

Ecological and evolutionary drivers of range size in *Coenagrion* damselflies

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Abstract

Geographic range size is a key ecological and evolutionary characteristic of a species, yet the causal basis of variation in range size among species remains largely unresolved. One major reason for this is that several ecological and evolutionary traits may jointly shape species' differences in range size. We here present an integrated study of the contribution of ecological (dispersal capacity, body size and latitudinal position) and macroevolutionary (species' age) traits in shaping variation in species' range size in *Coenagrion* damselflies. We reconstructed the phylogenetic tree of this genus to account for evolutionary history when assessing the contribution of the ecological traits and to evaluate the role of the macroevolutionary trait (species' age). The genus invaded the Nearctic twice independently from the Palearctic, yet this was not associated with the evolution of larger range sizes or dispersal capacity. Body size and species' age did not explain variation in range size. There is higher flight ability (as measured by wing aspect ratio) at higher latitudes. Species with a larger wing aspect ratio had a larger range size, also after correcting for phylogeny, suggesting a role for dispersal capacity in shaping the species' ranges. More northern species had a larger species' range, consistent with Rapoport's rule, possibly related to niche width. Our results underscore the importance of integrating macroecology and macroevolution when explaining range size variation among species.

Introduction

Interspecific variation in range size is a key topic in ecology and evolution (Gaston, 2009). However, the causal basis of interspecific variation in range size remains largely unresolved (Gaston, 2009; Blanchet *et al.*, 2013; Morin & Lechowicz, 2013), especially in invertebrates (Abellán & Ribera, 2011). Several ecological traits have been identified as contributing to species variation in range size, most notably dispersal capacity,

body size and the latitudinal position of the range and associated niche width (reviewed by Gaston, 2003, 2009). However, evolutionary history may also play an important role (Roy *et al.*, 2009).

Given appropriate habitat, a high dispersal capacity, which allows reaching and colonizing distant areas, has been linked to larger range sizes in diverse taxa (reviewed in Lester *et al.*, 2007; Gaston, 2009). Especially, species that were able to invade areas without congeneric species can be expected to be highly dispersive and hence display greater range size, analogously to intraspecific patterns during range expansion (Hill *et al.*, 2011). The importance of dispersal capacity as a driver of range size has been shown mainly through

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proxies such as wing length in birds and insects (e.g. Rundle *et al.*, 2007). For flying animals, other key morphological proxies of dispersal capacity, such as wing loading, wing shape and relative flight muscle mass, have remained largely unstudied. Species with a higher dispersal ability are expected to show a lower wing loading, which is more energetically efficient (Angelo & Slansky, 1984), and a higher wing aspect ratio (i.e. have long narrow wings; Berwaerts *et al.*, 2002). For example, wing aspect ratios are higher in migrating species of birds (Hedenström, 2008) and dragonflies (Johansson *et al.*, 2009). Furthermore, flight capacity has been associated with relative flight muscle mass in insects (Marden, 2000).

Positive relationships between body size and range size have been documented for diverse taxonomic groups of both terrestrial and aquatic organisms (Brown *et al.*, 1996). Several ecological mechanisms may underlie this pattern (Luiz *et al.*, 2013): larger species may be better colonizers as they are generally less susceptible to predation, use a broader range of food types and are more tolerant of environmental variability than small species. Moreover, in general, body size is positively correlated with longevity which may facilitate species establishment and persistence at new range outposts (Warner & Chesson, 1985).

A positive correlation between range size and latitude has been identified in several groups inhabiting the Palearctic (Rapoport's rule; Gaston, 2009; Blanchet *et al.*, 2013; Morin & Lechowicz, 2013). The most accepted hypothesis for this correlation is that only species with a broad niche, which typically have large ranges (Slatyer *et al.*, 2013), are able to persist in these northern areas where the climate is more variable than further south.

The evolutionary history of species may act as a potentially confounding factor and/or an evolutionary driver of range size. First, the ecological traits that determine species' range sizes may not be phylogenetically independent, hence, using methods that incorporate phylogenetic relationships into data analyses is an essential part of bridging the gap between macroecology and macroevolution (Hernandez *et al.*, 2013). Second, a species' longevity may play a role in shaping the size of its range (Roy *et al.*, 2009; Blanchet *et al.*, 2013), with older species potentially occupying larger ranges because they have had more time to colonize larger areas of suitable habitat (Roy *et al.*, 2009), as has been observed in plant species (Paul *et al.*, 2009).

