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Research

Interrelations of global macroecological patterns in wing and thorax size, sexual size dimorphism, and range size of the Drosophilidae

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Support for macroecological rules in insects is mixed, with potential confounding interrelations between patterns rarely studied. We here investigate global patterns in body and wing size, sexual size dimorphism and range size in common fruit flies (Diptera: Drosophilidae) and explore potential interrelations and the predictive power of Allen's, Bergmann's, Rensch's and Rapoport's rules. We found that thorax length ($r^2=0.05$) and wing size ($r^2=0.09$) increased with latitude, supporting Bergmann's rule. Contrary to patterns often found in endothermic vertebrates, relative wing size increased towards the poles ($r^2=0.12$), a pattern against Allen's rule, which we attribute to selection for increased flight capacity in the cold. Sexual size dimorphism decreased with size, evincing Rensch's rule across the family ($r^2=0.14$). Yet, this pattern was largely driven by the *virilis-repleta* radiation. Finally, range size did not correlate with latitude, although a positive relationship was present in a subset of the species investigated, providing no convincing evidence for Rapoport's rule. We further found little support for confounding interrelations between body size, wing loading and range size in this taxon. Nevertheless, we demonstrate that studying several traits simultaneously at minimum permits better interpretation in case of multiple, potentially conflicting trends or hypotheses concerning the macroecology of insects.

Keywords: Allen's rule, Bergmann's rule, clinal variation, Diptera, Drosophilidae, dispersal, Rapoport's rule, Rensch's rule, wing loading

Introduction

Convergent patterns of phenotypic variation across large-scale environmental gradients have long been recognized and have given rise to several macroecological 'rules' predicting such variation as putative adaptive responses to selection. These patterns are generally supported by empirical evidence and underlie theory. Until a more complete understanding of the selective mechanisms underlying the patterns is achieved, however, the predictive power of such rules must remain limited. This



is particularly true for insects, in contrast to mammals and birds (Blanckenhorn and Demont 2004, Chown and Gaston 2010, Shelomi 2012).

As in all organisms, insect body size is inherently linked to physiology, metabolic rate, survival and reproductive success and is thus thought to evolve in predicted ways if subjected to similar selective drivers (Blanckenhorn 2000, Chown and Gaston 2010). Large size typically increases mating success in males and fecundity in females, but can entail heightened mortality risks and reproductive costs due to prolonged juvenile development (Blanckenhorn 2000). Amongst the macroecological rules proposed to account for body size variation, Bergmann's rule, signifying an increase in size with latitude, is well supported in homeothermic vertebrates (Bergmann 1847, Meiri and Dayan 2003), but its absence and often its converse is prominent among invertebrates (Shelomi 2012). An increase in size with latitude has been attributed to temperature-dependent variation in growth and metabolic rates, a pattern congruent with the so-called temperature-size rule (Atkinson 1994, Atkinson and Sibly 1997) that generally predicts insects to grow bigger in the cold (Kingsolver and Huey 2008). However, a shortened active season with increasing latitude can also cause adaptive negative size clines through selection for fast development if development cannot be extended across one season, both within and between species (Chown et al. 1999, Blanckenhorn and Demont 2004, Zeuss et al. 2017). Small insects with rapid development are thus expected to follow Bergmann clines, whereas large insects may be limited in their development by season length at high latitudes, thus emerging smaller and showing converse Bergmann clines (Blanckenhorn and Demont 2004, Zeuss et al. 2017).

In contrast to body size, the relative size of appendages has been found to decrease with latitude in endothermic vertebrates (Nudds and Oswald 2007, Symonds et al. 2010). Termed Allen's rule, this pattern has received considerable attention and has been attributed to selection for a reduced surface-to-volume ratio to limit heat loss in the cold. Originally documented for warm-blooded animals (Allen 1877), qualitatively similar patterns have also been described in invertebrates (Alpatov 1929, Ray 1960). In insects, most appendages, including wings and antennae, are connected to the circulatory system (Chapman et al. 2013), and the constant flow of haemolymph through these appendages can contribute to thermoregulation, as in endotherms. Patterns equivalent to Allen's rule might thus be expected. Still, particularly for small insects, body temperature is unlikely to be strongly dependent on the relative surface area as an insect's body adjusts nearly instantly to the ambient temperature (Harrison and Roberts 2000), although many insect taxa are capable of considerable thermoregulation (social Hymenoptera in particular; Stabentheiner et al. 2010, Chapman et al. 2013). Consequently, small insects primarily regulate their body temperature by modifying their behavior (Clench 1966, Dillon et al. 2009), thus making best use of available microhabitats. The capacity to disperse, however, is greatly restricted at cool temperatures, which impedes take-

off in winged insects (Dillon and Frazier 2006, Frazier et al. 2008). As increased wing size relative to body size facilitates take-off at cooler temperatures (Frazier et al. 2008), a latitudinal increase of relative wing size (a pattern counter to Allen's rule) can be predicted. Such patterns have indeed been observed in insects (e.g. clinal population differentiation in *D. melanogaster*: Azevedo et al. 1998), but the repeatability of such clines awaits further scrutiny. Whether the relative size of insect appendages increases, decreases or shows any consistent latitudinal pattern at all thus remains unclear.

Whereas both Bergmann's and Allen's rules describe spatial variation in body and appendage size irrespective of sex, the widely-studied Rensch's rule focuses on variation in sexual size dimorphism (SSD; Abouheif and Fairbairn 1997, Fairbairn 1997). Rensch (1950) documented that, among closely related species of many disparate taxonomic groups, SSD increases with body size in species in which males are the larger sex but decreases when females are larger than males. Unlike Bergmann's and Allen's rules, which capture size variation that likely results from natural selection, differential variation between the sexes has been attributed to a combination of sexual selection on males (e.g. via male-male contest competition) and fecundity selection on females (Fairbairn and Preziosi 1994, Fairbairn 1997, Székely et al. 2004). However, evidence for Rensch's rule is mixed, particularly in taxa with female-biased size dimorphism, such as most insects and spiders (for which SSD tends to increase with body size: Blanckenhorn et al. 2007b, Webb and Freckleton 2007, Stuart-Fox 2009), undermining its predictive power.

