

Evolution of multivariate wing allometry in schizophoran flies (Diptera: Schizophora)

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Abstract

The proximate and ultimate mechanisms underlying scaling relationships as well as their evolutionary consequences remain an enigmatic issue in evolutionary biology. Here, I investigate the evolution of wing allometries in the Schizophora, a group of higher Diptera that radiated about 65 million years ago, by studying static allometries in five species using multivariate approaches. Despite the vast ecological diversity observed in contemporary members of the Schizophora and independent evolutionary histories throughout most of the Cenozoic, size-related changes represent a major contributor to overall variation in wing shape, both within and among species. Static allometries differ between species and sexes, yet multivariate allometries are correlated across species, suggesting a shared developmental programme underlying size-dependent phenotypic plasticity. Static allometries within species also correlate with evolutionary divergence across 33 different families (belonging to 11 of 13 superfamilies) of the Schizophora. This again points towards a general developmental, genetic or evolutionary mechanism that canalizes or maintains the covariation between shape and size in spite of rapid ecological and morphological diversification during the Cenozoic. I discuss the putative roles of developmental constraints and natural selection in the evolution of wing allometry in the Schizophora.

KEYWORDS

allometry, constraint, geometric morphometrics, pleiotropy, selection, wing morphology

1 | INTRODUCTION

Integration between phenotype expression—be it in morphology, physiology or behaviour—and overall body size represents a hallmark of living forms (Schmidt-Nielsen, 1984). The formation and evolution of such covariation, or allometry *sensu lato*, has received long-standing attention in evolutionary biology, not least because body size is closely linked to fitness, rapidly responds to selection (Blanckenhorn, 2000; Fairbairn, Blanckenhorn, & Székely, 2007; Peters, 1986), and

frequently elicits correlated evolutionary responses in integrated traits (Gould, 1966; Lande, 1979; Pelabon et al., 2014). However, even though covariation between traits may itself represent an adaptation shaped by natural selection (Cheverud, 1982, 1984), it can constrain or bias evolutionary trajectories of complex phenotypes (Gould, 1966; Klingenberg, 2014; Schluter, 1996). Therefore, in order to understand and predict evolutionary change, we not only need to understand how allometric variation arises and is maintained, but also how static scaling relationships hamper or bias the evolution of

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differentiation. Yet, despite more than a century of research on scaling relationships, their ultimate and proximate drivers as well as their evolutionary consequences remain an enigmatic problem (Casasa, Schwab, & Moczek, 2017; O'Brien, Katsuki, & Emlen, 2017; Pelabon et al., 2014; Shingleton & Frankino, 2018; Shingleton, Frankino, Flatt, Nijhout, & Emlen, 2007).

While many researchers emphasize the evolutionary constancy of static scaling relationships via various mechanisms (Bolstad et al., 2015; Gould, 1966; Kleiber, 1947; Voje, Hansen, Egset, Bolstad, & Pelabon, 2014), others highlight the potential of particularly rapid divergence (Casasa et al., 2017; Emlen, 1996; Frankino, Zwaan, Stern, & Brakefield, 2005; Puniamorthy, Blanckenhorn, & Schäfer, 2012; Rohner & Blanckenhorn, 2018; Wilkinson, 1993). At least some discrepancies about the speed of allometric evolution can be ascribed to methodological and conceptual differences in how scaling relationships are studied. Traditionally, allometry-related concepts were based on the (allometric) coefficient in an exponential equation (e.g., Gould, 1966; Huxley, 1932; here referred to as 'narrow-sense' allometry); however, many contemporary researchers use the term *allometry* to describe various forms of covariation between size and organismal shape (Crabtree, Macagno, Moczek, Rohner, & Hu, 2020; Larson et al., 2018), or, in fact, virtually any phenotype of interest (sexual dimorphism: Fairbairn, 1997; life history: Marbà, Duarte, & Agustí, 2007; behaviour: Dial, Greene, & Irschick, 2008). As some researchers apply a more inclusive concept of allometry than others, this necessarily causes disagreement over what extent allometries differ and hence on how fast they evolve. Irrespective of the concept of allometry, however, there is little doubt that static allometries can affect the course of evolution. Yet it remains unclear how often and to what extent this is the case, whether such effects vary among traits or forms of selection, and to what degree they depend on the conceptual/mathematical approach that is used to study allometry in the broadest sense.

The forewing of *Drosophila melanogaster* has been widely used to study the evolution of static allometry and developmental plasticity more generally with a variety of approaches (Bolstad et al., 2015; Debat, Begin, Legout, & David, 2003; Gidaszewski, Baylac, & Klingenberg, 2009; Gilchrist & Partridge, 2001; Houle, Jones, Fortune, & Sztepanacz, 2019; Robertson & Reeve, 1952; Weber, 1990). Despite the ecological and morphological diversity observed among drosophilids, static narrow-sense allometries of the length of one of the major longitudinal wing veins are very similar across species (Bolstad et al., 2015). Because the drosophilids rapidly diversified about 40 million years ago, this suggests a strongly conserved scaling relationship. While such stasis has traditionally been attributed to selection and/or constraints, (multivariate) stabilizing selection on (multivariate) pleiotropic effects has been shown to be the most likely explanation for the relative constancy of allometric scaling of this particular wing vein in drosophilids (Bolstad et al., 2015; Houle et al., 2019). Given that conserved genes involved in wing development have pleiotropic effects on potentially fitness-related traits (McKay & Lieb, 2013; Ruiz-Losada, Blom-Dahl, Córdoba, & Estella, 2018) and the typical polygenic nature of allometric

relationships, similar mechanisms are expected to act in other species or clades. Unfortunately, however, our understanding of wing allometry (and wing shape evolution in general) is primarily based on a few species of *Drosophila* with most research focussing exclusively on *D. melanogaster*. It is thus unclear to what extent the findings in drosophilids apply to other groups and if so, how far they can be extrapolated to other dipterans or insects in general.