Despite the many potential drivers of range size, most studies of species' range size have focused on one possible explanatory trait. This can be misleading when there is covariation between drivers (Reed, 2003; Cruz *et al.*, 2005). For example, the effects of latitudinal position and dispersal ability are hard to disentangle at high latitudes when only good dispersers were able to colonize these areas after the last glacial maximum (Rundle

et al., 2007). Similar interrelated patterns may exist for body size. For example, body size has been positively associated with both dispersal capacity (Rundle *et al.*, 2007) and latitudinal position (Bergmann's rule; Shelomi, 2012). Species at northern latitudes can hence occupy large ranges due to their larger body size at these latitudes or because of climatic history and differences in niche width (Rapoport's rule; Reed, 2003). A potential interplay between various candidate ecological and macroevolutionary determinants of range size highlights the importance of integrating traits within a single study (Böhning-Gaese *et al.*, 2006; Laube *et al.*, 2013).

Here, we present an integrated macroecological and macroevolutionary analysis of the contribution of dispersal capacity, body size, latitudinal position and evolutionary age in shaping variation in species' range size in *Coenagrion* damselflies (Odonata, Zygoptera). Odonata have an aquatic larval stage during which growth occurs and a flying terrestrial stage where/in which reproduction and dispersal occurs (Stoks & Córdoba-Aguilar, 2012). Odonates are well recorded with well-known species' range distributions (Boudot *et al.*, 2009). Further, correlates of flight ability have been well-documented in the genus *Coenagrion* (Therry *et al.*, 2014). This genus is predominantly Palearctic, with three species residing in the Nearctic (Westfall & May, 1996). We quantify the contribution to geographic range size of (i) four proxies of dispersal capacity in damselflies, wing length, wing loading, wing aspect ratio and relative flight muscle mass, (ii) latitudinal position and (iii) body size. Moreover, (iv) we use phylogenetic distance as an estimate of species' evolutionary age to control for phylogenetic nonindependence and to quantify any effect of species' age upon range size. Finally, (v) we test whether an invasion into the Nearctic that previously lacked *Coenagrion* species resulted in larger range sizes and the associated evolution of a better dispersal capacity.

Methods

Study taxa and phylogeny

We studied the 16 most common species of the genus *Coenagrion*, not including eight more local Asian species (Schorr *et al.*, 2009). For each species, morphometric and genetic data for between one and four individuals were taken, using individuals from different geographical areas whenever possible (Table 1, Fig. 1).

We sequenced 39 individuals that represented 16 species of the genus *Coenagrion* (Table 1), together with *Ischnura elegans*, *Paracercion melanotum* and *Enallagma cyathigerum* as outgroups. We genotyped animals at three nuclear markers and two mitochondrial markers. As nuclear markers, we studied the ITS region I and II (Dumont *et al.*, 2010), 28S (Hasegawa & Kasuya, 2006),

Table 1 Overview of the 16 studied *Coenagrion* species, sample size, geographic range sizes, mean morphological measurements (with standard deviation between brackets) and latitudinal position for the Palearctic species.

