Body size evolution in an old insect order: No evidence for Cope's Rule in spite of fitness benefits of large size

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We integrate field data and phylogenetic comparative analyses to investigate causes of body size evolution and stasis in an old insect order: odonates ("dragonflies and damselflies"). Fossil evidence for "Cope's Rule" in odonates is weak or nonexistent since the last major extinction event 65 million years ago, yet selection studies show consistent positive selection for increased body size among adults. In particular, we find that large males in natural populations of the banded demoiselle (*Calopteryx splendens*) over several generations have consistent fitness benefits both in terms of survival and mating success. Additionally, there was no evidence for stabilizing or conflicting selection between fitness components within the adult life-stage. This lack of stabilizing selection during the adult life-stage was independently supported by a literature survey on different male and female fitness components from several odonate species. We did detect several significant body size shifts among extant taxa using comparative methods and a large new molecular phylogeny for odonates. We suggest that the lack of Cope's rule in odonates results from conflicting selection between fitness advantages of large adult size and costs of long larval development. We also discuss competing explanations for body size stasis in this insect group.

KEY WORDS: Body size, comparative methods, natural selection, Odonata, phylogenetics, sexual selection, stasis.

Body size influences many aspects of an organism's life history (Cardillo et al. 2005), and larger body size is typically associated with fitness benefits (Blanckenhorn 2000). In spite of these fitness benefits, many populations and lineages remain small over long evolutionary time scales (Blanckenhorn 2000; Kingsolver and Pfennig 2004). On paleontological time scales, Cope's Rule states that within-lineage body size tends to increase over time (Gould 1997; Alroy 1998; Gotanda et al. 2015; Bokma et al. 2016). Support for Cope's Rule comes most famously from comparative analyses of extant mammalian taxa (Baker et al. 2015). Based on the results from meta-analyses of natural and sexual selection in natural populations (Kingsolver et al. 2001), it has been suggested that Cope's Rule might largely or entirely be a result of positive selection of large size at the intraspecific level and in local populations (Kingsolver and Pfennig 2004; Kingsolver and Diamond 2011).

Some lineages, however, do not show evidence for Cope's Rule and the expected evolutionary increase in mean body size, in spite of consistent directional selection for larger size (Gotanda et al. 2015; Rollinson and Rowe 2015). Body size evolution in some lineages is often slow, potentially being an example of evolutionary stasis (Gotanda et al. 2015; Rollinson and Rowe 2015). The problem of evolutionary stasis (or at least apparent stasis in the fossil record) has been discussed for a long time in the evolutionary biology literature, and a variety of explanations have been put forward to explain such patterns (e.g., Charlesworth et al. 1982; Merilä et al. 2001; Hansen and Houle 2004; Estes and Arnold 2007). Controversies remain whether stasis is a real phenomenon or a statistical artefact. The two main explanations for stasis are either based on some forms of genetic constraints or opposing selection pressures (Hansen and Houle 2004). A third pattern of body size evolution is punctuated change associated with cladogenesis as lineages split (Mattila and Bokma 2008). Understanding how and which of these different modes of body size evolution are operating on extant taxa and were operating on extinct taxa in the past remains a major challenge in evolutionary biology.
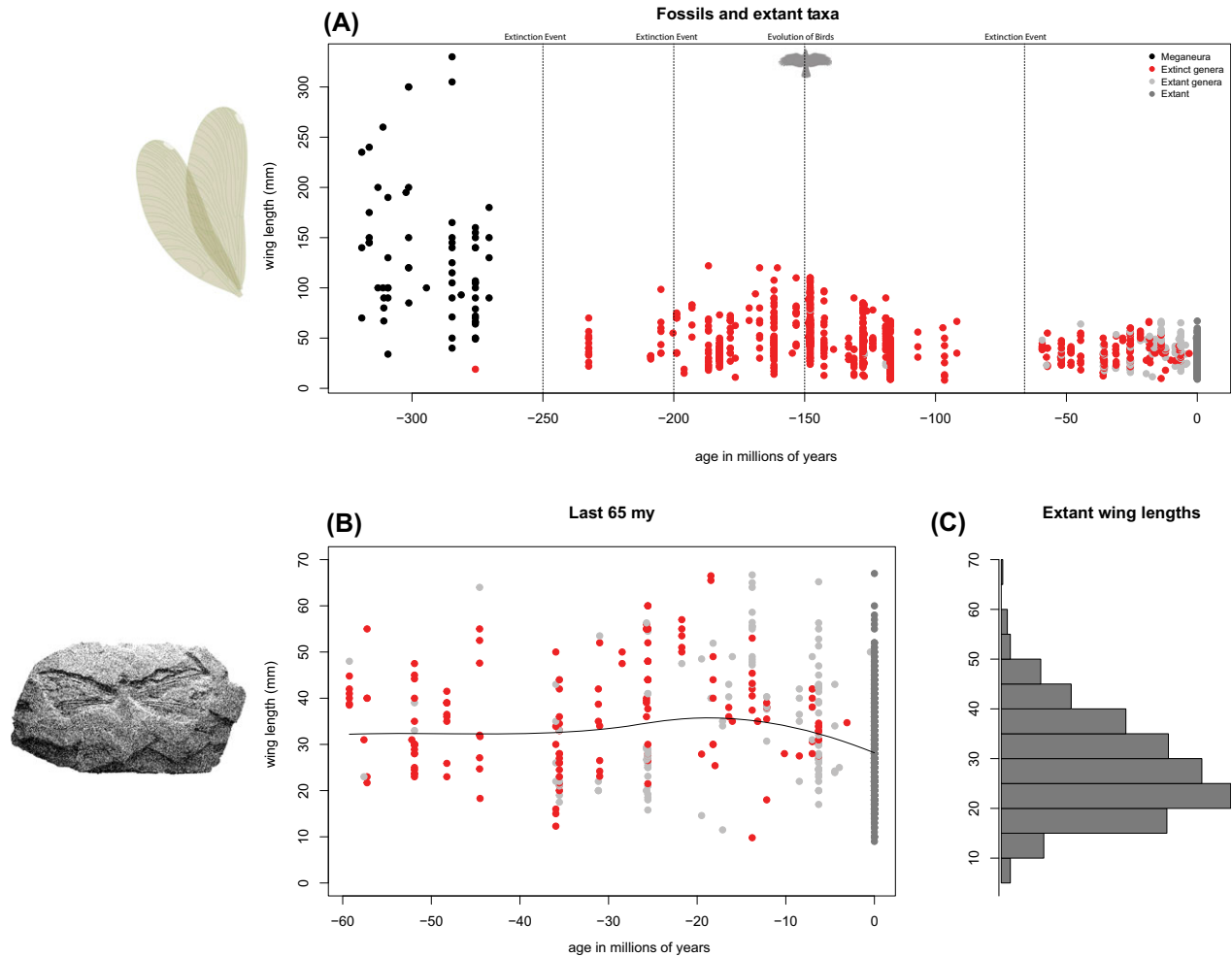
Figure 1. (A) Odonate fossil data over the last 350 million years. Wing measurements and dates were taken from Clapham and Karr (2012). The genus *Meganeura* are shown in black, extinct genera in red, extant genera in gray, and extant taxa in dark gray. Major extinction events are indicated with dotted lines. (B) Over the last 65 my, since the last major extinction event, mean wing length has remained relatively constant (indicated with a lowess curve). The down-tick seen in this graph is probably an artifact of lowess overfitting and not representative of real evolution of smaller wing length. Smaller extinct species may be underrepresented, since they might fossilize at a lower rate. (C) The histogram shows the distribution of extant wing sizes, which is slightly right-skewed, which might indicate size-dependent diversification in the presence of a lower bound (Clauset and Erwin 2008).

Hone and Benton (2005) outlined several reasons why lineages might remain small, despite selection for larger body size, and their possible explanations include increased development times and increased susceptibility to extinction of large taxa (Damuth 1981; Millar and Zammuto 1983; Damuth 1993).

The old insect order Odonata (Anisoptera: "dragonflies"; Zygoptera: "damselflies") consists of more than 6000 extant species. This insect order was present already during the lower Permian about 250 mya, and they might have evolved even earlier (Grimaldi and Engel 2005). Odonates have complex life cycles, with an aquatic larval stage and a terrestrial adult reproductive stage, and final adult structural body size is therefore fixed as they emerge from the larval stage (Corbet 1999; Stoks and Cordoba-Aguilar 2012). Environmental conditions in the larval stage have

been shown to have strong carry-over effects with important fitness consequences for the adult stage (Corbet 1999; Stoks and Cordoba-Aguilar 2012). The aquatic larval stage can last years in some species (Corbet 1999), and it is usually considerably longer than the adult stage, which can last as little as a few days or weeks (Corbet 1999).