Drosophilids belong to a division of Diptera called the Schizophora. This group originated around 65 million years ago and underwent rapid diversification. Today, with more than 50,000 species, the Schizophora accounts for more than a third of all fly species (Wiegmann et al., 2011; Wiegmann & Yeates, 2017). The clade is vastly ecologically diverse, is found in all terrestrial habitats and shows a striking diversity in wing morphology that relates to both natural and sexual selection (Dudley, 2002a). Therefore, by investigating static wing allometry across members of this diverse clade should help understand the ecological, developmental or genetic causes and consequences of the evolution of allometry, and whether the evolutionary stasis found in drosophilids also occurs in other groups of flies.

Using geometric morphometric methods, I here investigate the evolution of static and evolutionary allometry in the wings of higher Diptera. I first investigate overall variation in wing morphology among five species belonging to four different families and evaluate the relative contribution of species, sex and size differences. I then investigate the evolution of static allometries in more detail and investigate differences between species and sexes. Lastly, I assess whether static allometries within species are associated with evolutionary divergence across Schizophora by evaluating evolutionary allometry among 33 different families (belonging to 11 of 13 superfamilies following McAlpine (1989); Table S1). I discuss how the patterns across Schizophora relate to the evolutionary stasis found in drosophilids and evaluate the potential roles of selection and pleiotropy in driving static and evolutionary scaling relationships in Diptera.

2 | MATERIALS AND METHODS

2.1 | Dipteran wing morphology

Compared to other insects (e.g., odonates or stoneflies), the forewings of (higher) Diptera have a particularly derived and reduced wing venation pattern (Grimaldi & Engel, 2005; Shimmi, Matsuda, & Hatakeyama, 2014). Despite this reduction, fly wings represent complex functional structures lending dipterans their outstanding flight capacity that facilitates their vast degree of ecological diversification (Dudley, 2002a). Although wing shape varies strongly across all Diptera, wing venation is relatively invariant among Schizophora, allowing to trace the evolution of homologous characters (Ennos, 1989; Hennig, 1958, 1981; Redtenbacher, 1886). Wing vein nomenclature used here follows Oosterbroek (2006) (also see McAlpine, 1981).

To quantify static allometries in wing morphology, five species belonging to four distantly related dipteran families (Scathophagidae, Muscidae, Sepsidae and Drosophilidae) were reared under controlled

laboratory conditions. Although they all can be reared on decaying organic matter at the larval stage (as is common in flies), they vary strongly in the ecology of their adult forms. The yellow dung fly *Scathophaga stercoraria* (Linnaeus, 1758) is a large, predatory, cold-adapted species of Scathophagidae common across the Holarctic. The common housefly, *Musca domestica* Linnaeus, 1758, represents an extreme generalist common across the globe. The afro-tropical *Sepsis lateralis* Wiedemann, 1830 and the holarctic *Saltella sphondylii* (Schränk, 1803) both belong to Sepsidae, but strongly differ in their overall appearance, adult ecology and wing morphology (Pont & Meier, 2002). *Drosophila prolongata* (Singh and Gupta, 1977) is a cold-loving fruit fly found in South-East Asia. Laboratory cultures of all species were provided with a plastic dish filled with a food source that acted as an oviposition plate. These plates were removed after 24–48 hr. Eggs were retrieved and haphazardly distributed among plastic containers that held varying amounts of food (see Rohner, Teder, Esperk, Lüpold, & Blanckenhorn, 2018 for additional information). This resulted in varying degrees of larval competition for food and hence caused strong variation in adult body size. *Drosophila prolongata* was reared on standard *Drosophila* medium, whereas the other four species were reared in homogenized, previously frozen cow dung. The size variation within species and sex was extensive and approximates the maximal size range that can be achieved with food manipulation, facilitating accurate estimation of allometric slopes despite large differences in sample size per species and sex (*D. prolongata*: 166 females, 119 males; *M. domestica*: 56 females, 45 males; *Sa. sphondylii*: 37 males, 39 females; *Sc. stercoraria*: 213 females, 210 males; *Se. lateralis*: 52 females, 43 males). Upon adult eclosion and complete hardening, animals were killed, and their wings were removed and embedded in Euparal on glass slides. Slides were dried and later photographed using a LeicaDFC490 camera mounted on a Leica MZ12 microscope.

To investigate wing shape and its covariation with size, I integrated several methods of geometric morphometrics. Only eight landmarks that could unambiguously be identified in all specimens were used. These were placed at the intersections between the longitudinal veins R_1 , R_{2+3} and R_{4+5} and the costa, the most proximal edge of the wing cell r_{2+3} , as well as the anterior and posterior ends of both cross veins R-M and DM-Cu (see Figure 1). Together, these landmarks capture major axes of differentiation among families of the Schizophora (Ennos, 1989). Landmarks were digitized using TpsDig2 (Rohlf, 2009). All eight landmarks of all species (see below) were simultaneously subjected to a Procrustes analysis (or Procrustes transformation) using the R-package *geomorph* (Adams & Otárola-Castillo, 2013). These Procrustes-transformed coordinates were then used to investigate how wing shape changes with centroid size and sex within and across species.

2.2 | Variation in wing shape

First, a principal component analysis (PCA) was used to visualize the major axes of shape variation (using the *plotTangentSpace()*-function

as implemented in *geomorph*). To assess whether size contributes to the major axis of shape variation, Pearson correlation coefficients were used to investigate the relationship between PC1 and log-centroid size.