Finally, in addition to body size, populations or species have also been documented to vary in their range size. Specifically, Rapoport's rule (or 'effect': Stevens 1992) predicts that species occurring close to the equator will have more restricted ranges because they exhibit limited climatic tolerance as a result of adapting to a local environment with low climatic variability. Conversely, species occurring at higher latitudes (or altitudes), which feature ample climatic variability, are predicted to be better adapted to colonize and occupy more diverse habitats. Evidence for this rule is still controversial as it might be restricted to specific latitudes and certain regions and somewhat scale dependent (Rohde 1996, Ruggiero and Werenkraut 2007).

The four macroecological patterns described above are often studied in isolation from each other. Nevertheless, body size, wing morphology (which relates to dispersal capacity; Ray et al. 2016), SSD and range size are all likely to be ecologically and evolutionarily interrelated, if only because most adaptive explanations discussed above relate to climate. For example, although Rensch's rule has mostly been studied independently of environmental factors, latitudinal patterns of SSD have been observed (Blanckenhorn et al. 2006), and a relationship of Rensch's rule with the widely-observed temperature-size rule was postulated but not found after all (Hirst et al. 2015). Similarly, a large body of literature investigates relationships between range size and both dispersal capacity and body size (Gaston and Blackburn 1996, Malmqvist 2000, Lester et al. 2007, Rundle et al.

2007, Laube et al. 2013), thus raising the issue of whether clinal variation in size and dispersal might generally drive Rapoport's rule, possibly undermining its ecological relevance (Reed 2003). It is thus useful – if not imperative – to account for additional potentially confounding effects when investigating range-size variation (Laube et al. 2013).

Rigorous examination of macroecological patterns requires not only robust taxon sampling and coverage of a large geographic range, but also the ability to account for phylogenetic relationships among species. The Drosophilidae, a highly speciose and diverse family of fruit flies with a global distribution, should be highly suited to assess such patterns and their underlying mechanisms in insects. Many drosophilids are cosmopolitan generalists, whereas others are highly specialized and endemic to small geographic areas (Ashburner 1981). In this taxon, Bergmann's rule has received considerable attention at the intraspecific level along both latitudinal and altitudinal gradients (e.g. *D. melanogaster*: van't Land et al. 1999, Klepsatel et al. 2014, Fabian et al. 2015; *D. buzzatii*: Karan et al. 2000; *D. subobscura*: Gilchrist et al. 2001; *Zaprionus indianus*: Karan et al. 2000), but little information is available at the interspecific level. Similarly, Rensch's rule has been addressed in the *obscura* group (Huey et al. 2006), but the general pattern has not been investigated (but see Blanckenhorn et al. 2007a, b). The potential relationship of range size with body and appendage size also remains largely unexplored in this group.

We here assessed the ecogeographical patterns of thorax length and wing size, SSD, and range size in 151 drosophilid species from around the globe, including members of all three major *Drosophila* clades plus species of other genera belonging to this speciose family. Accounting for phylogenetic non-independence based on a reconstructed phylogeny, we investigated the geographical patterns described above and potential relationships between the four macroecological rules. Such relationships are expected under the prediction that they all relate (to some extent) to climate, but their covariation is poorly studied empirically. We thus aimed to better understand the causes and consequences of macroecological variation.

Material and methods

Phylogeny reconstruction

To reconstruct the drosophilid phylogeny, we obtained the sequences of six nuclear, three mitochondrial and three ribosomal genes from GenBank (see Supplementary material Appendix 1 Table A1 for accession numbers and sequence coverage). The gene coverage per species ranged between 1 and 12 (mean \pm SD = 7.2 ± 2.9 loci/species), with a total sequence length of 6269.2 ± 3267.6 bp (range = 337–14 449 bp). The nuclear sequences comprised the genes expressing the alcohol dehydrogenase (Adh), α -amylase-related protein (Amyrel), aromatic-L-amino-acid decarboxylase (DOPA decarboxylase; Ddc), glycerol-3-phosphate

dehydrogenase (Gpdh), and xanthine dehydrogenase (Xdh). The mitochondrial genes included the cytochrome c oxidase subunits I, II, and III (COI, COII and COIII, respectively) and the NADH dehydrogenase subunit 2 (ND2). The ribosomal genes included 28S and the large and small subunits of 12S and 16S (omitting the adjacent tRNAs as they were difficult to align and represented only a small amount of data). For each locus, we aligned the sequences of all species using multiple sequence alignment (MUSCLE) as implemented in MEGA ver. 7.0 (Kumar et al. 2016) and determined its best nucleotide substitution model using jModelTest ver. 2.1.7 (Darriba et al. 2012). The best substitution models were GTR + Γ + I for all nuclear genes and 16S, HKY + Γ + I for the mitochondrial genes and 12S, and HKY for 28S, respectively.