Crow-sized dragonfly relatives of modern odonates (Protodonata) existed between the late Carboniferous and Late Permian, but these large insects went extinct around 247 mya (Grimaldi and Engel 2005) (Figs. 1, S1). As atmospheric oxygen levels were considerably higher during this period (30–35%) compared to today (about 21%), such physiological limits have been suggested to limit body size evolution of tracheal-breathing insects (Harrison et al. 2010). This oxygen hypothesis is also partly

supported by experimental work on fruit flies (Drosophila), which if raised and exposed to experimentally increased oxygen levels for multiple generations evolve increased body sizes (Klok and Harrison 2009). However, it has also been argued that predation or niche filling by birds are major selection pressures that have counteracted the evolution of larger body sizes in insects during the last 150 mya (Clapham and Karr 2012).

While there have been large increases and decreases in maximum body size in the odonate fossil record within individual lineages, whole-lineage mean body size has changed very little since the end of the Cretaceous (Fig. 1; data taken from Clapham and Karr 2012). This lack of any directional trends suggests that body size evolution in odonates have largely followed an unbiased random walk process since the last mass extinction at the K-T boundary 65 mya. Such apparent stasis at the level of the entire order–-as suggested by the odonate fossil record–-is somewhat paradoxical in light of the common observation of positive selection for large body size in the adult stage in most odonate species studied to date (Honek 1999; Sokolovska et al. 2000). Hence, there appears to be body size stasis in this and many other animal groups, when we would rather expect to see Cope's Rule, based on the positive bias in the direction of selection on body size (cf. Kingsolver and Pfennig 2004), that is the idea that "bigger is better" (Blanckenhorn 2000; Kingsolver and Huey 2008).

In this article, we argue that slow body size evolution in odonates is likely to result from conflicting selection pressures that change sign during the life cycle, in line with some previous suggestions (Thompson and Fincke 2002; Nijhout et al. 2010). The problem of evolutionary stasis has been discussed at depth in the evolutionary literature (Merilä et al. 2001; Hansen and Houle 2004; Voje 2016). Here, we show using combination of field studies and phylogenetic comparative analyses that several of the proposed mechanisms that could generate stasis are unlikely to explain slow body size evolution in odonates. We generated a large new molecular phylogeny of odonates for this purpose and use modern comparative methods to investigate the mode and dynamics of body size evolution in this insect order. We especially evaluate five not mutually explanations for stasis: (1) stabilizing selection in the adult stage; (2) fluctuating selection in the adult stage; (3) conflicting selection in the adult stage (pleiotropic constraints between adult fitness components); (4) species-selection against large taxa, and (5) conflicting selection between the adult and larval stage.

Based on the results in our study, we suggest that conflicting selection between adult body size and costs of long development time is the most likely explanation for stasis in this insect order (cf. Schluter et al. 1991). Our favored explanation for slow body size evolution in odonates is potentially also applicable to other insect groups and ectotherms that show no evidence of "Cope's Rule" and no evidence for apparent directional evolutionary size

trends. Conflicting selection pressures can thus be divided into selection against long larval development time in opposition against selection for large body size in the adult stage through increased fecundity, survival, and mating success (Schluter et al. 1991). We briefly discuss the various other explanations for stasis in body size evolution that have been proposed, and the implications of our results for other organismal groups.

Materials and Methods
GENERAL PROCEDURES: FIELD WORK, SELECTION ANALYSES, LITERATURE DATA, AND PHYLOGENY RECONSTRUCTION

Unless otherwise stated, all statistical analyses in this article were performed using the R programming environment (R Development Core Team 2013). The phylogenetic tree that we use in this study was constructed from odonate DNA sequences downloaded GenBank (Fig. S2). The resulting phylogeny comprised 1322 taxa, about 21% of all known extant odonate species (about 6400 in total) (Schorr and Paulson 2015). See our Supplementary Material for a detailed description of the construction of phylogenetic trees used in this study (Figs. S2–S4), and how we accounted for phylogenetic uncertainty (Fig. S5).

Survival and sexual selection estimates come from a mark-recapture study of males in the banded demoiselle (*Calopteryx splendens*), that took place in southern Sweden from 2011–2015 (see Supplementary Material; Figs. S6, S7; Table S1). Briefly, we performed daily field work during the summer months (June and July) 2011–2015. We captured as many males as possible, marked them individually, and recorded survival (longevity) and mating success (fraction of males obtaining copulations). We also measured several morphological traits, of which we used forewing length and total body length (both measured in mm) in our analyses of natural and sexual selection in this study. We subsequently used individual variation in these two traits and fitness components (longevity and male mating success) to quantify natural and sexual selection in the field (Lande and Arnold 1983; Gosden and Svensson 2008; Stinchcombe et al. 2008). More detailed methodological information about general field work procedures, population locations, and the quantification of natural and sexual selection in this species can also be found in the Supplementary Material and in our previous publications (Svensson et al. 2004, 2006, 2014, 2016; Waller and Svensson 2016).

Body length and wing length data were extracted from the literature (mainly from national, regional and local field guides, complemented with scientific articles, See Table S2) for each odonate species in the World Odonata List (Schorr and Paulson 2015). All these trait data and ecological variables have been compiled into a database called the Odonate Phenotypic Database (opdb), which will become publicly available in the near future).

More detailed information can be found in the Supplementary Material (Fig. S8).

We collected data on larval development time in relation to latitude and body length data of European odonates from the literature (Sandhall 1987). To quantify the relationship between body size and development time (in years), we conducted a phylogenetic generalized least square (PGLS) regression using the corPagel function in ape (Paradis et al. 2004) with a lambda parameter estimated be 0.89. Latitudinal data for these species were obtained from the distribution maps in Dijkstra and Lewington (2006). More information can be found in the Supplementary Material.

We compiled and updated literature data from a previously published quantitative review about the relationship between body size and various fitness components across several odonate species (Sokolovska et al. 2000). To evaluate the possible role of stabilizing selection on body size from these correlation estimates, we simulated the distribution of correlation coefficients that we would expect to sample under a scenario where only stabilizing selection was operating and only a correlation coefficient was estimated (See Supplementary Material for explanation).

DIVERSIFICATION IN RELATION TO BODY SIZE

We estimated the speciation rate in relation to body size of each odonate family for which we had sufficient (> 10 taxa) phylogenetic and phenotypic data ($N = 21$ families), assuming a yule model or pure speciation model (Paradis et al. 2004). A yule model was fitted for each family tree with missing species added. We used mean body length per family, calculated using the body lengths from all our species in our dataset.

The procedure described above was preferred over fitting all the data in a single model, such as using the QuaSSE procedure (FitzJohn 2010). We had a large number of missing tip taxa, and QuaSSE can only deal with randomly missing tip taxa (Fitzjohn 2010). QuaSSE is also known to have high type I error rates when the rates on the tree vary (Rabosky and Goldberg 2015). However, for completeness, we also present the results of a fitted QuaSSE model (sigmoid), although we emphasize that these results should be interpreted with the above caveats in mind (Fig. S9). We were unable to reliably estimate extinction rates, as there were many missing terminal taxa in our phylogeny (FitzJohn et al. 2009). In these analyses, we therefore assumed that the extinction rates were constant across the phylogeny.

COMPARATIVE ANALYSES OF BODY SIZE EVOLUTION

First, we fitted a simple single-optimum Ornstein-Uhlenbeck (OU) model of evolution to our body size data. Our goal here was simply to detect if there was any evolutionary constraints on interspecific body size variance accumulation in this lineage (Butler and King 2004). This single-optimum OU-model was first compared to a Brownian Motion (BM) model where interspecific variance in body sizes is supposed to increase at a linear rate over time (Hansen 1997). A significant alpha parameter in an OU-model suggests the presence of a significant pullback force that constrains maximum or minimum body size, and is often interpreted as an indication that some constraints are operating on the lineage as a whole (Hansen et al. 2008; O'Meara and Beaulieu 2014). The fit of this simple OU-model with one optimum was subsequently compared to the fit of a BM-model using the Akaike Information Criterion (AIC).