2.3 | Static allometries

A Procrustes ANOVA was used to test for species and sex differences in allometry across the whole data set using the function *procD.lm()* as implemented in *geomorph*. Because this model indicated that allometries differed between species and sexes (significant log-centroid size \times sex \times species interaction; see Results), sex-specific multivariate regressions of shape on size were calculated for each species separately. The vectors of coefficients of these regressions represent the multivariate 'broad-sense' form of static allometry for each sex and species, respectively. These allometric vectors (A) were then used to compute allometric spaces using ordination of allometric vectors to illustrate variation in allometric scaling (Gerber, Eble, & Neige, 2008; Gerber & Hopkins, 2011; Strelin, Benitez-Vieyra, Fornoni, Klingenberg, & Cocucci, 2018). To this end, I used the R-function *prcomp()* based on the covariance matrix of all static allometric vectors. Such ordination resulted in an allometric space where each point represents an allometric vector (rather than an individuals' shape as in an ordinary morphospace), where distances between points relate to the similarity in this particular allometric space.

For a more detailed assessment of pairwise sex and species differences in allometric scaling, I computed pairwise vector correlations following:

$$r_{A_i, A_j} = \frac{|A_i \cdot A_j|}{\|A_i\| \times \|A_j\|}$$

That is, the dot product of the allometric vectors A_i and A_j was standardized by their norm (Claude, 2008; Pitchers, Pool, & Dworkin, 2013; Schäfer et al., 2018). Because correlations between sexes were much higher than interspecific correlations, pooled within-sex vectors were compared when calculating correlations between species. Confidence intervals surrounding these correlations were estimated using a nonparametric bootstrapping procedure (10,000 iterations). The norm of the allometric vector was used to quantify the strength of the allometric effect in unit Procrustes distance per log-millimetre.

The shape score method proposed by Drake and Klingenberg (2008) was used to visualize the allometric relationship across all species. That is, the shape data (y) were projected onto a vector in the direction of a common allometric slope (β , as derived from a multivariate regression with all data combined) as $s = y\beta'(\beta\beta')^{-0.5}$. The regression score s can then be used to visualize the strength and shape of the overall relationship between shape and size (Drake & Klingenberg, 2008).

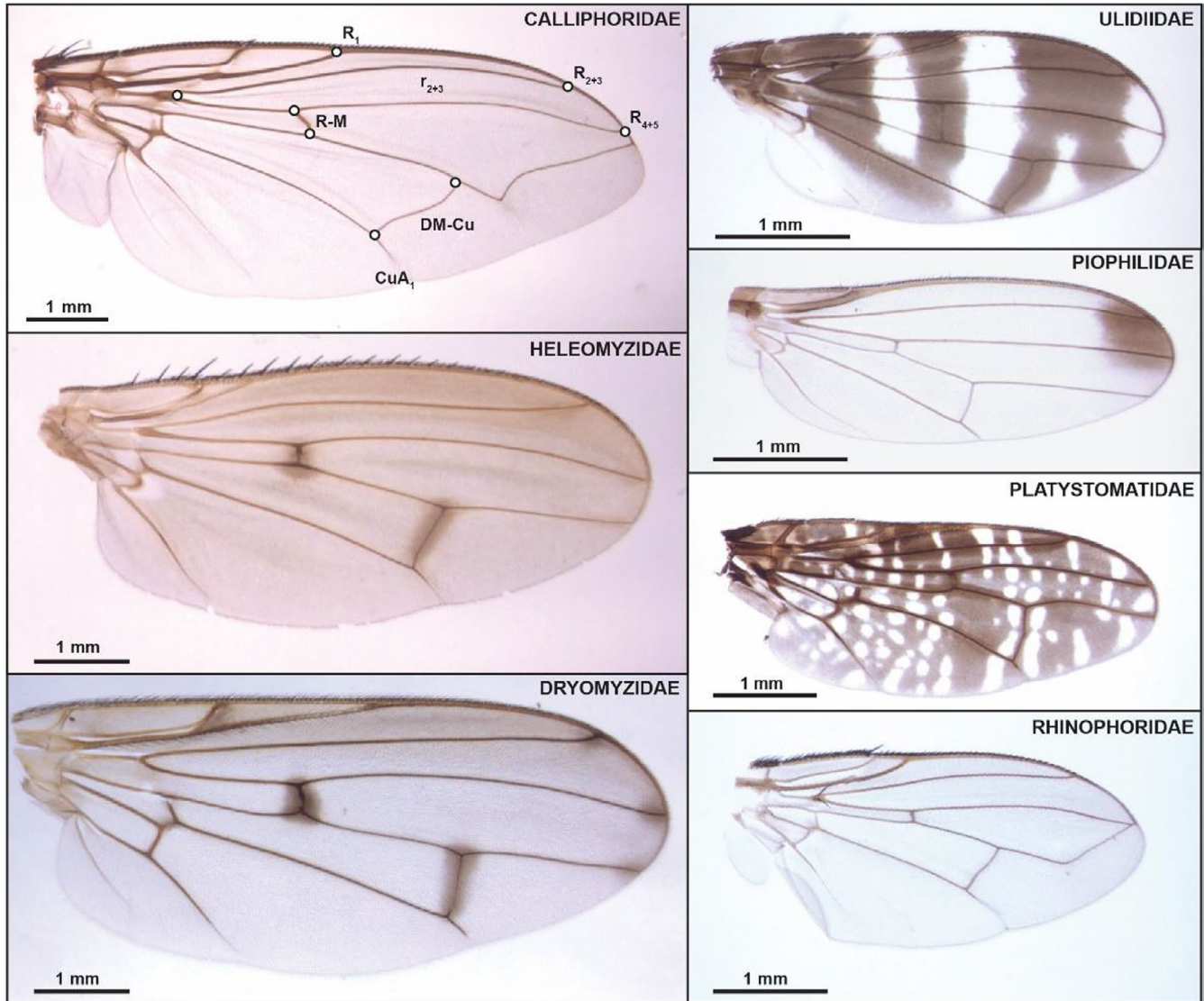


FIGURE 1 Wing morphology of selected species used in the comparative data set. Two-dimensional landmarks were placed at the intersections between the longitudinal veins R_1 , R_{2+3} and R_{4+5} and the costa, the most proximal edge of the wing cell r_{2+3} , as well as the anterior and posterior ends of both cross veins R-M and DM-Cu