Subsequently, we reconstructed the phylogeny based on Bayesian inference using BEAUTi and BEAST ver. 1.8.3 (Drummond et al. 2012), with unlinked substitution models, a relaxed uncorrelated log-normal clock, and a Yule speciation process. Due to a lack of well-defined fossil dates in our sample of species, and because the absolute timing of speciation events was deemed less important for our analyses than the relative branch lengths, we omitted the time calibration. We ran the Markov Chain Monte Carlo (MCMC) simulation on the CIPRES Science Gateway (<www.phylo.org>; Miller et al. 2010) for 100 million generations, sampling every 10 000th tree. We used Tracer ver. 1.6 (Rambaut and Drummond 2013) to examine the convergence of the Bayesian chain and the stationary states of all parameters, considering effective sample sizes (ESSs) greater than 200 to be adequate. Finally, we generated a maximum clade credibility tree with mean node heights and a 10% burn-in using TreeAnnotator ver. 1.8.3 (Drummond et al. 2012).

In addition to the full *Drosophila* phylogeny, we separately generated a phylogeny for the *Zaprionus* dataset (for which only body lengths and no thorax lengths were available). For these species, adequate sequence coverage was restricted to the Amyrel, COI, COII, and 28S genes, respectively (Supplementary material Appendix 1 Table A2). We used the same procedures as above, except that GTR + Γ + I was the best substitution model for all loci and the MCMC chain was run for only 30 million generations, with a tree sampled every 3000 generations.

Data collection

We measured sex-specific body size as thorax length of field-caught specimens (distance between the tip of the scutellum and the basis of the head, a standard measure) for 56 species of Drosophilidae stored at the Zoological Museum of the Univ. of Zurich. Whenever available, we measured at least 10 individuals per sex per species. We further obtained thorax length data for 111 additional species and data on total body length for 20 *Zaprionus* species from the literature (Supplementary material Appendix 1 Table A3).

For each of our 146 species available in the database TaxoDros (<www.taxodros.uzh.ch/>), we retrieved the geographic coordinates of every faunistic record. This database

comprises an enormous amount of information on the taxonomy and distribution of more than 6800 species of drosophilids. All coordinates are derived from published data or stem from museum catalogs in which the identification was verified by experts. Although these faunistic data do not stem from standardized collecting schemes but are somewhat haphazard in nature, TaxoDros represents one of the most powerful and comprehensive data sources for any insect taxon. Nonetheless, the sampling coverage might be biased towards certain regions and not be homogeneous across the globe, a common problem when handling such datasets. Thus, we concede that any inference based on these distribution data should be treated with some caution (see Conclusion).

We included only species with at least 20 unique sampling locations in our analyses (removing duplicate and nonsensical localities, resulting in over 25 000 unique coordinates; mean number of coordinates per species: 273.3, SE: 47.9, median: 137). Many drosophilids are distributed globally, but their range is often restricted within latitudinal bands such that they do not occur at the equator. We thus used the mean of the absolute latitudinal distribution to obtain a suitable estimate of the species-specific latitudinal distribution.

Although several major ecogeographic rules describe patterns of latitudinal trait variation, latitude itself remains a compound trait integrating various climatic factors. In this respect, the differential effects of temperature and seasonality are of particular interest. Using climatic data, we tried to decompose latitude into variates related to temperature or seasonality. However, due to the high collinearity among climate variables and latitude, we were unable to use multiple-regression approaches (variance inflation factor always greater than 5 and often greater than 10). When using a principal component analysis (with oblique rotation) to extract the major axes of variation, both temperature and seasonality variables loaded strongly on the first principal component (but in opposite directions, thus mirroring latitude), while all other dimensions did not explain significant proportions of variance and did not show any association to either temperature nor seasonality (Supplementary material Appendix 1 Table A1). For the sake of interpretation (as we were unable to disentangle temperature from seasonality and latitude), we decided to restrict our analyses to latitudinal patterns alone, although future studies disentangling the climate compounds of latitude are clearly desirable.

Thorax length

We analyzed the relationship between log thorax length (mean of male and female values) and median latitude, using phylogenetic generalized linear models (PGLS) as implemented in the R package ‘caper’ (Orme et al. 2012). We used the sexual dimorphism index (SDI) as an estimate of the strength and direction of SSD as proposed by Lovich and Gibbons (1992). We thus divided the thorax length of the larger sex (usually the female) by the smaller and subtracted 1 from this ratio, which arbitrarily defines the SDI positive if females are the larger sex and negative if males are larger.

Wing size

We retrieved wing-size data for 54 species from Bolstad et al. (2015). These wing sizes represent the square root of wing area derived from outline spline reconstructions. To assess clinal variation in relative wing size, we used PGLS with latitude as the predictor and thorax length as a covariate. In addition, we calculated wing loading, which is typically associated with wing-beat frequency and flight capacity (Pétavy et al. 1997, Frazier et al. 2008). Wing loading is usually defined as some ratio of body mass and wing area, where low values relate to better dispersal capacity as less weight is ‘loaded’ onto the wing. Because body mass estimates were lacking, we used thorax length³, which scales well with mass. We analyzed its relationship with latitude using PGLS.

Sexual size dimorphism

To test whether SSD scales iso- or allometrically with body size, we applied phylogenetic reduced major-axis regressions (as implemented in the R package ‘phytools’: Revell 2012) of log male against log female thorax length across all species (for justification see Fairbairn 1997, Blanckenhorn et al. 2006). Rensch’s rule is evident only if the slope of this relationship exceeds one. We repeated the analysis separately for all three major clades of *Drosophila* (*Sophophora* subgenus, *immigrans–tripunctata* radiation, *virilis–repleta* radiation) and the *Zaprionus* spp. data set, for which body size was measured as total body length. To quantify the predictive strength of Rensch’s rule in drosophilids, we further calculated r^2 from a PGLS of SDI against log mean size.