We complemented this single-optimum OU-model by using a newly developed phylogenetic comparative method that automatically detects evolutionary shifts in OU optima for a continuously varying trait (the l1ou R-package; Khabbazian et al. 2016). We used the estimate_shift_configuration function in this package to document the most likely shifts in optima in our body length data on the phylogenetic tree (Khabbazian et al. 2016). Shifts in body size optima would indicate a changing adaptive landscape over evolutionary time and suggest cases of positive or negative body selection pressures toward different optima. The l1ou R-package package offers several computational advantages for large trees (such as ours, with > 700 species), compared to similar methods such as surface and bayou (Ingram and Mahler 2013; Uyeda and Harmon 2014), which often take a long time to converge. For further details regarding our phylogeny and our comparative analyses, see Supplementary Material.

Results

NATURAL AND SEXUAL SELECTION ON BODY SIZE IN THE FIELD

Standardized linear univariate selection gradients (β) estimated from how forewing length of *C. splendens* males affected survival suggested that natural selection on wing length was positive in four out of the five seasons (Fig. 2). The strength of selection varied only marginally across years and did not change sign (ANCOVA; Forewing length: $F_1 = 13.8$, $P < 0.001$; Year: $F_4 < 0.001$, $P = 1.00$, Forewing length × Year: $F_4 = 2.08$, $P = 0.085$). Selection on body size was positive from 2011 to 2014 (Fig. 2). The exception to this positive survival selection was observed in 2015, when natural selection on forewing length was close to zero and not significant (Fig. 2). Combining these natural selection estimates for all years showed significant and consistent directional selection toward longer forewing length, but no evidence of significant quadratic selection (Fig. 2). A similar picture emerged when we estimated survival selection on total body length, where selection gradients were positive in all five years and highly significant when years were combined (β = 0.147; $P < 0.001$; Fig. 2). Similar to the situation for wing length, there was no significant
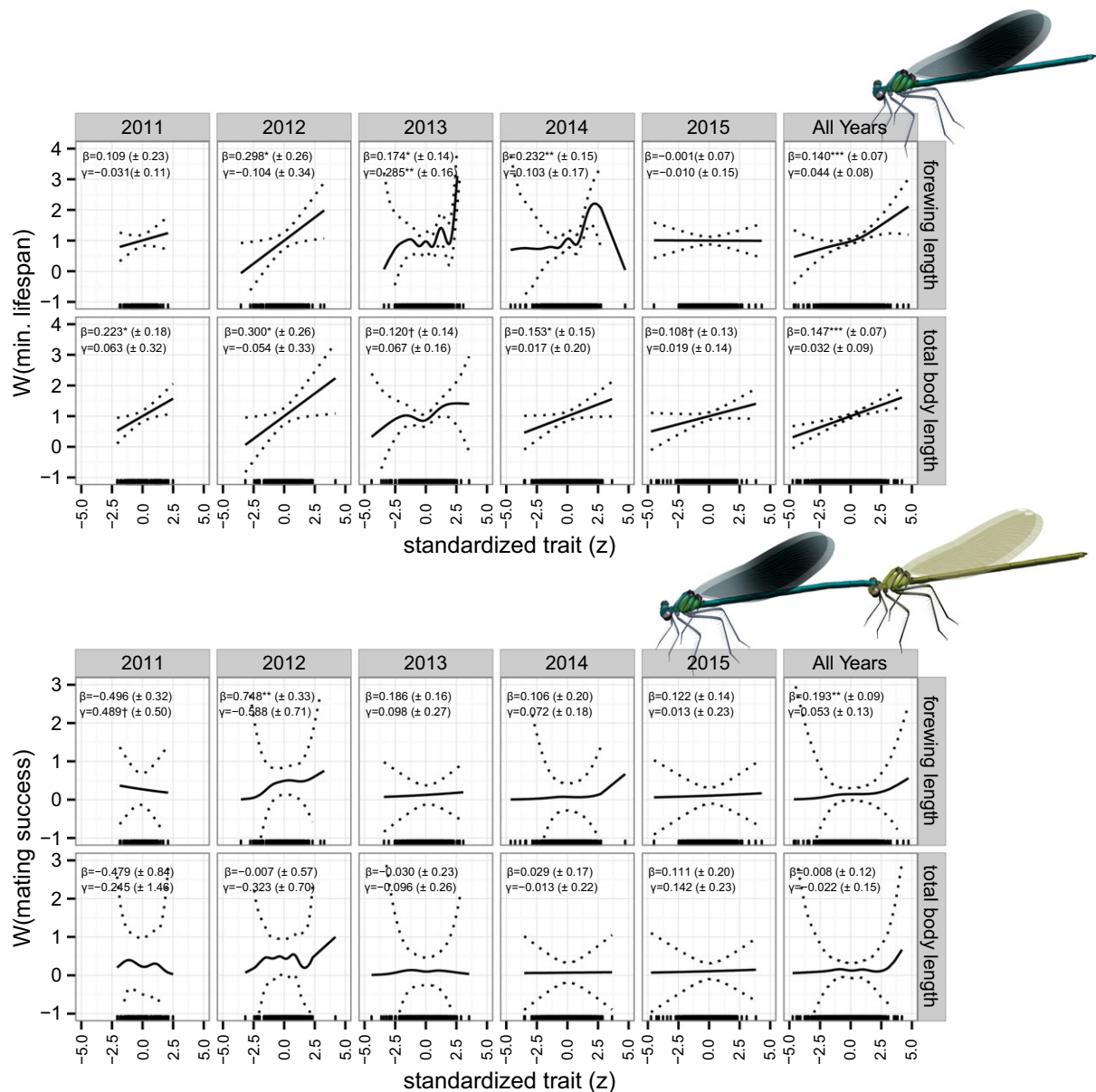
Figure 2. Natural and sexual selection in adult male banded demoiselles (*Calopteryx splendens*) from 2011 to 2015. Cubic splines were fit to each year's fitness data (Schluter 1988). Fitness functions for natural selection (minimum lifespan) are shown in the upper panel, and fitness surfaces for binary mating success (sexual selection) in the lower panel. Cubic splines were fit using the gam function in the package mgcv (Wood 2011) and smoothing with method GCV.Cp. R code for this figure is included in the supplement. Linear selection (β) and quadratic coefficients (γ) are listed in the top corner. Significance is marked († < 0.1, * < 0.05, ** < 0.01, *** < 0.001). In most years, selection is positive for wing length, both in terms of survival and mating success. There is very little evidence for stabilizing selection, as often our quadratic coefficients show even slight positive curvature. See supplement for more detailed statistics and a similar analysis with a composite body size measure (PC1).

evidence for any negative curvature in the fitness function of body length, that is no evidence for stabilizing selection (γ) on body size (Fig. 2).

For sexual selection (male mating success), we found that the univariate selection gradients for forewing length were also mainly positive in four out of five years (Fig. 2). The exception to this general pattern was in 2011, when we found a negative