2.4 | Evolutionary allometry across Schizophora

To quantify the scaling relationship among schizophoran species, I selected 33 pinned specimens belonging to 33 different families of both major subsections of the Schizophora (all specimens stem from the private collection of G. Bächli; families and representative species listed in Table S1). This taxon sampling covers 11 of the 13 superfamilies sensu McAlpine (1989). These species are ecologically highly diverse and differ markedly in their size (ranging from the usually 1–3 mm long leaf-miner flies (Agromyzidae) to the much larger flesh flies (Sarcophagidae) of more than 20 mm body length). Wing morphology, that is venation, colouration, thickness and size, also varied markedly (Figure 1).

Specimens were re-hydrated in a plastic container fitted with wet cotton overnight. Wings were carefully removed, embedded in Euparal, photographed and digitized as described above. After Procrustes transformation, wing shape was subjected to a

phylogenetic regression on log-centroid size to estimate evolutionary allometry taking into account the phylogenetic relationship among the dipteran families by Wiegmann et al. (2011) (*procD.pgls()* function implemented in *geomorph*). This vector was then compared to the static allometric vectors found within species. Sex could not be taken into account for this analysis. As a phylogenetically weighed estimate of the average static allometry, and to prevent multiple testing, I first computed pooled within-sex static allometries and then used a maximum-likelihood approach implemented in the R-package *phytools* (*fastAnc()*; Revell, 2012) to estimate the shared direction of static allometry among the five species. The correlation between this ‘ancestral’ static allometry and evolutionary allometry was computed as above, but its significance was tested using the approach proposed by Klingenberg and Marugan-Lobon (2013) using the closed-form expression for the area of a hypersphere cap by Li (2011). In brief, the sum of all vectors that have an angle of α or less relative to a fixed

vector can be represented as the cap of the hypersphere. Dividing the area of this cap by the total surface of the hypersphere (representing a random sample of a uniform distribution) equals the probability that a vector drawn at random from a uniform distribution has an angle $\leq \alpha$. This ratio then represents the p -value (see Klingenberg and Marugan-Lobon (2013) for a detailed description of the method).

3 | RESULTS

3.1 | Variation in wing shape

The major axis of variation (PC1) mostly related to the placement of the anterior cross vein (R-M) and an elongation of the proximal part of the wing relative to the distal part. PC2 mostly affected the posterior cross vein and landmarks at the tip of the wing (Figure 2a). PC1 and PC2 explained 59.4% and 21.8% of the total shape variation, respectively, and discriminate between species (Figure 2a), as is expected because they differ markedly in their wing shape (Figure 3). Intraspecific variation in shape was pronounced, but sexual dimorphism does not seem to constitute a major contributor. In contrast, PC1 was strongly related to log-centroid size ($r = .87, p < .001$; Figure 1b). As this correlation was also present within species (*Sc. stercoraria*: $r = .67, p < .001$; *D. prolongata*: $r = .73, p < .001$; *Sa. sphondylii*: $r = .83, p < .001$; *Se. lateralis*: $r = .86, p < .001$; *M. domestica*: $r = .45, p < .001$; Figure 1b), this suggests that the association between size and PC1 is not merely driven by interspecific differentiation but that a similar covariation holds within species. Repeating this principal component analysis based on the pooled within-species variance-covariance matrix leads to the same conclusion as the resulting first common principal component (explaining ~35% of the total variance) correlated strongly with log-centroid size ($r = .85$).

3.2 | Static allometries

A Procrustes ANOVA indicated that static allometries differed between species and sexes (significant log-centroid size \times sex \times species interaction in Table 1). Corroborating the overall patterns of the principal component analysis on wing shape, species differences ($\eta^2 = 0.50$) and log-centroid size ($\eta^2 = 0.46$) accounted for most of the variance in shape, whereas sex and the interaction terms had a much smaller effect size (all $\eta^2 \leq 0.03$; Table 1). To investigate differences in allometries in more detail, sex-specific multivariate regressions were fitted for each species separately. All species showed covariation between shape and size when analysed using multivariate regression, but the strength (i.e., the vector norm) of this effect differed between species and somewhat between sexes (Table 2). *Musca domestica* showed relatively weak deformations (average between sexes: 0.06), followed by *Sc. stercoraria* (0.10) and *D. prolongata* (0.12), whereas the two sepsids showed much stronger responses (*Se. lateralis*: 0.18; *Sa. sphondylii*: 0.17). Sex differences were particularly pronounced in *Sa. sphondylii* and *Se. lateralis* where females reacted much stronger to an increase in log-centroid size (Table 2).

Ordination of the sex-specific allometric vectors yielded an allometric space where the first two principal components explained 60.7% and 25.7% of the total variance, respectively. This is a large portion of the total variance, indicating that variation in wing shape allometries can be summarized in relatively few dimensions. PC1 and PC2 clearly distinguish between species (see Figure 2c), but in contrast to the morphospace (Figure 2a), the allometric space indicates comparably small interspecific variation relative to intraspecific variation in the form of sex differences in allometric slopes.

Pairwise vector correlations between static allometric vectors were moderate to strong between *Scathophaga*, *Musca*, *Saltella* and *Sepsis* (average correlation $r = .47$; Table 3). Only the pairwise comparison between *D. prolongata* and *Scathophaga* as well as *Saltella* was weak and not significantly different from zero (Table 3). When visualizing the common allometric variation using regression scores (Figure 2d), however, all species followed a similar allometric trajectory. The shape deformation associated with this regression score was very similar to the variation captured by PC1 (Figure 2a vs. d), reiterating the notion that a common allometric variation may explain a large portion of the total variance in wing shape.