Range size

When investigating variation in range size, we considered only species with 20 or more unique records, thus reducing the number of species with sufficient data to 110 (mean number of coordinates per species: 273.3, SE: 47.9; median: 137). To approximate range sizes directly from faunistic records, we derived range-size estimates using α -hulls (as in Gallagher 2016), which are more robust than simple minimum convex polygons, particularly when sampling is haphazard and not standardized (Burgman and Fox 2003). We used the Lambert azimuthal equal-area projection to generate appropriate range-size estimates in km² across the globe and restricted these range sizes to actual land masses using the R package ‘rangeBuilder’ (Davis Rabosky et al. 2016). A PGLS model was used to test for a relationship between log range sizes and latitude (median). In order to test for potential confounding effects of body size and relative wing length (as an estimate of short-distance dispersal), we also performed multiple PGLS regression analyses with thorax length and wing size as covariates. As wing sizes were available for only 54 species, the sample size for this analysis was drastically reduced (note, however, that the number of sampling coordinates per species was greater in this reduced data set: mean: 395.9, SE: 89.7; median: 176).

Data deposition

Data available from the Dryad Digital Repository: < <http://dx.doi.org/10.5061/dryad.b248v> > (Rohner et al. 2018).

Results

Thorax length and wing size

Log mean thorax length and wing size increased with median latitude (thorax length: $r=0.23$ [95% confidence limits: 0.04, 0.39], $\lambda=0.97$, $n=107$, $p=0.019$; wing size: $r=0.30$ [0.04, 0.51], $\lambda=0.96$, $n=54$, $p=0.026$; Fig. 1), although the unexplained variation in thorax and wing size was rather large (Fig. 1). Log wing size also showed a positive relationship with latitude when log thorax length was included as a covariate ($r=0.30$ [0.03, 0.50], $\lambda=0.55$, $n=54$, $p=0.031$), suggesting a disproportionate increase in wing size towards high latitudes. Accordingly, wing loading decreased with lati-

tude ($r=-0.35$ [-0.09, -0.54], $\lambda=0.00$, $n=54$, $p=0.009$; Fig. 1).

Sexual size dimorphism

Phylogenetic signals in male thorax length ($\lambda=0.86$, $p<0.001$), female thorax length ($\lambda=0.84$, $p<0.001$), mean body size ($\lambda=0.85$, $p<0.001$) and SSD ($\lambda=0.75$, $p<0.001$, Fig. 2) suggest phylogenetic inertia of body size and SSD in drosophilids (Fig. 2). When testing Rensch's rule across all species, RMA slopes were significantly steeper than unity ($\beta_{\text{phyRMA}}: 1.10$ [1.06, 1.15], $p<0.001$, $n=151$; Fig. 3), and body size explained 14% of the total variation in SDI. RMA slopes did not significantly differ between radiations (log female size \times radiation interaction: $F_{2,114}=2.31$, $p=0.104$), however, when testing Rensch's rule within the three major radiations, we did not find consistent support. Rensch's rule was evident in the *virilis-repleta* radiation ($\beta_{\text{phyRMA}}: 1.08$ [1.01, 1.14], $p=0.021$, $n=48$; Fig. 3), but