sexual selection gradient for forewing length, although this was far from significant (Fig. 2). Sexual selection on body length showed a less consistent pattern, with negative directionality the first three years, and positive the last two years, although notably these differences were not significant, neither alone or when years were combined (Fig. 2). Just like in the case for natural selection (see above), there was no evidence for any significant stabilizing
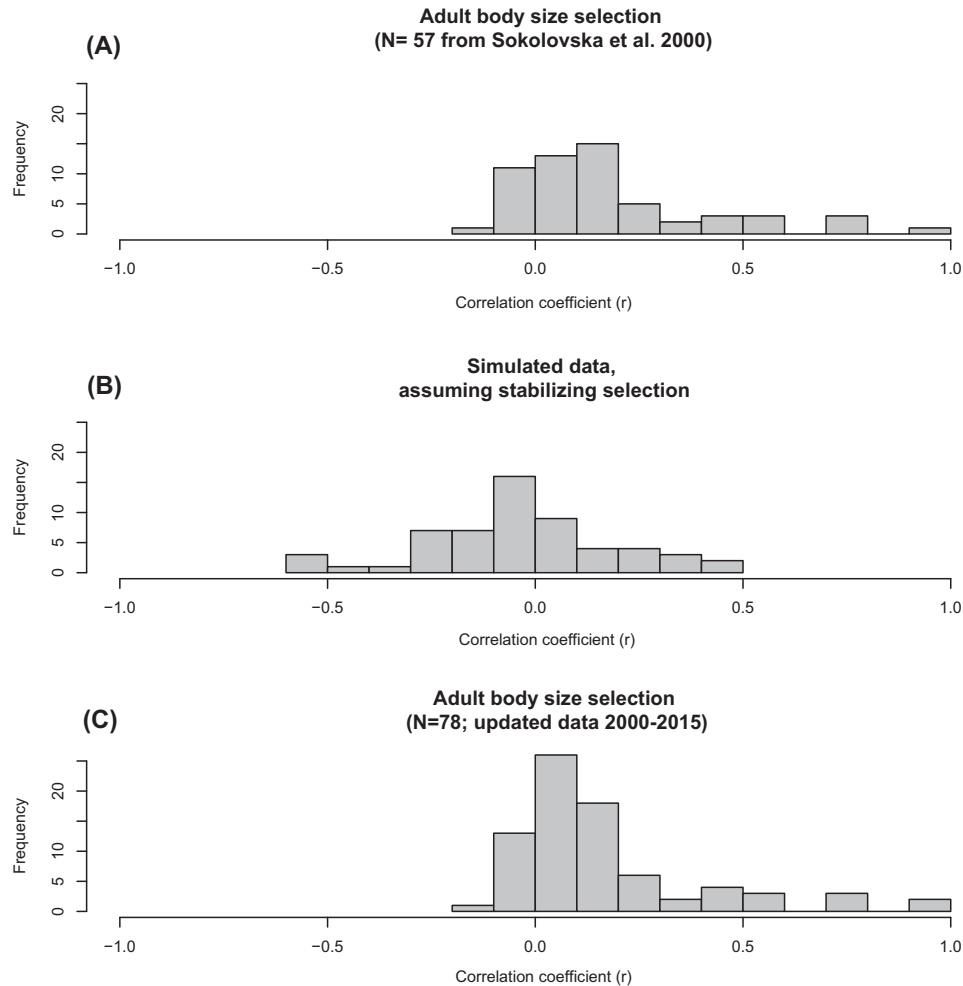
Figure 3. Correlation coefficients from studies obtained from a literature survey of body size effects on fitness in odonates (mating success, survival, and fecundity). (A) The distribution coefficients from the original review by Sokolovska et al. (2000) that contained 57 estimates. (B) The symmetrical distribution of correlation coefficients that would be expected if all populations were sitting on their adult optima (adaptive peaks), assuming a moderate level of stabilizing selection (i.e., $\beta \sim N (0, 0.1)$ and $\gamma \sim N (–0.15, 0.1)$, See R code in supplement for details). (C) Updated dataset since Sokolovska et al. (2000) ($N = 78$). Rather than a symmetric distribution around zero, the distribution is strongly right-skewed, revealing that most populations studied to date are currently not occupying their adult adaptive peaks. Instead, adults are on average smaller than their adult fitness optima.

sexual selection on either forewing length or body length (i.e., no significant negative γ). If anything, there was a small but non-significant positive curvature in our fitness function, consistent with disruptive rather than stabilizing selection (Fig. 2). Similar results were obtained when we instead estimated selection on the first principal component (PC1) of five morphological traits (fore- and hindwing length, total body length, abdomen length, thorax length), where PC1 was used as a composite measure of selection on overall size (Fig. S7).

LINKING BODY SIZE TO ADULT FITNESS

Our updated literature review on odonate body size and fitness produced 78 estimates (correlation coefficients) of the relationship between body size and various male and female fitness components (Fig. 3). Of the 78 correlation coefficients, 33 come from independent studies, 26 from different species, and 48 of the coefficients from study sites in temperate regions. These fitness components include the relationship between male mating success, survival, and female fecundity on body size (Fig. 3). Overall, this analysis confirmed the pattern found in the original analysis from 2000 (Sokolovska et al. 2000). The distribution of these 78 correlation coefficients reported in the literature was strongly and positively skewed to the right, with 67 estimates being positive and only 11 estimates being negative. This differs significantly from the expectation if we would assume stabilizing selection in most taxa, which instead predicts that the values should be symmetrically distributed around zero (sign test: $P < 0.001$; Fig. 3B). Similarly, the original dataset reported by Sokolovska

et al. (2000) shows a significant positive skew, compared to the null expectation (48 out of 57 estimates were positive; $P < 0.001$, using a sign test). Note that these positive relationships between large body size include several different fitness components and come from both sexes (i.e., male mating success, female fecundity, and adult survival). Although not all estimates were positive, those that were negative were typically low in magnitude (Fig. 3).

Our simulated dataset (Fig. 3B) showed that if these correlation coefficients had been sampled from the same underlying fitness functions with a stable optimum (i.e., all populations in the literature would be occupying their adult optima or fluctuating around those optima), we would instead have expected to find a symmetrical pattern of correlation coefficients around zero (Fig. 3B). Similarly, disruptive selection would also have produced a symmetrical pattern (Fig. 3B). The strongly right-skewed distribution (Fig. 3 A, C) therefore suggests that stabilizing selection on body size in the adult life-stage can be rejected, or it is at least not very common. It does not rule out the possibility, however, that some populations could be experiencing stabilizing selection in the adult stage, but these are exceptions to the general rule. Stabilizing selection on adult body size is thus not common, since large body size in odonates, and many other ectotherms is almost always associated with higher fitness, irrespective of fitness components and sex (cf. Kingsolver and Huey 2008).

DEVELOPMENT TIME AND BODY SIZE

We found a significant and positive relationship between development time and body size in the European odonates ($N = 64$ species; Fig. 4). This relationship was not confounded by latitude or phylogeny, both which were accounted for in a phylogenetic generalized least square (PGLS) analysis (Lambda: 0.89; Effect of size $= 0.040$, SE $= \pm 0.01$, $P = 0.002$; effect of latitude $= 0.053$, SE $= \pm 0.01$, $P = 0.0003$). Thus, after controlling for phylogeny, European odonates increased their developmental time at higher latitudes and with larger adult body size. We emphasize that at a given latitude, development time increases with body size, and the pattern we see in Fig. 4 is not simply due to confounding variation in mean range latitude.

OU-MODELS: ONE OR MULTIPLE BODY SIZE OPTIMA?

Using phenotypic data and our novel molecular phylogeny of extant odonate species, we found evidence for a significant constraining force on maximum and minimum body sizes (Fig. 5A). A single-optimum OU-model (fit to the wing length dataset) had a significantly better fit than a simple BM-model (BM: AIC $=$ 4807.86, $\sigma^2 = 1.08$, root $= 30.9$, log-likelihood $= -2401.93$; OU: AIC $= 4774.10$, $\alpha = 0.008$, $\sigma^2 = 1.36$, root $= 31.3$, log-likelihood $= -2384.05$) (Fig. 5A). Note that not only were body
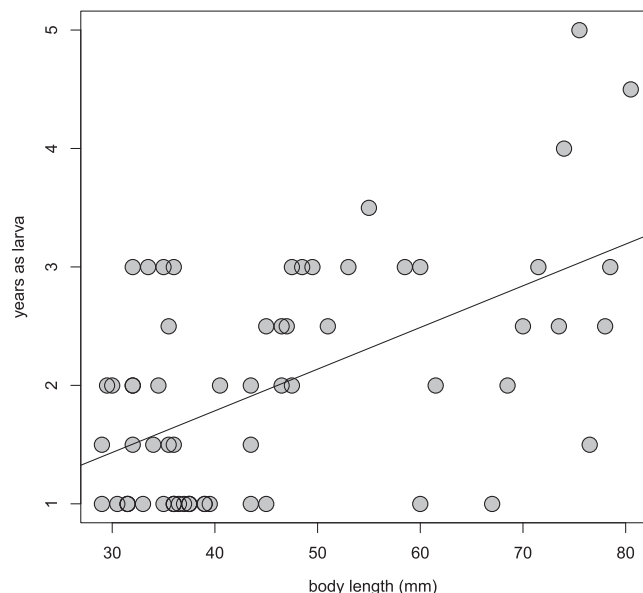

Figure 4. The positive relationship between development time and body size (body length) in the European odonates (Phylogenetic Least Square analysis (PGLS): $N = 64$; Effects of body size: size $= 0.054$, $P < 0.001$; effects of latitude: lat $= 0.065$, $P = 0.0002$). Thus, there is a significant effect of adult body size on larval development time that holds even when we control for phylogenetic nonindependence and geographical variation due to latitude. Species that have noninteger developmental times (e.g. 1.5 years) are those where the literature gives ranges in years of the development time. See Dryad supplement for access to the full dataset.

size (wing length) variance constrained across the last 65 million years since the Cretaceous (Fig. 5A), but there was no evidence for any directional change in mean wing length, either upwards or downwards (Fig. 1). Hence, these data suggest some constraining force keeping interspecific wing length variance lower than expected from a random diffusion process (Figs. 1, 5A). These results are supported by comparing wing length trends within genera with both fossil representatives and extant taxa (Fig. 5B). Of these 25 taxa, 10 (40%) show wing length declines up to the present, whereas 15 (60%) show wing length increases. Hence, evolutionary wing length increases and decreases were approximately equally common (Fig. 5B).