3.3 | Evolutionary allometry across Schizophora

Wing shape varied strongly among families (Figure 4). The multivariate extension of Blomberg's Kappa ($K_{\text{mult}} = 0.49, p = .024$; Figure 4) was smaller than expected under Brownian motion ($K_{\text{mult}} = 1.00$), indicating little phylogenetic inertia (Adams, 2014). There was, however, evidence for evolutionary allometry in wing shape ($r = .38, p = .005$) which mostly entailed changes in the aspect ratio and the positioning of the two cross veins (Figure 4). The vector of this evolutionary shape change correlated significantly with the ancestral (i.e. phylogenetically weighed average) static relationship ($r = .49, p = .023$).

4 | DISCUSSION

Studying multivariate static allometries in five distantly related species of flies led to three main findings: Firstly, although species strongly diverged in wing shape (Figures 2a and 3), size-related changes represent a major contributor to overall variation, both within and among species (Figure 2b). Secondly, static allometries differ between species and sexes, yet allometric changes are correlated across species, suggesting a shared developmental programme underlying size-dependent shape variation (Figure 2c and Table 3). Lastly, static allometries also relate to the evolutionary divergence across the Schizophora. Together, these findings point towards a general mechanism that canalizes or maintains the covariation between shape and size despite rapid ecological and morphological diversification during the Cenozoic. I in turn discuss the putative roles of selection and developmental constraints in the evolution of wing allometry in the Schizophora.

Flight is the primary means of dispersal in (most) dipterans and is therefore critically involved in mate finding, courtship,

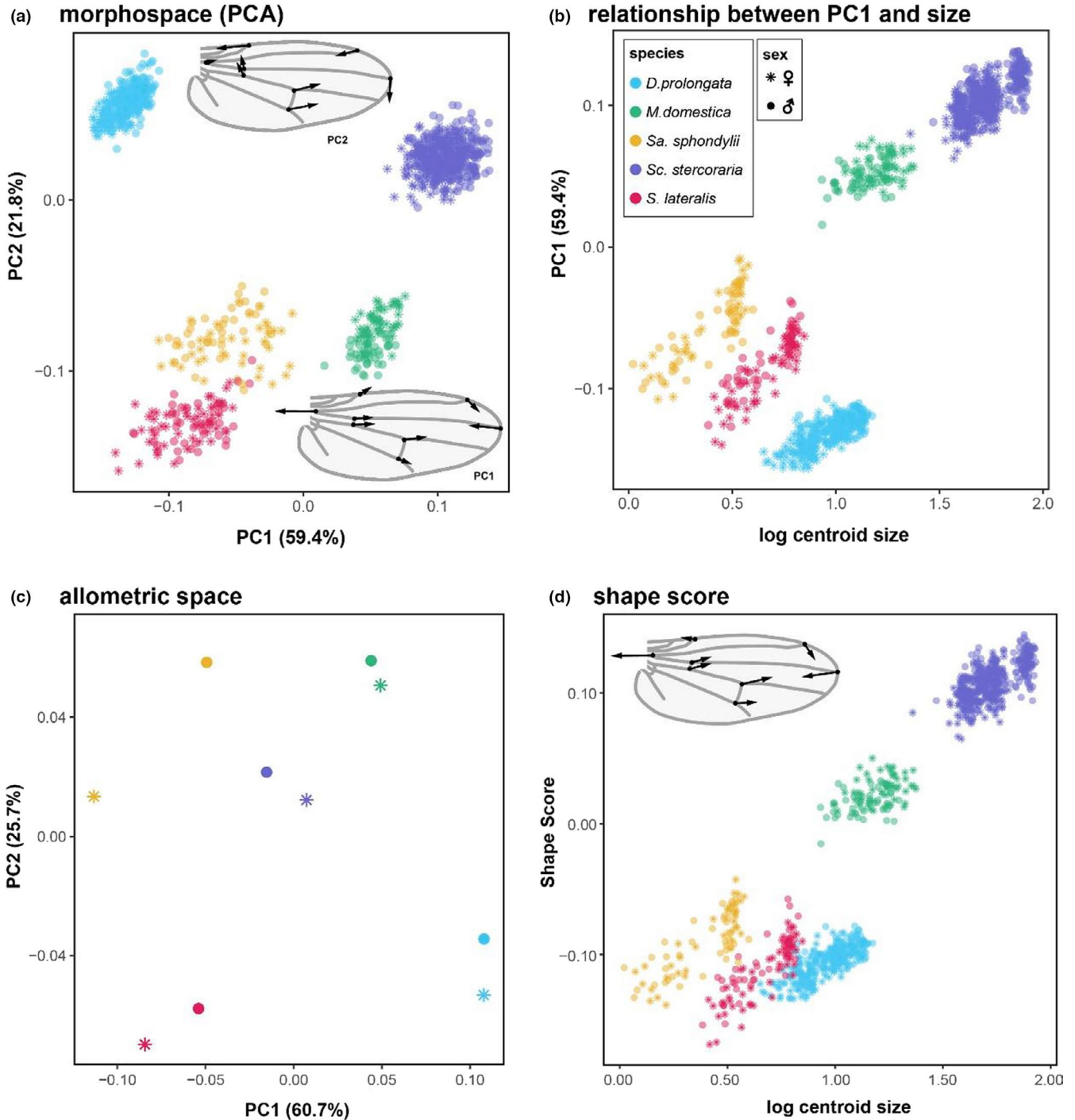


FIGURE 2 (a) The first two principal components cumulatively explain ~ 80% of the total variation in shape and differentiate between the five species investigated here. Shape change associated with an increase in PC1 and PC2 is indicated in sketches. (b) Plotting PC1 against log-centroid size reveals that the first principal component is strongly related to variation in size, both within and among species. (c) Ordination of sex-specific multivariate allometries in an allometric space shows clear differences in allometric relationships among species; this, however, seems small compared to intraspecific variation in the form of sex differences. (d) Drake & Klingenberg's regression score, a metric representing a projection of the individual wing shape data on the allometric vector across all samples. The shape deformations associated with an increase in the shape score correspond well to the shape changes captured by PC1 (a)