Species	Sample size	Locations	Range size (km ²)	Body mass (mg)	Wing loading (mg mm ⁻²)	Aspect ratio	Wing length (mm)	Relative flight muscle mass	Mid-range latitude (°N)	Latitude group
<i>Coenagrion angulatum</i>	3	Canada	3 441 728	4.737 (0.309)	8.04 (0.187)	19.718 (0.324)	13.697 (0.533)	0.477 (0.007)	49.533	Northern
<i>Coenagrion armatum</i>	2	Russia	18 656 275	4.063 (0.587)	10.523 (1.368)	19.115 (0.903)	14.765 (0.318)	0.422 (0.041)	54.717	Northern
<i>Coenagrion caeruleescens</i>	4	Morocco, Spain	1 512 954	3.52 (0.571)	9.987 (2.213)	19.541 (0.6)	13.015 (2.009)	0.443 (0.02)	39.733	Southern
<i>Coenagrion glaciale</i>	3	Russia	9 062 717	3.03 (0.542)	13.305 (1.641)	20.131 (0.42)	14.137 (0.537)	0.383 (0.017)	56.046	Northern
<i>Coenagrion hastulatum</i>	4	Italy, Sweden	26 197 941	5.663 (1.015)	8.21 (1.558)	19.69 (0.794)	14.91 (0.342)	0.44 (0.068)	55.088	Northern
<i>Coenagrion hylas</i>	4	Russia	13 477 522	6.825 (1.347)	7.819 (2.611)	20.739 (0.463)	16.215 (1.37)	0.476 (0.06)	52.927	Northern
<i>Coenagrion interrogatum</i>	4	Alaska, Canada	21 298 652	3.59 (0.805)	9.89 (2.667)	21.338 (0.634)	13.46 (0.412)	0.398 (0.07)	56.681	Northern
<i>Coenagrion johanssoni</i>	4	Finland, Russia	26 436 302	3.11 (0.591)	11.106 (1.175)	19.887 (0.565)	13.018 (1.031)	0.396 (0.044)	52.287	Northern
<i>Coenagrion lanceolatum</i>	1	Russia	8 194 813	3.67	10.99	18.622	13.267	0.365	43.244	Southern
<i>Coenagrion lunulatum</i>	4	Sweden, Russia	26 098 645	4.943 (1.252)	9.107 (1.784)	19.722 (0.272)	14.618 (0.468)	0.442 (0.033)	55.937	Northern
<i>Coenagrion mercuriale</i>	3	Italy, Spain	2 329 334	4.403 (0.277)	9.789 (0.878)	18.755 (0.788)	14.033 (0.315)	0.412 (0.025)	44.499	Southern
<i>Coenagrion ornatum</i>	2	Slovenia	4 926 793	6.4 (0.382)	8.678 (0.275)	18.408 (0.593)	15.98 (0.481)	0.383 (0.02)	44.529	Southern
<i>Coenagrion puella</i>	4	Croatia, Spain	16 060 224	5.17 (0.15)	8.614 (1.286)	21.327 (0.665)	15.363 (0.952)	0.445 (0.039)	47.705	Central
<i>Coenagrion pulchellum</i>	4	Bosnia, Macedonia, Russia	15 817 361	4.27 (1.2)	10.833 (0.896)	20.428 (0.987)	15.21 (1.759)	0.428 (0.026)	51.017	Central
<i>Coenagrion resolutum</i>	4	Alaska, Canada	28 633 340	4.635 (0.839)	8.012 (0.749)	20.206 (0.767)	13.68 (1.853)	0.42 (0.009)	53.303	Central
<i>Coenagrion scitulum</i>	4	Italy, Morocco, Portugal, Spain	4 295 999	4.943 (0.538)	6.741 (1.197)	20.055 (1.322)	12.828 (0.72)	0.453 (0.053)	40.967	Southern