Even though average body length did not change across all odonate taxa, we were able to infer multiple likely shifts in body length optima on this phylogenetic tree (Fig. 6) (see Supplementary Material for comparison with simpler models presented above). Thus, body length has therefore changed significantly during evolution, both upwards and downwards in different families compared to an assumed neutral background rate of body length evolution (Fig. 6). This suggests the presence of multiple body size optima in odonates, which are likely to be related to local
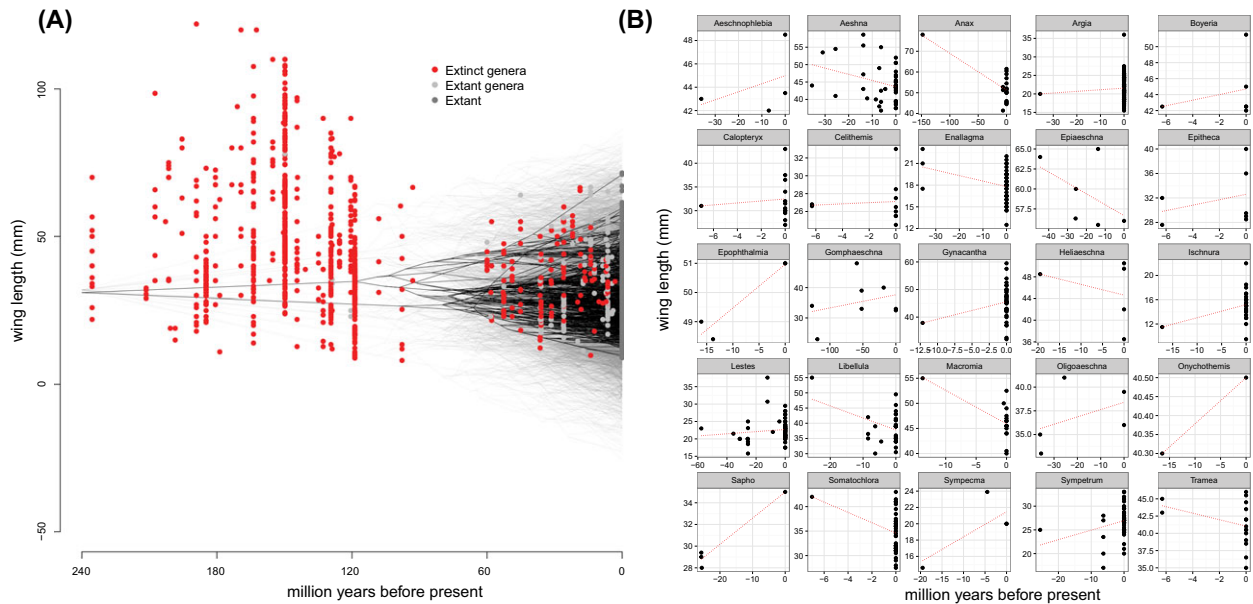
Figure 5. (A) Observed data (black) compared to expected evolutionary increase in interspecific body size variance, obtained from simulations of a Brownian Motion (BM) process (gray). The simulated pure BM-process predicts significantly more interspecific variance in wing length than we observe in this dataset. This suggests the presence of a significant constraining force that limits maximum and minimum body size in odonates. This was further confirmed by an Ohrnstein-Uhlenbeck (OU)-model that had a significantly better fit to these data than a simple BM model (BM: AIC = 4807.86, σ^2 = 1.08, root = 30.9, log-likelihood = –2401.93, ntaxa = 780; OU: AIC = 4774.10, α = 0.008, σ^2 = 1.36, root = 31.3, log-likelihood = –2384.05, ntaxa = 780). Fossils are shown as points (red: extinct fossils, gray: fossil from extant genera, dark gray: extant taxa). A completely unconstrained model, however, still does not reach the sizes observed 150 my ago, as many fossils are larger than the extent of the unconstrained simulations. (B) Body size trends within extant odonate genera for which we also have fossil data. Here, simple linear relationships were fit to each of the extant genera for which we have fossil data. Of the 25 genera that are shown, 10 (40%) decline in body size up to the present, whereas 15 (60%) increase in body size. Hence, there is no obvious evolutionary trend for a directional bias toward either smaller or larger wing size over evolutionary time (i.e., no support for Cope's Rule).

environmental conditions, correlated with latitude, temperature, humidity, or other abiotic and biotic factors (Fig. 6).

DIVERSIFICATION IN RELATION TO BODY SIZE

Across odonate families, we found a negative, although nonsignificant relationship between speciation rate and mean family body length (linear regression: body length effect = –0.00019, SE = ± 0.00013, P = 0.18; Fig. 7). In this analysis, we did not control for phylogenetic nonindependence (Fig. 7), but the above conclusion remained when we included suborder (i.e., dragonfly or damselfly) as a random effect (linear-mixed effect model: $\beta_{bodyLength}$ = –0.00012 SE = ±0.00014, P = 0.40). Dragonflies showed a positive relationship and damselflies a negative relationship between body length and speciation rate (Fig. 7). However, a formal statistical test for any such interaction revealed no significant difference between the two suborders in this relationship (linear regression: $\beta_{bodyLength}$ = 0.00009, SE = ±0.0004, P = 0.75; $\beta_{Suborder}$ = 0.023, SE = ± 0.020, P = 0.26; $\beta_{bodyLengthxSuborder}$ = –0.00025, SE = ± 0.00033, P = 0.46). Finally, we note that the species distribution of wing lengths in odonates shows a slightly right-skewed pattern

(as shown in the histogram in Fig. 1C). Such right-skewed distributions have been suggested to indicate species selection against larger body size (Clauset and Erwin 2008).

We also fitted a QuaSSE-model (FitzJohn 2010) to these data. This analysis suggested a trend of decreasing speciation rate with body length (QuaSSE: l.y0 = 0.25, l.y1 = 0.23, l.xmid = 45.62, l.r = 0.97, m.c = 0.19, drift = 0.00, diffusion = 2.64), as indicated by a positive and significant l.r term (full: log-likelihood = –22720, AIC = 45452; constant_lr: log-likelihood = –22732, AIC = 45469) (Fig. S9). However, we note that QuaSSE-models have high type I error (false positives) when diversification rates vary across the tree (Rabosky 2009; Rabosky and Goldberg 2015), which is especially likely to be the case in large phylogenetic trees, as we studied here.