thermoregulation, foraging and various other behaviours (Dillon, Wang, Garrity, & Huey, 2009; Dudley, 2002a). Because the physical forces involved in flight directly relate to size, and natural as well as sexual selection act on performance and manoeuvrability, selection

is expected to act on size-dependent compensatory adjustments of wing morphology (Dudley, 2002b; Unwin & Corbet, 1984) and flight behaviour (Lehmann, 1998, 2002; Sane, 2003). Stabilizing selection on a shared developmental programme that accommodates size-related

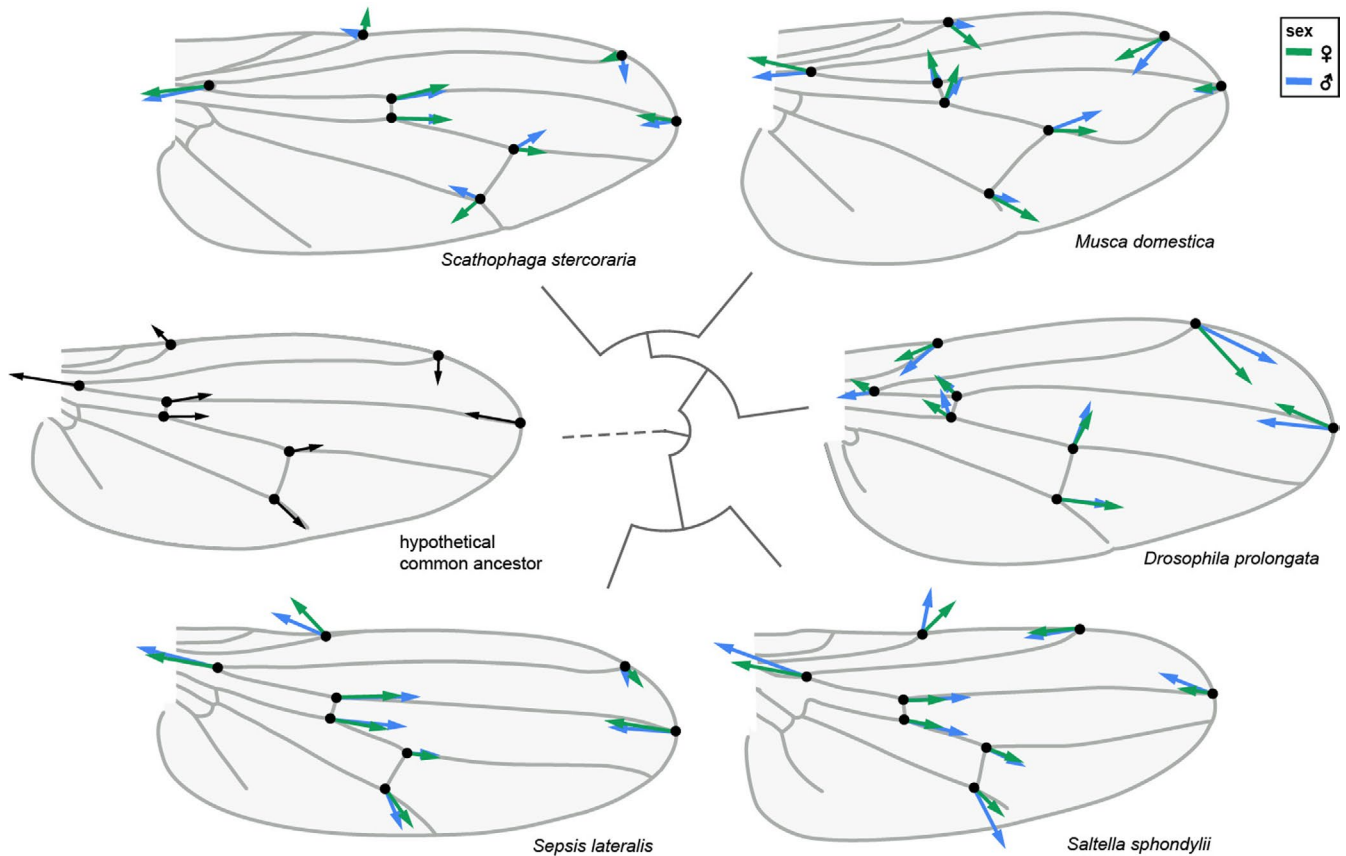


FIGURE 3 Sex-specific static allometric variation in the five species that were reared under laboratory conditions. A maximum-likelihood based reconstruction of the ancestral pooled within-sex static allometry is shown in black. The grey cladogram in the centre illustrates the phylogenetic relationships

TABLE 1 Procrustes ANOVA table summarizing the effects of size, sex and species differentiation on wing shape

	<i>df</i>	<i>SS</i> × 10 ²	<i>MS</i> × 10 ²	η^2	<i>F</i>	<i>Z</i>	<i>p</i>
Log-centroid size	1	861.72	861.72	0.46	13,536.3	10.3	<.001
Sex	1	2.00	2.00	<0.01	31.4	4.1	<.001
Species	4	920.90	230.23	0.50	3,616.5	16.1	<.001
Log-centroid size × sex	1	0.41	0.41	<0.01	6.5	2.4	<.001
Log-centroid size × species	4	8.15	2.04	<0.01	32.0	7.6	<.001
Sex × species	4	3.20	0.80	<0.01	12.6	5.7	<.001
Log-centroid size × sex × species	4	0.57	0.14	<0.01	2.2	2.0	.022
Residuals	960	61.11	0.06	0.03			
Total	979	1,858.06	1.90				

physical constraints therefore appears as a likely general mechanism that canalizes allometry in a wide range of species. Such an adaptive scenario would not only explain the similarities in static allometries between species, but also the association between static allometries and evolutionary divergence. Because the allometric changes mostly involve the positioning of the cross veins and the overall aspect ratio, which most probably relate to the stiffness of the wing blade and lift production, respectively, such an adaptive scenario seems likely.