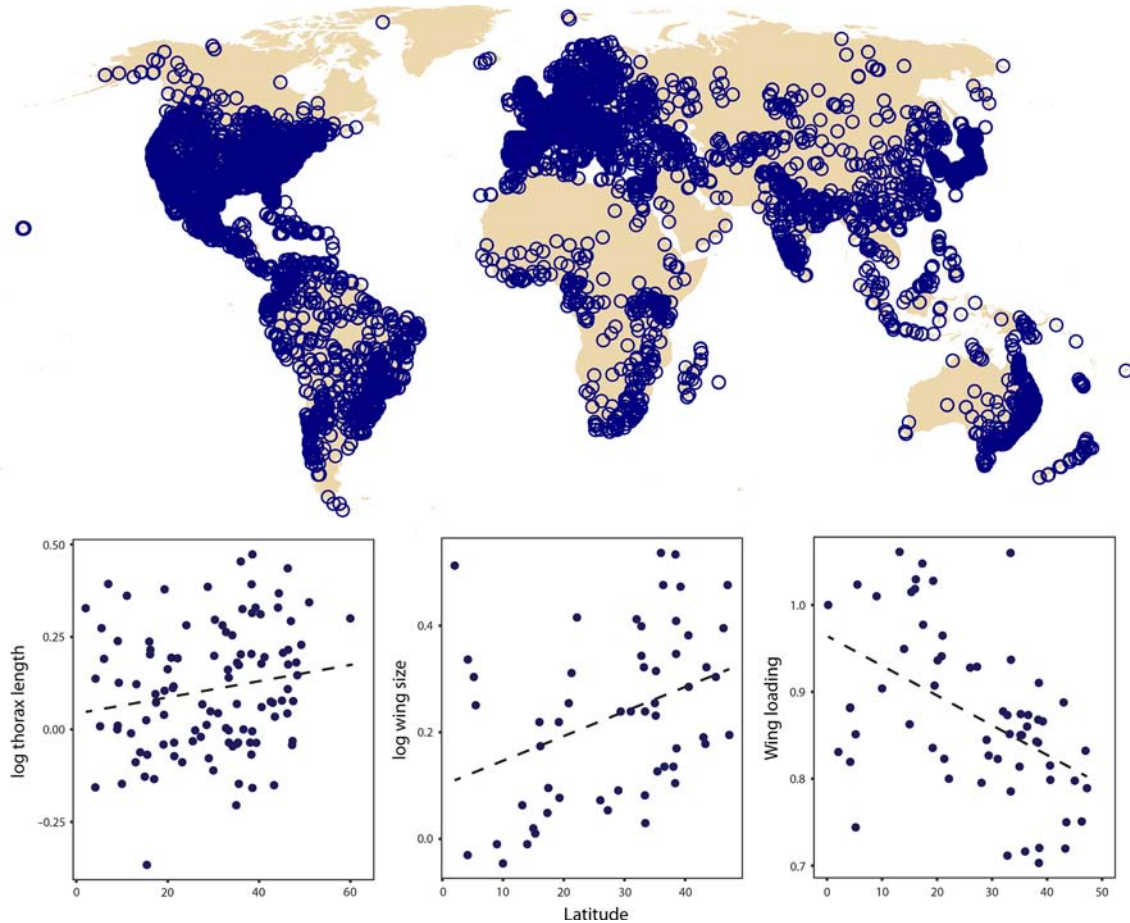


Figure 1. Top: species-specific range size estimates and mean latitudinal distributions were derived from over 25 000 unique sampling locations depicted here. This global dataset was retrieved from TaxoDros, a large and detailed database on taxonomy and diversity of drosophilids (< www.taxodros.uzh.ch/ >). Bottom: thorax length and wing size increases with mean absolute latitude, demonstrating a weak interspecific Bergmann cline in drosophilids. Wing size increased more strongly with latitude than thorax length, resulting in lower wing loading (thorax length³/wing area) towards the poles. These plots showing simple linear regressions are for illustrative purposes only. All analyses were done using PGLS (phylogenetically corrected correlation coefficients are given in the text).

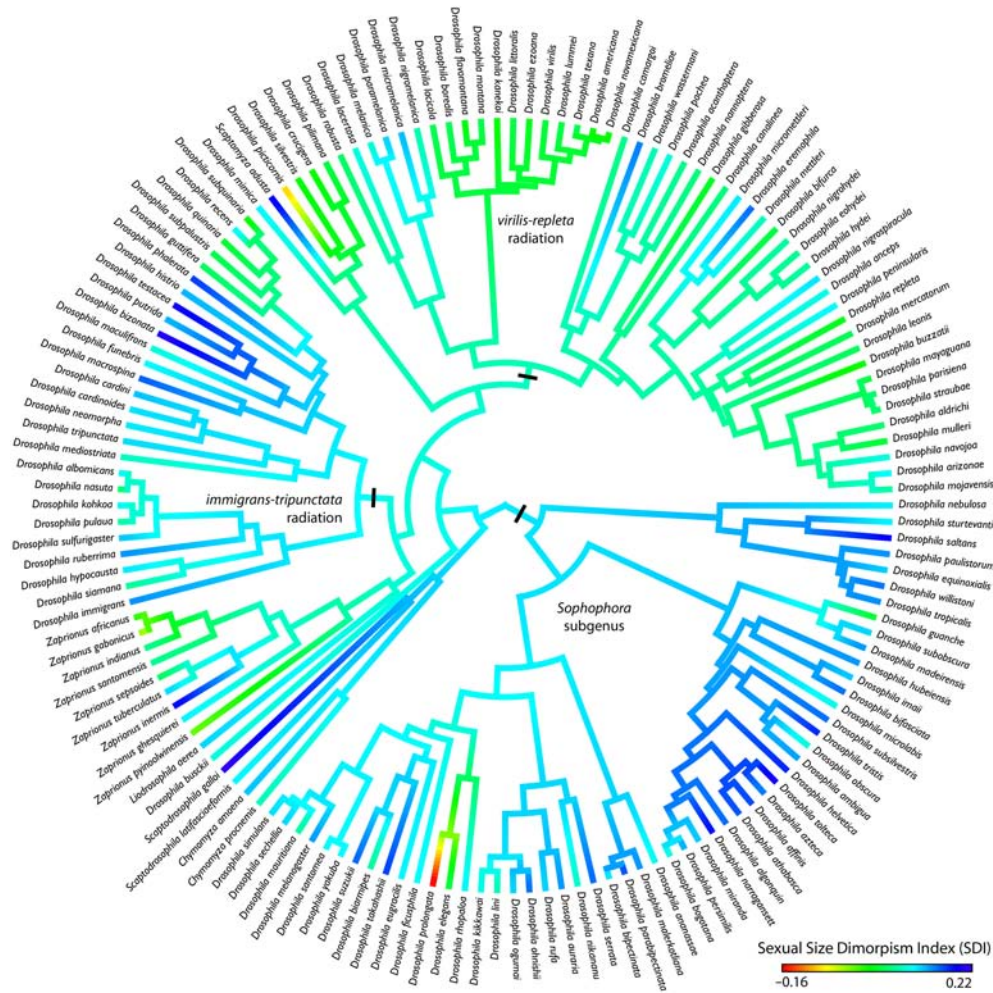


Figure 2. Ancestral state reconstruction of sexual size dimorphism ($SDI = (\text{thorax length of larger sex} / \text{thorax length of smaller sex}) - 1$; arbitrarily defined negative if males are the larger sex), for illustration purposes only. SSD shows strong phylogenetic signal and in most species females are the larger sex (positive SDI values). The conspicuous exception is *Drosophila prolongata*, which shows pronounced male-biased SSD.

not in the *immigrans-tripunctata* radiation ($\beta_{\text{phyIRMA}}: 1.02 [0.92, 1.13], p=0.650, n=22$; Fig. 3). In the *Sophophora* subgenus, the RMA slopes were very steep ($\beta_{\text{phyIRMA}}: 1.18 [1.06, 1.31], p=0.002, n=51$; Fig. 3), but this pattern was driven exclusively by *Drosophila prolongata*. This species is by far the largest member of this subgenus and the only one showing male-biased SSD. When excluding *D. prolongata*, Rensch's rule was no longer supported in this clade ($\beta_{\text{phyIRMA}}: 0.99 [0.92, 1.08], p=0.996, n=50$; Fig. 3). The relationship of male and female body length also did not deviate from isometry in *Zaprionus* spp. ($\beta_{\text{phyIRMA}}: 1.06 [0.83, 1.28], p=0.577, n=16$; Fig. 3). There was also no evidence for a correlation between sexual size dimorphism and latitude ($r=0.09, [-0.10, 0.27], \lambda=0.67, n=107, p=0.369$).