Discussion

Here, we have combined fossil data (Figs. 1, 5B), phylogenetic comparative analyses (Figs. 4–6) and measurements of various fitness components from extant odonate species and several
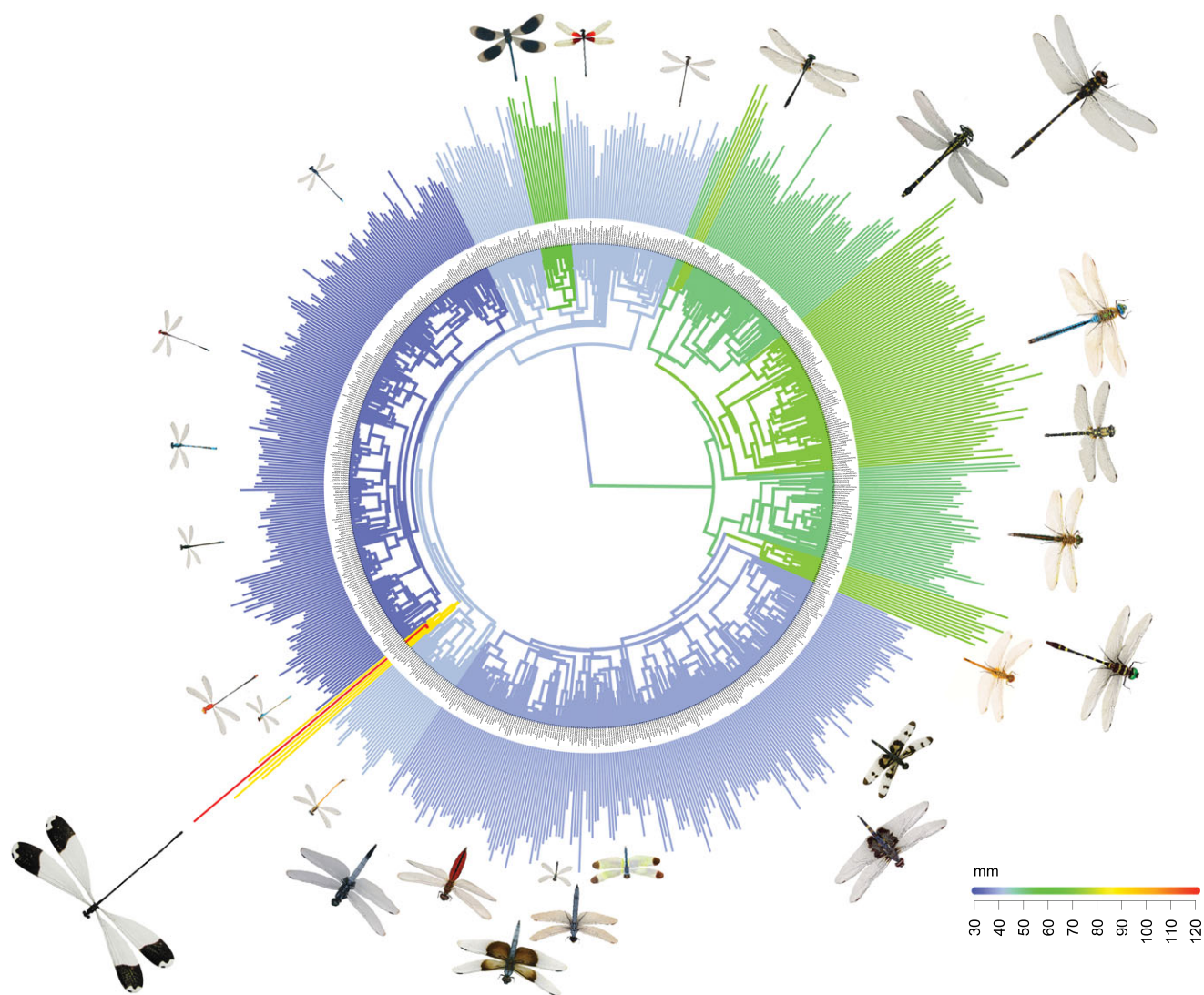
Figure 6. Body size evolution in odonates inferred from a multiple-optima Ornstein-Uhlenbeck (OU: optima shifts = 10, number of taxa = 728) model. Bars on the tips represent total body lengths. Warm colors (red, green, yellow) represent significant positive shifts from a neutral background process, whereas cold colors (blue, green, violet) represent shifts toward smaller body sizes and warmer colors (red, orange, yellow) represent shifts toward larger body sizes. These body size shifts are indicative of past positive and negative evolution toward either increased or decreased body size, revealing that evolution in this group has been completely neutral. We used the estimate_shift_configuration function of the l1ou package (Khabbazian et al. 2016) to fit our body size data to our phylogenetic tree. Photos of some selected taxa are roughly to scale (See Supplementary Material for more statistical details).

populations in the field (Figs. 2 and 3) (Weber and Agrawal 2012). Our general goal was to understand the causes of body size evolution in this ancient insect order. Our combined results suggest consistent directional selection (for these fitness components, at least) toward larger adult body size in the majority of extant populations and species studied to date (Figs. 2 and 3).

As a case in point, we found that selection on *Calopteryx splendens* male body size (as measured by both forewing length and total body length) was positively biased for both mating success and survival (Fig. 2). Although both natural and sexual selection varied marginally in magnitude between years, the long-term

trend was clearly and significantly positive over the five seasons that this study took place (Fig. 2). The estimated strengths of natural and sexual selection on body size in this study are similar in magnitude and sometimes larger than estimates of the strength of selection in natural populations from previous meta-analyses (Fig. 2; cf. Kingsolver et al. 2001).

The narrow-sense heritability (h^2) of morphological traits in natural populations (like wing length and body size) is on average 0.40–0.50 (Mousseau 1987), but see Hansen et al. 2011 for a critical discussion about the relationship between heritability and evolvability. Morphological traits typically have higher
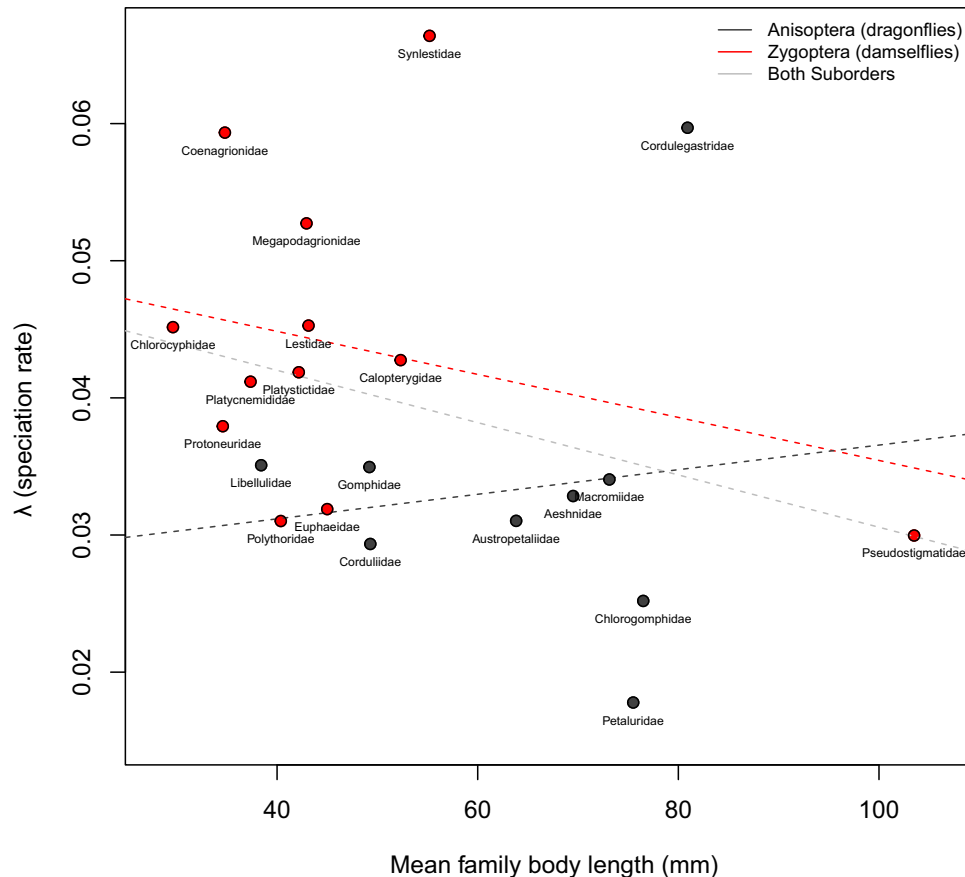
Figure 7. Speciation rate (λ) and body size (mm) for two odonate suborders: Anisoptera (dragonflies) and Zygoptera (damselflies). A pure birth model (i.e., assuming no extinction) was fit for each family. The estimate from each model was compared to mean family body length. λ decreases with increasing mean family body size, but this decrease is not significant within or across suborders, whether phylogenetic nonindependence was controlled for or not. Extinction rates cannot be estimated reliably using this phylogeny because of too many missing tip taxa. However, missing tip tax was added to their families before estimating λ for each family.

heritabilities than physiological, behavioral, and life-history traits (Mosseau 1987). Assuming similar heritabilities for the traits included this study (or even more moderate levels), we would expect rapid increases in body size over a few thousand generations, given the strengths of natural and sexual selection we have documented in *C. splendens* (Fig. 2; cf. Lande and Arnold 1983). However, such expected rapid increases from a univariate selection model would assume that selection in the adult stage is not counteracted at the larval stage. Since adult and larval phenotypic traits are likely to be at least partly correlated with each other, there is potential for antagonism between the sign of selection on body size during the adult and the juvenile life-stages (cf. Walsh and Blows 2009).

Although conflicting selection *between* life stages can potentially be important in odonates, we have not found any strong evidence for conflicting selection *within* the adult life-stage (Fig. 1, 5). For example, we have found no evidence of any trade-off between fitness components within the adult life-stage, which would manifest itself in the form of different signs of sexual

selection and natural selection on body size (cf. Fig. 2). Instead, natural and sexual selection on male body size was typically concordant and of the same sign (Fig. 2). In other words, large males had both higher survival and higher mating success and there was no sign of any trade-off between natural and sexual selection in these data (Fig. 2).