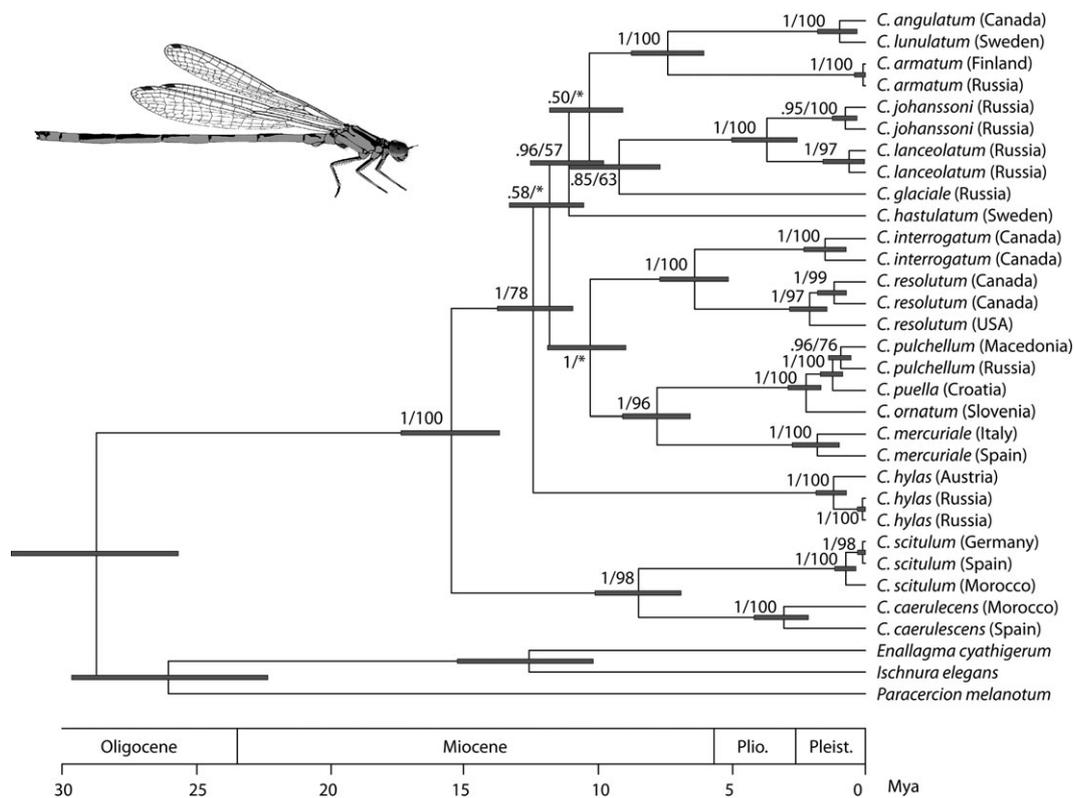


Fig. 1 Bayesian maximum-likelihood tree of *Coenagrion* species constructed with BEAST using concatenated genes ITS, 28S, PGI, 16S and CO₂. Bayesian posterior probability values ≥ 0.5 and bootstrap values ≥ 50 are respectively shown above nodes separated by /. Nodes indicated with * are not supported. The thick bars indicate the 95% confidence intervals of the approximate dating. Countries of origin of samples are listed within brackets along with the ecozone of the species and the latitudinal group of the Palearctic species. *Ischnura elegans*, *Paracercion melanotum* and *Enallagma cyathigerum* are used as outgroups. The scale bar at the bottom indicates the estimated timescale. Picture with courtesy of Viktor Nilsson-Örtman.

and an intron region of PGI, PGI-8 (Ferreira *et al.*, 2014). As mitochondrial markers, we studied CO₂ (476 bp, Swaegers *et al.*, 2014) and 16S (Palumbi *et al.*, 1991) (see Appendix S1B for details). Genomic DNA was isolated from legs or thorax using a nucleospin extraction kit (MacheryNagel, Düren, Germany). PCR amplifications were completed using primers and thermal cycling conditions described in Appendix S1B. Sequences were aligned using ClustalW in Geneious v.7.0.4 (Biomatters, Auckland, New Zealand) and then manually edited to minimize the amount of homoplasy among sequences. A total of 2619 bp were retained for analyses. Generated DNA sequences were deposited in GenBank (Appendix S1C).

Most appropriate evolutionary models for each sequence were determined in jModeltest (Posada, 2008) using Akaike's information criterion. Phylogenetic trees and associated posterior probabilities were obtained from a Bayesian analysis conducted with MrBayes v.3.1.1 (Huelsenbeck & Ronquist, 2001). A partition homogeneity test (implemented in PAUP*4.0b10a; Swofford, 2003) was used to evaluate whether the data

matrices provide different signal in the combined analyses of the different nuclear and mitochondrial markers.

Using BEAST v.2.0 (Bouckaert *et al.*, 2014), an approximate dating was performed with prior evolutionary rates for every gene based on insect estimates by Kaya *et al.* (2013) for the ITS gene, by Papadopoulou *et al.* (2010) for 28S and the mitochondrial genes and by Dunn *et al.* (2001) for PGI. The age of divergence of the lineages was calculated using the Bayesian relaxed phylogenetic approach, allowing variation in substitution rates among branches. A GTR+I+G substitution model was implemented with four rate categories with *a priori* rates used for every partition. We used an uncorrelated lognormal relaxed molecular clock model to estimate substitution rates and the Yule process of speciation as the tree prior (Heled & Drummond, 2012). Two independent analyses for each group were run sampling each 1000 generations. TRACER v1.4 (Rambaut *et al.*, 2014) was then used to determine convergence and to calculate the mean and 95% highest posterior density interval for divergence times. The evolutionary age of each species was defined as the

estimated age of the most recent node that connects it to any other species or clade and was presented with 95% confidence intervals.

Geographic range size and ecological traits

We defined range size as the area of occupancy (Gaston & Fuller, 2009). Range sizes were determined using the most recent distribution maps (V.J. Kalkman, unpublished) by measuring the continuous areas using cell[^]P 2.7 (Olympus Soft Imaging Solutions© 2007, Münster, Germany). Range position was defined using the mid-range latitude of each species, which was measured as the mid-point between the most northern and most southern latitude of the distribution area.

Each animal was photographed together with a scale using a digital camera. Pictures were analysed for wing morphometry. Eighteen landmarks were digitized on hind wing pictures (Appendix S1A). Wing length (distance between landmarks 1 and 9), wing width (maximum width perpendicular to the line between landmarks 1 and 9) and wing area were measured. The wing aspect ratio was the square of wing span divided by the area of the wing, with wing span being twice the wing length, and wing loading was calculated as the ratio of body mass on wing area (see Gyulavári *et al.*, 2014).