Range size

Log range size did not correlate with latitude in the simple linear model using the full data set ($r=0.03 [-0.16, 0.22]$,

$\lambda=0.03, n=105, p=0.777$, Fig. 4). However, in a phylogenetic multiple regression including thorax and wing size as additional explanatory variables, range size increased towards the poles ($r=0.37 [0.11, 0.56], p=0.007, \lambda=0.98$) whereas thorax and wing length had no effect on range size (thorax: $r=0.10 [-0.17, 0.36], p=0.469, \lambda=0.98$; wing size: $r=0.04 [-0.23, 0.30], p=0.770, \lambda=0.98$). Note that the data underlying this multiple regression represent only a subset of the data because wing size, thorax length and range extent data were available for only 54 species.

Discussion

Our study of the morphology and global distribution patterns of Drosophilidae lends support to several macroecological phenomena. In accordance with Bergmann's rule, thorax length and wing size increased with latitude, and the same was

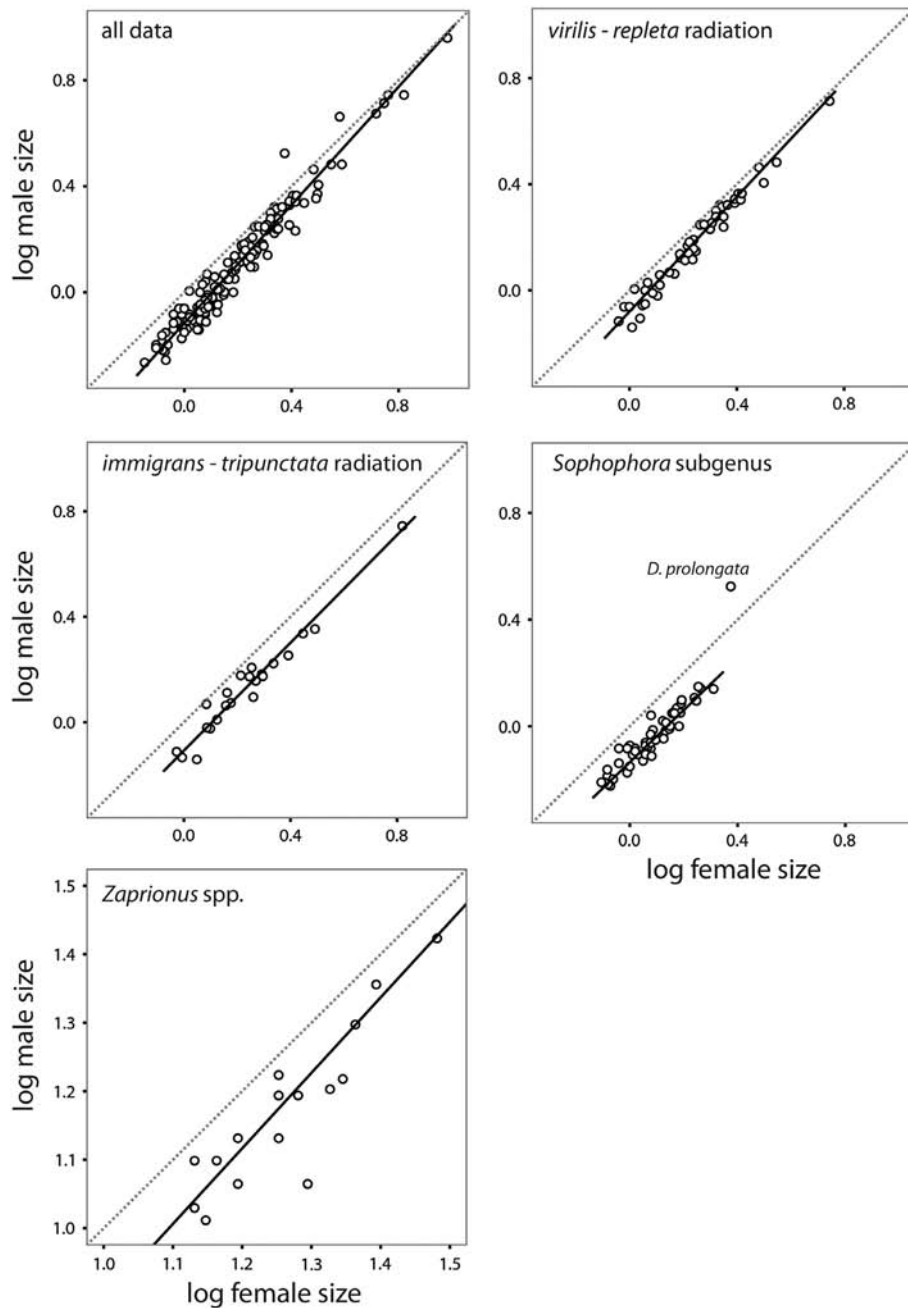


Figure 3. Male size increases more with body size than female size, supporting Rensch's rule for drosophilids. This pattern is however mostly driven by the *virilis-repleta* radiation and absent in all other major clades. For *Zaprionus* spp., only total body length was available while for all other species thorax length was used. Note that regression lines are derived from non-phylogenetic major axis regressions and for illustrative purposes only.

true for relative wing size (contrary to Allen's rule). Our data on SSD also support Rensch's rule overall, but this pattern was mostly driven by the *virilis-repleta* radiation, with weak support in three other major clades. We found no further evidence for a latitudinal cline in SSD. Range size did not vary with latitude across all species of our study, not generally supporting Rapoport's rule. However, when controlling for the

potentially confounding effects of body size and shape (and thus reducing our dataset), we found a significant increase in range size with latitude. In the following, we link our results to the ecology and physiology of drosophilids and discuss potential causes and consequences of these macroecological patterns and their apparent idiosyncrasy depending on which species are analyzed.