However, direct evidence for a functional relevance of wing shape and its allometry are scarce. While repeatable plastic and genetic responses, as well as functional implications have been demonstrated for wing size (Azevedo, James, McCabe, & Partridge, 1998; Gilchrist, Azevedo, Partridge, & O'Higgins, 2000; Rohner, Pitnick, et al., 2018; Rohner, Roy, Schäfer, Blanckenhorn, & Berger, 2019; Starmer & Wolf, 1989; Frazier, Harrison, Kirkton, & Roberts, 2008), the functional relevance of the relative positioning of individual wing veins remains

TABLE 2 Summary table providing information about the strength of sexual shape dimorphism (in unit Procrustes distance), the correlation between sex-specific static allometries and the length (=norm) of the sex-specific allometric vector ($|A|$)

	Sexual shape dimorphism	Sex-specific allometries		
	Procrustes distance $\times 10^3$	Correlation between sexes	$ A $ males $\times 10^2$	$ A $ females $\times 10^2$
<i>Drosophila prolongata</i>	17.9 [15.3, 20.0]	0.93 [0.89, 0.98]	11.6 [10.1, 13.1]	12.8 [11.4, 14.1]
<i>Musca domestica</i>	21.4 [17.2, 25.2]	0.89 [0.84, 0.97]	5.5 [3.4, 6.9]	6.9 [4.3, 9.2]
<i>Saltella sphondylii</i>	21.4 [15.5, 25.0]	0.94 [0.90, 0.99]	13.8 [10.6, 17.0]	20.3 [16.5, 23.5]
<i>Scathophaga stercoraria</i>	12.0 [9.6, 13.9]	0.92 [0.86, 0.98]	9.8 [7.9, 11.7]	9.3 [7.2, 11.4]
<i>Sepsis lateralis</i>	11.1 [7.0, 14.2]	0.97 [0.94, 0.99]	16.8 [13.8, 19.7]	19.6 [17.1, 22.1]

Note: Complementary 95% bootstrap intervals are given.

TABLE 3 Pairwise vector correlations between the vectors of allometric shape change (upper off-diagonal) and corresponding 95% confidence intervals (lower off-diagonal)

	<i>Drosophila prolongata</i>	<i>Musca domestica</i>	<i>Saltella sphondylii</i>	<i>Scathophaga stercoraria</i>	<i>Sepsis lateralis</i>
<i>Drosophila prolongata</i>	–	0.28	0.02	0.08	0.34
<i>Musca domestica</i>	[0.05, 0.53]	–	0.67	0.49	0.40
<i>Saltella sphondylii</i>	[0.00, 0.07]	[0.38, 0.87]	–	0.79	0.80
<i>Scathophaga stercoraria</i>	[0.01, 0.20]	[0.11, 0.77]	[0.71, 0.88]	–	0.85
<i>Sepsis lateralis</i>	[0.24, 0.44]	[0.07, 0.67]	[0.71, 0.88]	[0.79, 0.91]	–

poorly understood. As the biomechanics of flight are dependent on environmental conditions (such as temperature and atmospheric pressure; Dudley, 2002a; Lehmann, 1999; Unwin & Corbet, 1984), and in light of the ecological diversity among schizophorans, it remains unclear whether allometry is under selection at all and whether similar forms of stabilizing selection act in different species and ecological contexts.

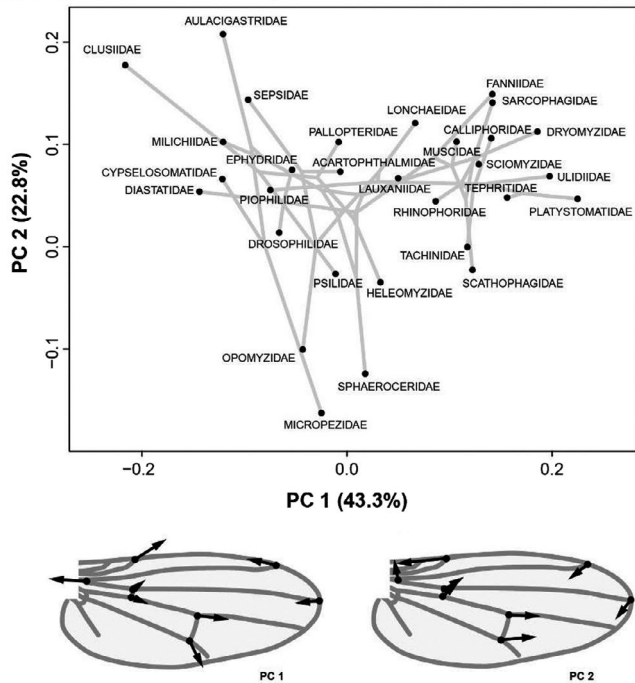
Alternative mechanisms that could lead to shared patterns of static allometry are genetic and/or developmental constraints. Wings might be particularly prone to pleiotropy because of rather few major signalling pathways that govern the patterning of imaginal discs giving rise to the adult wings, halteres and legs (e.g., hedgehog, decapentaplegic, EGFR; Ruiz-Losada et al., 2018). These discs further share temporal pattern of expression of the same regulatory elements (McKay & Lieb, 2013). Pleiotropy may thus be more common than previously expected and influence organismal evolution more than currently acknowledged (Sabarís, Laiker, Preger-Ben Noon, & Frankel, 2019). However, venation patterns do not seem to be particularly constrained to evolve per se given abundant additive genetic variation for selection to act upon (Mezey & Houle, 2005; Schäfer et al., 2018). Multivariate static allometries also do not seem to be hampered by absolute constraints. The two closely related black scavenger flies *Sepsis fulgens* and *Se. punctum*, for instance, show high vector correlations between multivariate allometric shape changes, yet this correlation is clearly distinct from unity ($r = .87$ [0.76, 0.92] 95% CI). Wing shape changes associated with rearing temperature differed even more between the two sepsids (vector correlation based on 15 landmarks amount to 0.33 or 0.60 depending on the temperature range used; Rohner et al., 2019), demonstrating that also other forms of plasticity evolve in comparably short periods of time. Evidence for rapid evolution of plasticity also comes from the yellow dung fly, for