We quantified body dry mass (as a measure of size) and flight muscle mass based on a protocol described in Swillen *et al.* (2009). We removed the wings and legs and separated the head, thorax and abdomen. Thoraces and abdomens were weighed after being dried for 48 h at 60 °C and then fat was extracted by adding 1.5 mL dichloromethane (99%) to the samples. After 24 h (with automatic shaking), the dichloromethane with the dissolved fat was removed and the body parts were dried (48 h at 60 °C) and weighed to obtain the body dry mass. To determine flight muscle mass, we added 1.5 mL NaOH (0.35 M) to the samples that were then shaken for 24 h. Flight muscle mass was the difference between the thoracal exoskeleton mass and the total fatless thorax mass (Marden, 1989). The relative flight muscle mass (or flight muscle ratio; Marden, 1989) was the flight muscle mass divided by the body dry mass. Thorax muscle mass is a good predictor of power output in Odonata (Schilder & Marden, 2006), and the relative investment in flight muscles is positively correlated with flight performance in butterflies (Berwaerts & Van Dyck, 2004).

Statistical analyses

To determine the influence of the proxies of dispersal capacity, body size, mid-range latitude and evolutionary age on range size of *Coenagrion* damselflies, we ran general linear models and performed phylogenetic generalized least squares models to correct for phylog-

eny (Hernandez *et al.*, 2013) as implemented in the R package Caper (R Development Core Team 2009; Orme *et al.*, 2012). When analysing the proxies of dispersal capacity and body size, we used species means. We used a hierarchical strategy. First, we performed a separate analysis for each trait (set) linked to a certain hypothesis to explain range size variation: (i) proxies of dispersal capacity, (ii) body size, (iii) latitude and (iv) evolutionary age of the species. Next, we included all significant variables from the first step in a common analysis. In addition, we tested for effects of latitude on the proxies for dispersal capacity and on body size.

Within the Palearctic, we divided the species into northern species, central species and southern species (Table 1) to test whether the phylogenetic tree topology reflected species' geographic distributions using the package Geiger (Harmon *et al.*, 2008). We thereby fitted latitude group as a discrete trait on the phylogenetic tree using Pagel's lambda and compared this to the null model of random association of species across latitude groups using a χ^2 -test. To test whether the colonization of the Nearctic by species of *Coenagrion* resulted in evolution of a better dispersal capacity and associated larger range sizes, we also compared the range sizes and dispersal proxies between the three Nearctic species and the remaining species using a phylogenetic generalized least square method as above.

Results

Phylogeny

For ITS (822 bp), the best evolutionary model was GTR; for 28S (590 bp), this was Trn+I+G; for PGI (419 bp), Trn+G; for 16S (303 bp), Tim1 + I+G; and for CO₂ (476 bp), GTR+I+G. The partition homogeneity test did not detect a significant ($P > 0.05$) conflict between mitochondrial and nuclear data sets, and the data were concatenated to construct a single tree based on all five partial gene sequences (Fig. 1).

The phylogeny shows one major clade containing the southern Palearctic species *Coenagrion scitulum* and *Coenagrion caeruleum*, and another major clade containing all other species with *Coenagrion hylas* at its base (Fig. 1). This second clade is subdivided into a mixed clade with one Nearctic and six northern Palearctic species (*Coenagrion angulatum* to *Coenagrion hastulatum*) and a clade that comprises subclades with (i) two Nearctic species (*Coenagrion interrogatum* and *Coenagrion resolutum*) and (ii) two southern Palearctic species (*Coenagrion mercuriale* and *Coenagrion ornatum*) and two central Palearctic species (*Coenagrion puella* and *Coenagrion pulchellum*). Bayesian posterior probabilities (indicating topology support) are high (> 0.9) for most nodes, with notable exceptions of (i) the basal split between the latter two subclades and (ii) the split within the northern subclade dividing the clades *Coenagrion armatum*–*Coenagrion anglatum*–*Coenagrion*

lunulatum and *Coenagrion lanceolatum*–*Coenagrion johansoni*–*Coenagrion glaciale* (Bayesian posterior probabilities = 0.58 and 0.50, respectively).

A phylogenetic signal was detected for the latitude group variable within the Palearctic (χ^2 test compared to $\Lambda = 0$, $P = 0.029$), indicating that species defined by latitude significantly clustered within the tree.

Ecological and macroevolutionary drivers in range size

Of the four proxies for dispersal capacity (Table 2), only wing aspect ratio had a significant effect on species' range sizes, albeit with statistical significance ($P < 0.05$) achieved only after correcting for phylogeny (Table 2a). Species with a higher wing aspect ratio (i.e. longer and slender wings) had larger ranges (Fig. 2a).