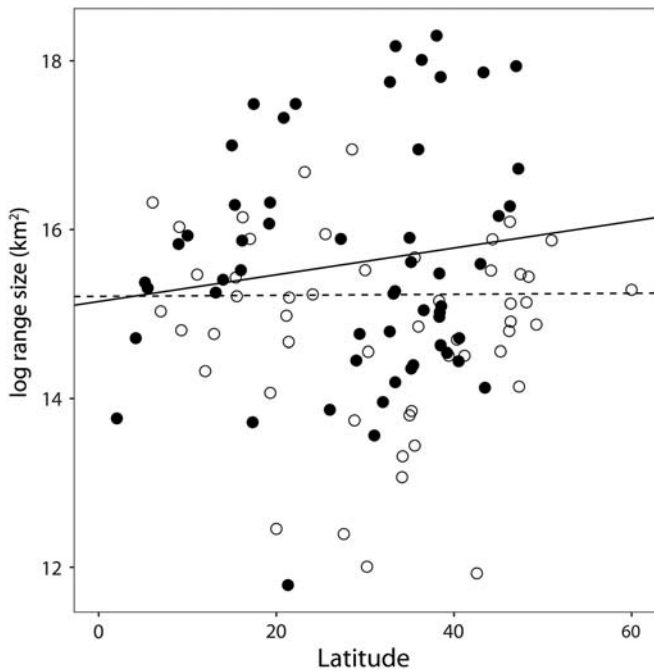


Figure 4. Alpha-hull derived range sizes did not correlate with latitude when all species were analyzed (broken regression line). However, when applying a multiple PGLS regression controlling for thorax length and wing size, range size increased with latitude (solid regression line). Based on merely a limited number of species, this does not seem to be a general pattern across the family, although species for which wing size data were available are dispersed well across the family. The plot shows simple linear regressions for illustrative purposes only. All analyses were done using PGLS (phylogenetically corrected correlation coefficients are given in the text).

Latitudinal effects on body size, shape and range size evolution

Bergmann's rule is thought to be driven by variation in temperature (Atkinson and Sibly 1997, Shelomi 2012), whereas its converse represents an adaptive response to season length (Blanckenhorn and Demont 2004). Drosophilids generally follow a weak positive Bergmann cline, thus at best suggesting only minor effects of temperature and no role of season length in this family. As most drosophilids are small, fast-developing and therefore strongly multivoltine (although some univoltine species and populations exist; Lakovaara et al. 2009), this could be expected (Blanckenhorn and Demont 2004). Compared to the strength of interspecific clinal variation in other insects, the variation explained by latitude in wing ($r^2=0.09$) and thorax length ($r^2=0.05$) is below average, though not particularly low (cf. r^2 for similar interspecific comparisons from Shelomi (2012): mean = 0.22, median = 0.10, SD = 0.25, n = 18). Given that most individuals measured for this study were collected in the field and not raised under controlled environments, a considerable amount of body size variation must be attributable to phenotypic plasticity. Although this typically also applies to other

studies of various taxa, it is possible that we underestimate the strength of the latitudinal pattern.

Even though processes acting within species (sometimes termed neo-Bergmannian rule or James's rule) do not necessarily coincide with among-species patterns (Blackburn et al. 1999), the interspecific clinal variation observed here is consistent with analogous intraspecific variation in drosophilids (Chown and Gaston 2010). Due to this qualitative consistency, it is reasonable to assume a common underlying mechanism. However, following the temperature-size rule (Atkinson 1994), drosophilids tend to grow larger in cool environments in general (Ray 1960), and experimental laboratory rearing would be required to test whether this between-species pattern is driven by evolutionary or purely plastic (i.e. physiological) responses. Note, however, that intra-specific common-garden experiments suggest a strong genetic component (James et al. 1995).

Along with thorax length, wing size increased with absolute latitude, though its steeper increase resulted in disproportionately larger wings at higher latitudes and consequently lower wing loading. Because log wing length showed an isometric relationship with log thorax length across species (evolutionary allometric coefficient derived from a phylogenetic reduced major axis regression: $\beta=0.94$, $p=0.528$), allometric scaling relationships cannot explain the relative increase in wing size with latitude. In contrast to warm-blooded animals, in which latitudinal variation in appendage size has been attributed to selection for thermoregulatory efficiency (e.g. reduced bill size in birds: Symonds et al. 2010), such mechanisms seem unlikely to act in insects. Yet, thermoregulation may still be involved in shaping the observed pattern. Being unable to control body temperature endogenously, small insects such as drosophilids regulate body temperature mostly by modifying their behavior (Dillon et al. 2009, Kjærsgaard et al. 2010). Since flight is hampered in the cold and larger wings lower the temperature threshold for take-off (Dillon and Frazier 2006, Frazier et al. 2008), relatively larger wings near the poles could represent an adaptation to large climatic variability or low temperatures (Angelo and Frank 1984, Pivnick and McNeil 1986, Azevedo et al. 1998, Dillon et al. 2009). Such correlations between dispersal capacity and latitude or altitude have been documented in several species (Hassall 2015, Kjærsgaard et al. 2015, Rohner et al. 2015), including latitudinal clines for wing loading in *D. melanogaster* (Azevedo et al. 1998, Klepsatel et al. 2014), and again suggest a common underlying mechanism of intra- and interspecific clines. Yet, greater dispersal capacity may be an essential prerequisite for colonizing habitats at high latitudes in the first place (e.g. following colonization after the last glacial period). Invading less predictable habitats may then in turn promote the evolution of physiological adaptations that are linked to the climate experienced. This alternative explanation seems unlikely, however, given that wing loading did not correlate with range size (see below), and therefore a direct link between colonization success and short-range dispersal ability appears questionable. Increased relative wing size could thus indeed be associated with cold temperature or

increased climatic variability per se and, consequently, a common pattern in small pterygote insects could be expected. Whether this pattern constitutes evidence against Allen's rule as originally formulated or whether such patterns should be discussed in this context at all is certainly debatable, but also not the main point here.