which populations on different continents show different patterns of thermal plasticity and static allometry (Schäfer et al., 2018). While surely biasing the phenotypic variation that is exposed to selection in general, developmental and/or genetic constraints do not seem to put an absolute limit on the evolution of wing allometry and thus are unlikely to account for the significant similarities that persisted despite rapid ecological diversification throughout the Cenozoic.

In drosophilids, the slow evolution of allometric scaling has been previously attributed to stabilizing selection on pleiotropic effects, causing evolutionary stasis (Bolstad et al., 2015; Houle et al., 2019). The multivariate approach followed here at least superficially recapitulates the patterns found for narrow-sense allometries in the Drosophilidae (Bolstad et al., 2015; Houle et al., 2019) and might indicate that similar processes act on a much larger phylogenetic scale. However, although the results found here indicate a shared axis of plasticity among species overall, it also shows that interspecific variation in allometry differs between landmarks. The length of vein R_{4+5} for instance shows similar covariation with size in all five species (e.g., R_{4+5} ; Figure 3), whereas other measurements show much more interspecific variation (R_1 ; Figure 3). This suggests that not all landmarks are under the same evolutionary forces and implies that the choice of measurements used for bivariate allometries must heavily influence the evolutionary inference. It is further worth pointing out that *D. prolongata* had the lowest vector correlations compared with the others (Table 3). Whether this is a species-restricted effect or whether this applies to drosophilids in general remains unclear at this point.

Sexual shape dimorphism and sex-specific allometric scaling have been documented in several species and seem to be the rule, rather than the exception at least among flies (Gidaszewski

(a) phylogenetic morphospace



(b) evolutionary allometry

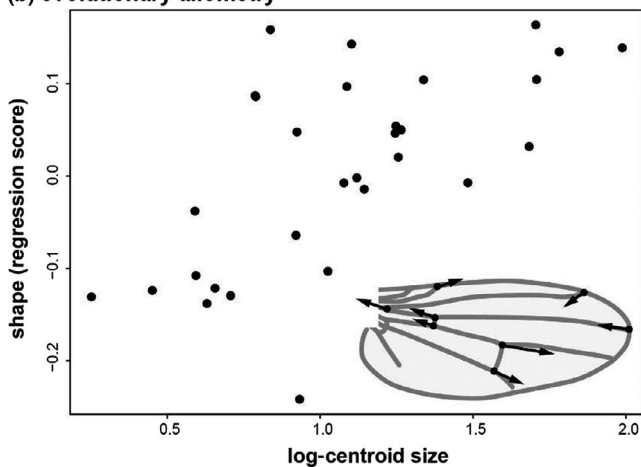


FIGURE 4 (a) Ordination of wing shape reveals strong variation among different dipteran families with little (if any) phylogenetic clustering (the phylogenetic relationship, following Wiegmann et al., 2011, is indicated in grey). (b) Despite the evolutionary lability of wing shape, wings show significant evolutionary allometry. Shape deformations associated with an increase in PCs and log-centroid size are indicated with vectors

& Klingenberg, 2009; Pitchers et al., 2013; Rohner et al., 2019; Schäfer et al., 2018). Sexual shape dimorphism was variable and almost twice as strong in *Sa. sphondylii* compared to *Se. lateralis*, suggesting that sexual shape dimorphism can differ rather strongly within families. However, the overall effect of sex seems small compared with species differentiation and allometry (Figure 2). Similar patterns were found for the direction of sex-specific allometric slopes (high between-sex correlations in Table 2). However, in contrast to sex differences in the direction of allometry, the strength of

the respective allometric change varied (leading to relatively large sex differences in allometric spaces, as the latter also incorporates direction and strength of the allometric effect). Whether the strength and direction of allometry relate to sex-specific differences in function remains unclear but warrants further scrutiny.

5 | CONCLUSIONS

How often and by which means the mechanisms that underlie static size-dependent trait expression can bias evolutionary change remains an open question. Comparing static allometric slopes in a phylogenetic framework suggests a shared developmental programme despite rapid ecological diversification among schizophoran flies. Presupposing functional implications of wing shape, strong stabilizing selection on a common allometric relationship is the most likely explanation. However, pleiotropy may represent a previously underestimated source of constraint. Whether selection or pleiotropy is more important in driving similarities in allometric relationships is, at this point, difficult to assess due to the lack of functional data. The broad comparative framework applied here may, however, prove useful for exposing variation in allometric scaling that can then be used to assess the functional relevance of wing shape and its allometry.

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AUTHOR CONTRIBUTIONS

PTR designed the study, analysed all data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data will be available in Dryad upon acceptance. <https://doi.org/10.5061/dryad.xd2547ddc>.

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Additional supporting information may be found online in the Supporting Information section.

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