The latitudinal position of a species' range, the mid-range latitude, was significantly ($P < 0.001$ in both the uncorrected and phylogenetically corrected analyses) associated with range size: species residing at higher latitudes occupy larger geographical areas (Table 2c,

Fig. 2b). However, neither body mass nor evolutionary age was associated with range size (Table 2b, d, Appendix S1D).

In the combined analysis of both wing aspect ratio and mid-range latitude, only mid-range latitude was returned as a significant ($P = 0.0012$ and $P = 0.0003$ in the uncorrected and phylogenetically corrected analyses, respectively) predictor of range size (Table 2e).

Geographic patterns in dispersal capacity and body mass

No significant ($P > 0.09$ in all tests) effects of latitude on the proxies for dispersal capacity or body mass were detected, although there was a trend for high-latitude species to have higher wing aspect ratios (Table 3). Nearctic species show significantly smaller range sizes than Palearctic species (mean ± 1 SE, Nearctic species $2 \times 10^6 \pm 1 \times 10^6$; Palearctic species: $10 \times 10^6 \pm 8 \times 10^6$ km²; $t_{13} = -2.752$, $P = 0.016$). Only wing length (of the four proxies of dispersal ability) significantly differed between the Nearctic and the Palearctic *Coenagrion*

Table 2 General linear models and phylogenetic generalized least squares models for the association between range size and proxies for dispersal capacity (a), body mass (b), latitudinal position (c), evolutionary age (d) and the combined analysis of significant predictor variables (e).

	General linear model				Phylogenetic generalized least squares model			
	Estimate	SE	t_{11}	P	Estimate	SE	t_{11}	P
(a) Dispersal								
Aspect ratio	0.2809	0.1322	2.124	0.0596	0.4121	0.1128	3.6539	0.0038
Wing length	0.111	0.0989	1.1224	0.2879	0.2246	0.1204	1.8666	0.0888
Wing loading	1.7756	6.9204	0.2566	0.8027	-1.0241	6.5563	-0.1562	0.8787
Relative flight muscle mass	-5.6595	4.7298	-1.1966	0.2591	-9.6183	4.4599	-2.1566	0.054
	Estimate	SE	t_{14}	P	Estimate	SE	t_{14}	P
(b) Body mass								
Body mass	0.0142	0.1003	0.1412	0.8898	-0.0402	0.1369	-0.2936	0.7733
	Estimate	SE	t_{14}	P	Estimate	SE	t_{14}	P
(c) Latitude								
Mid-range latitude	0.0576	0.0116	4.9592	0.0002	0.0845	0.0146	5.7851	< 0.0001
	Estimate	SE	t_{14}	P	Estimate	SE	t_{14}	P
(d) Evolutionary age								
Evolutionary age	-0.0093	0.0295	-0.3138	0.7583	0.0006	0.0575	0.0098	0.9923
	Estimate	SE	t_{13}	P	Estimate	SE	t_{13}	P
(e) Combined model								
Aspect ratio	0.0591	0.086	0.687	0.5041	0.0391	0.076	0.5147	0.6154
Mid-range latitude	0.0538	0.0131	4.1211	0.0012	0.0808	0.0166	4.8672	0.0003

Significant P -values are indicated in bold.

