter authors, however, found no consistent relationships between latitude and body size for European species, which might be a consequence of the authors' mid-point approach and the division of Europe into coarse latitudinal bands. A decrease in

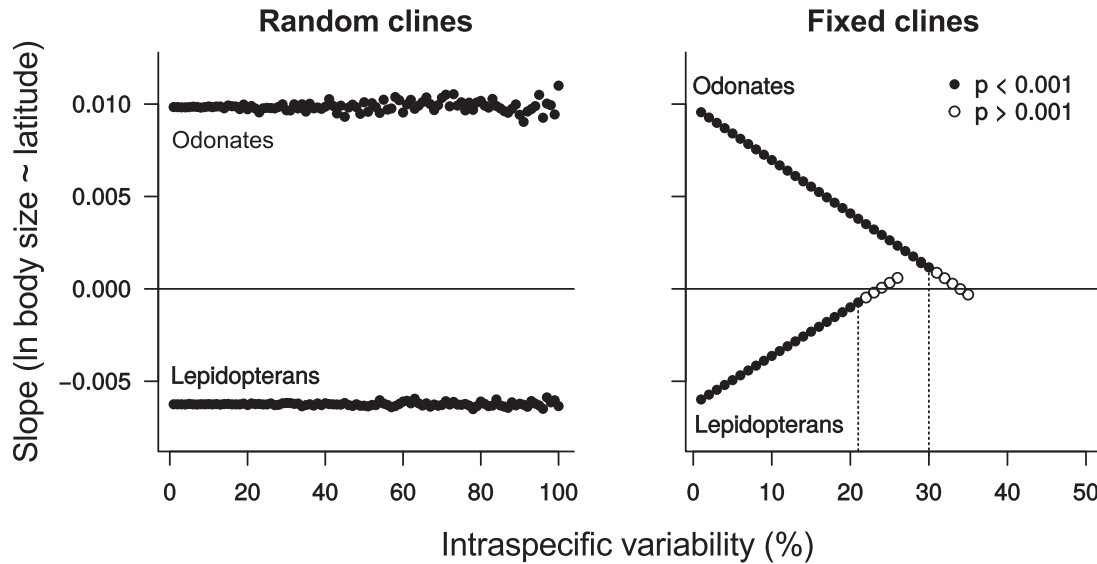


Figure 5 Slopes of regressions between body size and latitude for different levels of simulated intraspecific variability. Left panel: the north–south direction of intraspecific clines was selected randomly for each species as either increasing or decreasing with latitude. Right panel: intraspecific clines were set to be fixed and opposite to the observed macroecological trends for lepidopterans and odonates, i.e. increasing body size with latitude for lepidopterans and decreasing body size with latitude for odonates. Note that our observed geographical trends in body size are robust ($P < 0.001$, no change in the direction of the relationship) to intraspecific variability when the direction of intraspecific clines is randomly selected. Note also that our observed geographical trends in body size are robust to intraspecific variability up to 21% for lepidopterans and 30% for odonates (vertical lines) when all intraspecific variability of each species is simulated to be directly opposite to the observed macroecological patterns.

lepidopteran wingspan with increasing latitude in Sweden was reported by Nylin & Svård (1991) in an intraspecific analysis of nine species. By contrast, Karl & Fischer (2008) showed that the body size of *Lycaena tityrus* increases with decreasing developmental temperatures when reared in the laboratory without food and time limitations. The authors' explanation for this result is increased food intake and higher efficiency in converting food into body matter at low temperatures. This intraspecific trend contrasts with the decrease in lepidopteran body size with increasing latitude found in our interspecific study of species assemblages. However, the absence of food and time limitations may be the reason for their results. Our finding that the geographical clines in voltinism and body size match in sign for lepidopterans shows that butterflies and moths cannot always compensate for food and time limitations in northern areas because their life history does not allow them to grow for more than one season. We suggest that this is the major reason for the small average body size in northern assemblages of lepidopterans compared with southern areas, where lepidopterans tend to have both more generations per year and larger body sizes.

To the best of our knowledge, no macro-scale analysis of the body size of odonates is currently available. There are, however, several intraspecific studies, for example the study of Hassall *et al.* (2008), who found that the body mass of *Coenagrion puella* increases with latitude. Similarly, Hassall *et al.* (2014) found a positive correlation between latitude and body size of *Erythromma viridulum*, and Bried (2009) concluded from an intraspecific analysis of four species in Mississippi,

USA, that on average larger and heavier species of odonates are found in the northern parts of the state. By contrast, Śniegula *et al.* (2012) found that individuals of *Coenagrion puella* and *Coenagrion pulchellum* collected in Poland are larger than those from Sweden, which indicates a negative intraspecific relationship between body size and latitude. The differences in the findings for *C. puella* between Hassall *et al.* (2008) and Śniegula *et al.* (2012) may lie in different collection times during the year, as the body size of odonates declines with the date of emergence (summarized in Corbet, 2004). However, the above-mentioned studies on odonates differ from our study as they exclusively deal with intraspecific variability, whereas our analyses were at the assemblage level.

Finally, although the average number of generations per year within lepidopteran and odonate assemblages consistently decreased with decreasing temperature, the average body size showed contrasting geographical patterns for both groups (Fig. 2). We suppose that species with the ability to extend their generation time over multiple years can overcome this constraint, which allows a relatively large body size in cold areas. In addition, our finding that the average body size of species with more than one generation per year strongly decreased with latitude in assemblages of both lepidopterans and odonates underlines the importance of voltinism–body size trade-offs for the large-scale geographical variation in insect body size.

Our study also has implications for the consequences of global warming – with increasing temperature, large aquatic insect species should retreat to colder areas and species with

the ability to increase their annual number of generations should benefit and extend their distribution to higher latitudes. These shifts in the distribution and probably also abundance of species will lead to a change in the phenology and overall body size structure of terrestrial and aquatic ecosystems. Attempts to forecast effects of climate warming must hence consider the strong temperature dependence of insect voltinism and its relationship to body size of terrestrial and aquatic species.

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Additional references to the data sources used in this study can be found in Appendix S1.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 List of species.

Appendix S1 Data sources.

Appendix S2 Comparability of body size values.

Appendix S3 Male versus female body size.

Appendix S4 Spatial autocorrelation profiles.

Appendix S5 Structural equation modelling.

Appendix S6 Randomization tests.

Appendix S7 Generations per year and body size distributions.

Appendix S8 Environmental drivers of body size by volinism levels.

BIOSKETCHES

Dirk Zeuss is a biologist and geographer whose research interests are primarily the quantification of macroecological patterns of species traits with special emphasis on the understanding of the underlying mechanisms causing these patterns. His research focus at the moment is the size and colour of insects.

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Author contributions: D.Z. designed the study, conducted all analyses, prepared the figures and wrote the text. R.B. supervised the study. R.B. and S.B. commented on the text.

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