Body size evolution could also potentially be constrained by intralocus sexual conflict (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009), for example if males and females are selected toward different body size optima (Calsbeek and Sinervo 2004). However, we consider such intralocus sexual conflict on body size also an unlikely explanation for stasis in the case of odonates. Both male and female fitness components are typically positively correlated with body size, across most odonate species that have been studied to date (Sokolovska et al. 2000) (Fig. 3). Moreover, a long-term study of sexual selection in males and fecundity selection in females of the Coenagrionid damselfly *Ischnura elegans* found no evidence for ongoing intralocus sexual conflict on body size (Gosden and Svensson 2008). Instead,

selection gradients in the two sexes of *I. elegans* were positively correlated with each other at the local population level, suggesting sexually concordant selection rather than intralocus sexual conflict over body size (Gosden and Svensson 2008). In summary, available data in this study (Figs. 2 and 3) as well as previous studies (cited above) do not provide any empirical support for either stabilizing or conflicting selection on body size within the adult life-stage, nor any important role for intralocus sexual conflict.

Fluctuating selection pressures between generations around a stable long-term body size optimum is another potential explanation for stasis (cf. Estes and Arnold 2007). Although year-to-year variation in selection pressures could potentially result in a long-term stable adult body size optimum, such fluctuating selection is also unlikely to explain stasis on body size either for odonates in general (Fig. 3) or for *C. splendens* in particular (Fig. 2). Fluctuating selection around a long-term optimum is expected to produce a symmetrical distribution of correlation coefficients centered around zero, which contrasts strongly with the observed right-wing skew and bias toward positive selection for size (Figs. 2 and 3). In *C. splendens*, natural and sexual selection favors large body size, and adults are typically smaller than their fitness optima in most years (Fig. 2).

Therefore we can, based on these results, largely rule out conflicting selection between adult fitness components or stabilizing selection during the adult life-stage (see above). Instead, we suggest that selection for large adults is opposed by conflicting selection from predation, resource competition, or other ecological factors operating during the larval stage. Growing a larger body size will, all else being equal, require a longer developmental period (Fig. 4). A longer development period will result in a longer period of predator exposure and increased mortality risk. Such conflicting selection pressures that vary in sign between different ontogenetic stages during the life cycle have been found in studies of birds, fish, and insects (Schluter et al. 1991; Barrett et al. 2008). In the case of odonates, we note that there is considerable scatter around the regression line of how the length of the larval period affects adult body size (Fig. 4). Thus, although the length of the larval period within a given latitudinal band and controlling for phylogeny increases with adult body size, different taxa show considerable deviations from the overall positive relationship (Fig. 4). We suggest that such deviations from the overall positive relationship reflect species-specific trade-offs and differences in larval ecology and predator avoidance behaviors. For instance, larval burrowing behaviors in some long-lived dragonfly species (Baird 2014) could reduce mortality rates from predation during the larval period and thus partly mitigate the costs of a long developmental period. Moreover, some odonate species have invaded novel habitats with either reduced overall predation risk or changed predator faunas, which could also obscure the overall

positive relationship across species. One example is *Enallagma*-damselflies that moved from lakes with primarily fish predators to lakes with primarily dragonfly predators (McPeek et al. 1996).

Stabilizing selection on pleiotropically linked traits, either between or within life-stages have been suggested as a potential explanation for stasis (Hansen and Houle 2004). Here, we argue that such pleiotropic constraints between fitness components in the adult stage are unlikely to explain stasis in odonates, although we see a potential role of such pleiotropic constraint between life-stages, in the form of conflicting selection between the adult and the larval stages. Estes and Arnold (2007) argue that pleiotropic constraints are unlikely to explain long-term stasis for two reasons: (1) Populations often respond rapidly to changing environmental conditions and (2) Pleiotropic constraints can they themselves evolve and so are unlikely to account for long periods of stasis that characterize many fossil lineages. In Figures 1 and 7, we show that there has indeed been signficant past evolutionary shifts in body size, implying that any pleiotropic constrainsts have been overcome several times in the past.

Recently, Rollinson and Rowe (2015) proposed another model to explain stasis in organismal size that is also based on conflicting selection between life-stages. Using a meta-analysis from a large dataset of selection estimates on body size, they showed that (1) juveniles are typically under strong directional selection for larger size, (2) there are typically negative genetic and/or phenotypic correlations between juvenile size and parental fecundity, suggestive of parent-offspring conflict, and (3) directional selection on adult size is often weak or close to zero (Rollinson and Rowe 2015). While their model seemingly might contradict our explanation for stasis, we note that their dataset only included vertebrates and plants, but no insects (Rollinson and Rowe 2015). In contrast to their study and conclusions, here we have shown that adult body size is typically under strong positive directional natural and sexual selection (Figs. 2 and 3). Fitness benefits of large size is likely to be similar in many other insect species (Bonduriansky 2001). Moreover, organisms with complex life-cycles typically experience radically different juvenile and adult environments, especially semi-aquatic groups like odonates that change from aquatic to a terrestrial environments after emergence. Given the conflicting selection between fitness benefits of large adult size and the costs of a long development time (Fig. 4), we suggest that our explanation of stasis might be more generally applicable to organisms with complex life-cycles, than the parent-offspring conflict model favored by Rollinson and Rowe (2015).

Temperature (latitude and altitude), food resources, and seasonality have all been experimentally demonstrated to affect larval growth rate and final adult body sizes in odonates (Nylin and Gotthard 1998; McPeek 2004; Van Doorslaer and Stoks 2005; De Block et al. 2008; De Block and Stoks 2008; Örtman et al. 2012). Larger European odonate species spend more time in the

larval period than smaller species, and this positive relationship between adult body size and development time holds even after controlling for phylogenetic nonindependence and latitudinal position (Johansson 2003; Sniegula et al. 2016) (Fig. 4). In Fig. 4, we note some small species taking several years to develop (*Coenagrion armatum*, *Leucorrhinia albifrons*), and some rather large odonates developing quickly within a year (*Anax imperator*, *Aeshna mixta*; Sandhall 1987), but this variation is largely driven by differences in mean latitude, with r-squared increasing from 0.29 (without incorporating latitude) to 0.44 (when incorporating latitude). Body sizes in other insects has been shown to increase at an exponential rate during ontogeny, meaning that even a small increase in development time should lead to much larger size (Nijhout et al. 2006), with as much as 90 percent of the body size increase being gained in the last larval stage (Nijhout et al. 2010).

The odonate fossil data indicate relative stasis in mean body size at the order level over the last 65 million years (Fig. 5A, B). For continuous characters, a simple model of evolution is an unbounded random walk process (Brownian motion; BM) (Hansen 1997). BM-models assume that there are no evolutionary constraints on the upper and lower bounds of phenotypic traits (Hansen 1997). Evolutionary constraints can however be quantified and detected by adding an extra term (α), which measures a pullback force as traits move further away from an inferred optimum (Hansen 1997; Butler and King 2004) (Fig. 5B). The existence of such a pullback force has often been interpreted as a signature of long-term stabilizing selection (O'Meara and Beaulieu 2014).

Using data from our time-calibrated odonate phylogeny from extant odonate taxa and body size data, we detected a significant pullback force constraining the accumulation of interspecific body size variance (Fig. 5A), with maximum body size among extant odonate taxa being 10–15 mm smaller than predicted by Brownian motion (Fig. 5A). This suggests the existence of either selective or genetic constraints on maximum and minimum body size in extant odonates (Fig. 5A). We note, however, that the approach to an adaptive optimum in quantitative genetic models of phenotypic evolution is almost instantaneous on a geological time scale, whereas OU models typically capture more long-term constraining forces that are integrated over millions of years (Hansen 1997; O'Meara and Beaulieu 2014). Moreover, OU models cannot, in themselves, distinguish between "true" stabilizing selection within a life-stage, which has been suggested as an explanation for long-term stasis (Charlesworth et al. 1982) from conflicting selection between life stages (Schluter et al. 1991). As we have argued above, conflicting selection between life stages is a more likely explanation for relative stasis in this insect order than is stabilizing selection within the adult life-stage (Figs. 1–5). To distinguish true stabilizing selection from conflicting selection between life stages, phylogenetic comparative methods are clearly not sufficient but should ideally be complemented with experimental and mechanistic studies of carefully chosen species (Weber and Agrawal 2012).