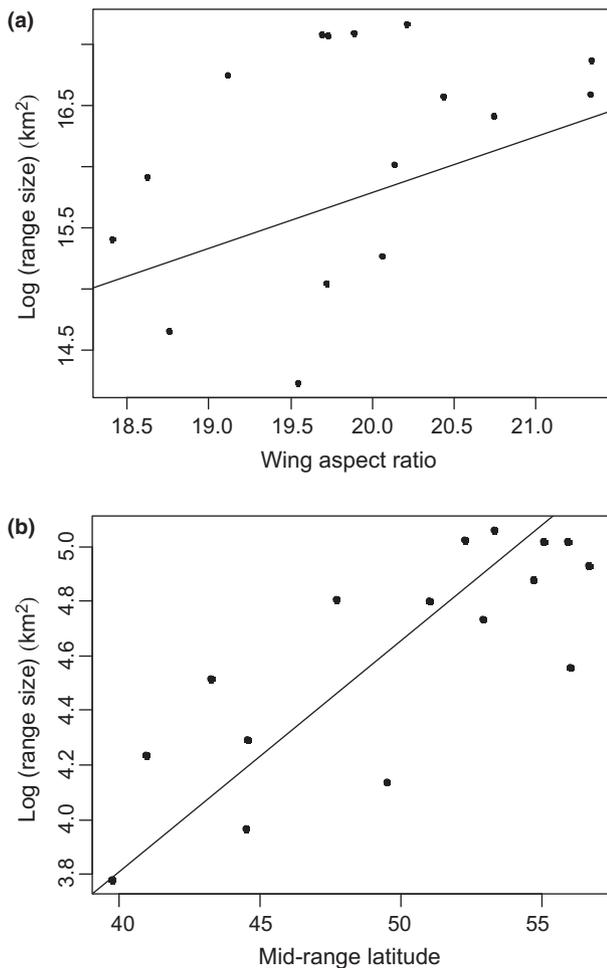


Fig. 2 Relationships between (log-transformed) range size and wing aspect ratio (a) and mid-range latitude of the distributions (b).

(Appendix S1E). However, in contrast with our prediction, Nearctic species have smaller wing lengths (mean \pm 1 SE, Nearctic species: 13.16 ± 0.37 mm; Palearctic species: 14.09 ± 0.56 mm; $t_{13} = -2.51$, $P = 0.026$).

Discussion

Ecological and macroevolutionary drivers of range size

When testing only for proxies of dispersal capacity, species with higher wing aspect ratios (with longer and slender wings) had larger range sizes, suggesting that dispersal capacity influenced the amount of habitat a species colonized after the last glacial maximum. In line with this, migrating birds (Hedenström, 2008) and dragonflies (Johansson *et al.*, 2009) have high wing aspect ratios and hence this phenotype is thought to be important for long distance dispersal. That dispersal capacity is a driver of range expansion has been shown in a study on the related *Enallagma* damselflies (Rundle *et al.*, 2007). Yet, in *Enallagma* damselflies, wing lengths were longer in species with larger range sizes, which was not upheld in current study. Given that wing aspect ratios were not included in the study by Rundle *et al.* (2007), it cannot be excluded that also in *Enallagma* damselflies they are a determinant of range size.

Also, we found a strong signal of mid-range latitude on range size with larger range sizes in northern areas, which is in concordance with Rapoport's rule (Gaston, 2009). As climatic changes were more severe in the northern than in the southern Palearctic region, this might have resulted in selection for species with a broad ecological niche and consequently a larger range size (Dynesius & Jansson, 2000). Although a common explanation of Rapoport's rule is that the range size has been determined by the environmental conditions (and hence latitude) under which a species evolved, it could be that species with broader ecological niches were better able to colonize new habitats after the last glacial maximum (Hausdorf, 2006). The observed patterns are then assumed to have a post-glacial origin (Shelomi, 2012).

Notably, we show that only mid-range latitude, and not wing aspect ratio, associates with range size when the candidate drivers of range size were combined into a single analysis. This may suggest that the positive relation between dispersal capacity and range size may not have been causal, yet rather a consequence of the

Table 3 General linear models and phylogenetic generalized least squares models of mid-range latitude on the proxies of dispersal capacity.

Predictor	Dependent variable	General linear model				Phylogenetic generalized least squares model			
		Estimate	SE	t_{14}	P	Estimate	SE	t_{14}	P
Mid-range latitude	Aspect ratio	0.0640	0.0368	1.7407	0.1037	0.0936	0.0527	1.7749	0.0977
	Wing length	0.0525	0.0481	1.0914	0.2935	0.0475	0.0422	1.1263	0.2790
	Wing loading	0.0659	0.0750	0.8775	0.3950	-0.0012	0.0009	-1.4425	0.1712
	Relative flight muscle mass	-0.0002	0.0015	-0.1576	0.8771	-0.0006	0.0017	-0.3666	0.7194
	Body mass	0.0142	0.1003	0.1412	0.8898	-0.0453	0.0509	-0.8903	0.3883

latitudinal structuring of wing aspect ratios. This is further suggested by a trend of increasing wing aspect ratios at higher latitudes. The latter may be interpreted as a compensating mechanism to maintain high flight and dispersal capacities at cooler northern latitudes where flight muscles work less efficiently (Hassall *et al.*, 2008). Other compensatory morphological mechanisms to maintain flight capacity, such as increases in flight muscle mass, may have been too costly. Changes in wing morphology rather than flight muscle ratio at colder temperatures have been shown in intraspecific studies (Azevedo *et al.*, 1998; Frazier *et al.*, 2008). Yet, the direction of causality may as well be opposite as at higher latitudes species may show higher wing aspect ratios because only good dispersers were able to colonize these areas after the last glacial maximum (Rundle *et al.*, 2007; review for intraspecific studies: Hill *et al.*, 2011).