When considering all data, we found no significant correlation between range size and absolute latitude, suggesting no support for Rapoport's rule overall. Our multivariate analysis further suggests that wing size (a proxy for short-range dispersal potential) and thorax length do not confound this relationship, even though both traits have been shown to play major roles in range-size evolution (Malmqvist 2000, Lester et al. 2007, Rundle et al. 2007, Gaston 2009, Swaegers et al. 2014). Surprisingly, the subset of species for which both wing and thorax data were available showed a significant increase of range size with latitude. This discrepancy is unlikely explained by phylogeny, as the species used in the multivariate analysis are well distributed across all major clades (Supplementary material Appendix 1 Fig. A1). At the same time, there was more faunistic information available for these 54 species (based on the number of coordinates), thus likely increasing the precision of our range-size estimation. Alternatively, this deviating pattern could also be caused by the ecology of these particular species. Unlike many other drosophilids (Markow and O'Grady 2006), these species can be cultured easily in the laboratory, so they may be particularly flexible and undemanding in their ecological preferences. If so, their range size might be less affected by ecological barriers such as the distribution of substrate host species. Given this non-random subset and the non-standardized sampling scheme used to derive range size, future research should test these patterns at the global scale.

Sexual size dimorphism and Rensch's rule

Females were the larger sex in nearly all species investigated. There is, however, one particularly conspicuous exception to this trend: *Drosophila prolongata*. This species is not only the largest-bodied species in the *Sophophora* subgenus, but also the only species exhibiting pronounced male-biased sexual size dimorphism (also see Rohner et al. 2017). This species adds further evidence to the notion that there is great potential for rapid evolution of reversed SSD in Diptera, and its apparent association with increased male–male contests (Rohner et al. 2016), which are also common in *D. prolongata* (Kudo et al. 2015). Given its large size and male-biased SSD, *D. prolongata* strongly affected the statistical appraisal of Rensch's rule here (Fig. 3), reemphasizing potential issues with the classic assessment of Rensch's rule when male- and female-biased taxa differ in size (Webb and Freckleton 2007). Nevertheless, the mating system, including the evolutionary drivers of SSD and body size, of *D. prolongata* is likely to be derived, such that this single extraordinary species may obscure rather than testify to Rensch's rule in *Sophophora*.

Although we found support for Rensch's rule across the entire family, this pattern did not hold within some of the

major (sub)radiations. Nevertheless, in most cases the reduced major-axis slope between males and females was steeper than one. In fact, empirical research demonstrates frequently that support for Rensch's rule depends strongly on the taxonomic level with considerable variation among closely related clades (Webb and Freckleton 2007). Even if supported in interspecific comparisons, Rensch's rule does not necessarily hold among or within populations of these species (Blanckenhorn et al. 2007a). Within-population variation in SSD is likely driven, at least in part, by ontogenetic processes and thus not necessarily linked to selective forces driving Rensch's rule across species (Teder and Tammaru 2005). In theory, Rensch's rule should nonetheless hold across populations and species. Sexual selection on male size tends to be the strongest and most consistent evolutionary driver of large male size (Székely et al. 2004, Rohner et al. 2016), and Rensch's rule is arguably more prominently supported in taxa with male-biased SSD due to this selective homogeneity (Stuart-Fox 2009). In contrast, fecundity selection mediates female-biased SSD to a much lesser extent than expected, as selection for small male size or other evolutionary scenarios are also common (Pincheira-Donoso and Hunt 2015). Blanckenhorn et al. (2007b) and Huey et al. (2006) found evidence for Rensch's rule in *Drosophila* based on 23 and 42 species, respectively. Our data suggest that this result is robust, but driven mostly by the *virilis–repleta* radiation, while tests in other clades do not support deviations from isometry.

Conclusions

Macroecological rules are sometimes considered to be weak and idiosyncratic, partly because their predictive strength and manifestation varies across taxa, but possibly also because they might be interrelated or confounded. While our comparative analyses largely corroborate previously reported intraspecific patterns for thorax length and wing size, support for Rensch's rule was inconsistent among clades (although slopes did not significantly differ between clades). Moreover, we found no support for Rapoport's rule overall and showed that this pattern is not necessarily associated with thorax length or wing size of high-latitude species. Although entirely correlational, we further suggest that increased relative wing size at higher latitudes may be driven by selection for more efficient flight and thermoregulatory behavior.

We conclude that studying the relationships between several prominent macroecological patterns can shed more light on broad ecogeographic patterns. However, we here found only little evidence for confounding effects. Nevertheless, given that their putative underlying causes are often linked to climatic factors, considering several macroecological patterns simultaneously at minimum permits better interpretation in case of multiple, potentially conflicting trends or hypotheses, as was the case here for wing size and its potential relationship with Allen's rule. Future research should focus on the underlying physiological mechanisms to definitively discern the causes and consequences of various macroecological patterns in Drosophilidae and other taxa.

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Supplementary material (Appendix ECOG-03382 at <www.ecography.org/appendix/ecog-03382>). Appendix 1.