Body size optima could shift, however, particularly in conjunction with speciation (Gould 1988). Using a multiple-optima OU-model we were able to locate several significant shifts in body size optima among extant odonate lineages (Fig. 6). These significant shifts imply past selection toward smaller or larger body size in some lineages. These comparative results from extant taxa are also consistent with our analyses on the dynamics of body size evolution among fossil data (Fig. 5B). The putative ecological causes driving these shifts will be discussed in more detail in a forthcoming article. These shifts suggest either that selection for large body size among adults in not always positive (*contra* Figs. 2 and 3), or that selection pressures during the larval stage have in some cases opposed the fitness benefits of large adult size. Future studies of both larval and adult fitness costs and benefits of size and development times, particularly in tropical odonates, would be needed to quantify the net selection pressures in different lineages. We emphasize that these different optima are unlikely to represent optimal body sizes for single species or populations. Rather, these optima should be interpreted as broad adaptive zones that cannot be well explained by a single-mean multivariate Gaussian distribution or an OU-model assuming a single optimum shared by multiple species (cf. Fig. 5). Adding additional taxa to our incomplete phylogeny is likely to reveal additional optima shifts in the future.

Several ecological factors might put an upper limit of body size in odonates. First, because the demand for oxygen in flying insects is likely large, atmospheric oxygen has been suggested as a major factor controlling maximum body size in insects (Harrison et al. 2010). The peak atmospheric oxygen in historical reconstructions corresponds well to the peak seen in giant insects 300 mya (Clapham and Karr 2012). High atmospheric oxygen levels during the Carboniferous have been suggested to be important in the energetically intensive process of flight (Harrison et al. 2010). However, body size was subsequently decoupled from atmospheric oxygen levels after the initial oxygen peak (Clapham and Karr 2012), although other oxygen-record reconstructions might show a different pattern (Harrison et al. 2010). Another selective agent that has been suggested to operate against large size is size-dependent predation by flying predators such as birds, which evolved during the last 150 million years (Clapham and Karr 2012). Interestingly, two independent studies of an extant populations of Calopterygid odonate species (*Calopteryx splendens* and *C. virgo*) have shown that individuals with longer wings are more likely to be predated by birds (Svensson and Friberg 2007; Kuchta and Svensson 2014). Previously, odonates and other large insects may also have occupied niches that are now occupied by vertebrates such as birds and bats (Harrison et al. 2010) (Fig. S1).

Body size distributions within taxonomic groups are often asymmetrical, with many more smaller species than larger ones (Clauset and Erwin 2008; Rainford et al. 2016). Such skewed body size distributions can indicate size-dependent differences in diversification rates (Clauset and Erwin 2008) or simply increases in variance in the presence of a lower bound. The distribution of odonate wing length data shows such a typical right-skewed distribution (as shown in the histogram in Fig. 1C), as one would expect if species selection was operating against larger body size in odonates. Species selection is gaining increased interest as potential macroevolutionary force (Jablonski 2008; Rabosky and Mccune 2010; Chevin 2016). Species selection has also been suggested as an explanation for the lack of phyletic size increases in many organismal groups, despite consistent positive directional selection and fitness benefits of being large (Kingsolver and Pfennig 2007). Species selection against large size (e.g., increased extinction rates of large taxa) could potentially counteract any evolutionary trend in body size that would be expected if individual selection was operating alone (Kingsolver and Pfennig 2007). A tension between species-level selection causing extinction of large taxa that is counteracted by within-species selection for large size could arise if large size is important in intraspecific competitive interactions. Such a tension could result in oscillating and frequency-dependent macroevolutionary dynamics of body size (Smith and Brown 1986; see also Frank and Slatkin 1992).

Differential rates of extinction and speciation might prevent niche filling of large lineages (Maurer et al. 1992), and could therefore counteract evolutionary increases in body size. In insects, Rainford et al. (2016) found little evidence for body size dependent diversification rates. In the case of odonates, we found no statistically significant support for a negative effect of body size on speciation rates in odonates (Fig. 7). Neither our study nor Rainford et al. (2016), however, estimated extinction rates. In our case, this was due to many missing tip taxa, which are needed to reliably estimate extinction rates (Nee et al. 1994; FitzJohn et al. 2009). Body size could of course still affect diversification rates through extinction (since diversification rate = extinction rate – speciation rate). It seems unlikely that differences in diversification rate alone, however, would be enough to balance the positive directional selection for large adults (Figs. 2 and 3) and lead to complete stasis in body size, although we cannot reject such a scenario completely based on our current data. We also note that speciation rates and extinction rates might be positively correlated with each other, resulting in different lineages having differential turnover rates (Rosenblum et al. 2012; Schluter 2016). This might imply that estimated speciation rates could partly also reflect extinction rates. Explicit evolutionary models of the interactions between individual and species level selection are still few (for a recent exception see Chevin 2016).

Conclusion

Despite consistent positive selection in the adult stage (Figs. 2 and 3; Figs. S6 and S7), odonate body size evolution appears to be constrained at both the micro- and macroevolutionary level. Since the last mass extinction event 65 mya, body size evolution in odonates has not been positively biased. Hence, there is no evidence for Cope's Rule in this insect order. Rather multiple body size shifts have been detected in both upwards and downward directions (Fig. 6), and these shifts were presumably driven by local ecological conditions.

We have suggested that the positive selection on adult body size in odonates is counteracted by conflicting selection at the larval stage (Fig. 4). We stress that other explanations for the apparent stasis in this insect order are not mutually exclusive to our favored explanation and might still be plausible. Such explanations include the possibility of species selection favoring small body size. However, we argue that pleiotropic fitness costs of large body size that are paid in the form of a lengthened larval period are likely to at least partly counteract the pervasive positive selection for large size that we have documented for adult fitness components (Figs. 2 and 3). We are fully aware that conflicting selection due to such pleiotropic costs in the form of a longer juvenile period are unlikely to result in stasis in an absolute sense, except in the unlikely case where such conflicting selection pressures are completely equal in magnitude and opposite in sign (Hansen and Houle 2004). However, we suggest that conflicting selection is one of the most likely explanation as to why positive selection in the adult stage does not lead to the rapid increases body size that would otherwise be expected.

The data and analyses in this study are certainly not incompatible with other models for stasis, including population structure, coevolutionary interactions, and gene flow between contemporary populations (Eldredge et al. 2005), niche conservatism (Wiens and Graham 2005), parent-offspring conflict (Rollinson and Rowe 2015) or selection on the environmental, rather than the heritable component of phenotypic traits (Price et al. 1988). However, our results contradict some previously suggested explanations of stasis that have been widely discussed. For instance, we find no support for stabilizing selection within life-stages (see Charlesworth et al. 1982). Neither do we find any support for trade-offs between sexual and natural selection (Figs. 2 and 3). Finally, there is no evidence that intralocus sexual conflict (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009) would constrain males and/or females from attaining their body size optima, as both sexes seem to benefit from large body size in odonates (Fig. 3). Thus, while our study cannot prove that conflicting selection is the sole explanation for stasis, our data are incompatible with several explanations for stasis that are based on

stabilizing, fluctuating, or conflicting selection within the adult life-stage.

AUTHOR CONTRIBUTIONS

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DATA ARCHIVING

Data are archived on https://doi.org/datadryad.org/ and https://doi.org/treebase.org/treebase-web/home.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Mark-recapture (MR) analysis of our survival selection data, using male forewing length as a trait.

Table S2. Field guides and other sources of body size (wing lengths and body lengths) data from extant taxa.

Figure S1. Atmospheric oxygen (GEOCARBSULF model) and min and max wing length over the last 350 my.

Figure S2. Current state of odonate sequences on GenBank. Ordered plot of all downloaded sequences from GenBank for use in the construction of our phylogenetic tree.

Figure S3. Posterior node support output from BEAST for the suborder Anisoptera (dragonflies).

Figure S4. Posterior node support output from BEAST for the suborder Zygoptera (damselflies).

Figure S5. Effect of phylogenetic uncertainty for the comparative tests run in this study.

Figure S6. Survival and sexual selection (on wing length) in adult *Calopteryx splendens* males from 2011-2015.

Figure S7. Selection gradients using the first principal component (PC1) rather than forewing length as is used in the main text Figure 1.

Figure S8. The strong correlation ($r = 0.90$; $P < 0.001$) between wing length and body length in odonates (males shown). Each point represents measurements from one species.

Figure S9. Fitted QuaSSE-model (FitzJohn 2010) values for the dependence of speciation rate and male body length (mm).

Supporting Material