Irrespective of the exact pattern of causality, our data indicate that besides dispersal capacity, unmeasured latitude-associated factors possibly related to niche width (see above) also contributed in shaping range size. We can exclude a role for body mass as this trait did not show the expected increase with latitude (Bergmann's rule; Shelomi, 2012), nor did it shape range size. The absence of a Bergmann cline in body size may be explained by differences in voltinism among *Coenagrion* species (Corbet *et al.*, 2006). Indeed, theory predicts complex, intraspecific sawtooth patterns of size against latitude in case of changes in voltinism (Roff, 1980; Johansson, 2003). Notably, the opposite pattern has been documented in *Sylvia* warblers where an increase in range size with latitude was the result of correlations between latitude and dispersal capacity (Böhning-Gaese *et al.*, 2006).

Phylogenetic patterns, species' ages and the invasion of North America

The phylogenetic tree topology supports the relationships of 13 species of *Coenagrion* included in a wider phylogenetic analysis of Zygoptereans (Dumont *et al.*, 2010). Within the Palearctic, species with similar distribution areas are significantly clustered within the tree, suggesting speciation events have occurred locally in the Palearctic rather than in a sympatric fashion following a range shift (Losos & Glor, 2003). The phylogeny confirms the species identity of the isolated European *C. hylas* population situated more than 5000 km away from the main range area in Asia. A similar remarkable disjunct distribution was found in the azure magpie (Fok *et al.*, 2002). Surprisingly, the genetic distance between the individuals of the isolated European population in the Alps with the populations in the central core range of the species is similar to the genetic distances among closely located populations in other species. The observed pattern of shallow genetic divergence over a large area across the Eurasian conti-

nent has been shown in other species and may be explained by recurrent recolonization of northern areas from southern refugia after glacial periods in the Quaternary (Saitoh *et al.*, 2010).

The dating of the phylogenetic tree suggests all species formed from the end of the Miocene onwards with considerable variation among different clades. The absence of an effect of evolutionary age on range size, however, suggests no clear directionality of range size through time in *Coenagrion*. Yet, it should be considered that, besides the uncertainty of mutation rates, an incomplete tree (due to extant or extinct missing species) may overestimate the evolutionary age of certain species potentially leading to the removal of the signal of evolutionary age on range size (Webb & Gaston, 2000). The observed higher estimates of mid-range latitude and wing aspect ratio on range expansion when phylogeny was accounted for shows the importance of correcting for evolutionary history when analysing the ecological drivers of species' ranges (Blanchet *et al.*, 2013).

The phylogeny confirms the Palearctic origin of the genus and suggests the independent invasion of the Nearctic by the ancestor of *C. angulatum* and by the ancestor of the two sister species *C. resolutum* and *C. interrogatum*. Interestingly, the genetic similarity between the North American *C. angulatum* and the northern Palearctic *C. lunulatum* matches their morphological resemblance (Westfall & May, 1996). Colonizing the Nearctic, however, was not associated with the evolution of larger range sizes or a larger dispersal capacity. This interspecific pattern thereby not follows the intraspecific pattern where during range expansion species evolve a higher dispersal capacity (Hill *et al.*, 2011; for *Coenagrion* damselflies: Therry *et al.*, 2013).

According to the phylogenetic tree, *C. angulatum* originated later than *C. resolutum* and *C. interrogatum*. Interestingly, *C. angulatum* has a smaller range size compared to the latter species. Possibly, this could be caused by the earlier presence of congeneric species, with interspecific competition limiting its ability to invade new areas.

Conclusions

We identified two ecological traits contributing to species differences in range size in *Coenagrion* damselflies: species range size was larger in northern areas (supporting Rapoport's rule) and larger in species with a higher dispersal capacity (measured as wing aspect ratio). Additionally, there was a trend for a higher dispersal capacity at northern latitudes. Instead, the macroevolutionary trait species' age had no discernable effect upon range size. These ecological effects were only significant or stronger when correcting for phylogeny. The genus *Coenagrion* invaded the Nearctic twice independently from Eurasia, yet this was not associated with the evolution of larger range sizes or higher dispersal capacity. Our results underscore

the importance of integrating macroecology and macroevolution when explaining range size variation among species and illustrate that studies only focusing on single traits may mistakenly conclude causality in explaining macroecological patterns.

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Supporting information

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

Appendix S1 Detailed methods.

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