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Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae)

Paula Arribas^{1*}, Josefa Velasco¹, Pedro Abellán², David Sánchez-Fernández³, Carmelo Andújar⁴, Piero Calosi⁵, Andrés Millán¹, Ignacio Ribera⁶ and David T. Bilton⁵

¹Departamento de Ecología e Hidrología, Universidad de Murcia, Murcia 30100, Spain,

²Department of Biological Sciences, Aarhus University, Aarhus DK-8000, Denmark,

³Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid 28006, Spain,

⁴Departamento de Zoología y Antropología Física, Universidad de Murcia, Murcia 30100, Spain,

⁵Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth PL4 8AA, UK,

⁶Departamento de Biodiversidad Molecular y Evolución, Instituto de Biología Evolutiva (UPF-CSIC), Barcelona 08003, Spain

ABSTRACT

Aim In aquatic ecosystems, standing (lentic) and running (lotic) waters differ fundamentally in their stability and persistence, shaping the comparative population genetic structure, geographical range size and speciation rates of lentic versus lotic lineages. While the drivers of this pattern remain incompletely understood, the suite of traits making up the ability of a species to establish new populations is instrumental in determining such differences. Here we explore the degree to which the association between habitat type and geographical range size results from differences in dispersal ability or fundamental niche breadth in the members of the *Enochrus bicolor* complex, an aquatic beetle clade with species across the lentic–lotic divide.

Location Western Mediterranean, with a special focus on North Africa, the Iberian Peninsula and Sicily.

Methods DNA sequences for four loci were obtained from species of the *E. bicolor* complex and analysed using phylogenetic inference. Dispersal and establishment abilities were assessed in lentic–lotic species pairs of the complex, using flight wing morphometrics and thermal tolerance ranges as surrogates, respectively.

Results There were clear differences in range size between the lotic and lentic taxa of the complex, which appears to have had a lotic origin with two transitions to standing waters. Only small differences were observed in temperature tolerance and acclimation ability between the two lotic–lentic sister species studied. By contrast, wing morphometrics revealed clear, consistent differences between lotic and lentic *Enochrus* species pairs, the latter having a higher dispersal capacity.

Main conclusions We hypothesize that there have been two habitat shifts from lotic to lentic waters, which have allowed marked expansions in geographical range size in western Mediterranean species of the *E. bicolor* complex. Differences in dispersal rather than in establishment ability appear to underlie differences in geographical range extent, as transitions to lentic waters were associated with changes in wing morphology, but not in thermal tolerance range. In this lineage of water beetles, selection for dispersal in geologically short-lived lentic systems has driven the evolution of larger range sizes in lentic taxa compared with those of their lotic relatives.

Keywords

Aquatic Coleoptera, colonization capacity, *Enochrus bicolor* complex, fundamental niche breadth, geographical range size, geological persistence, habitat constraints, habitat stability, western Mediterranean.

*Correspondence: Paula Arribas Blázquez, Departamento de Ecología e Hidrología, Universidad de Murcia, 30100 Espinardo, Murcia, Spain.
E-mail: pauarribas@um.es

INTRODUCTION

Despite a long history of the study of factors determining the geographical distribution of organisms (e.g. Stevens, 1989; Brown, 1995; Gaston, 2003; Lester *et al.*, 2007), some fundamental questions remain unresolved, such as why closely related species often have widely differing range sizes. Although a number of ecological and evolutionary processes may determine geographical range size (Gaston, 2003), habitat selection has been shown to be of key importance in several empirical studies (e.g. Duncan *et al.*, 1999; Gregory & Gaston, 2000; Marsden & Whiffin, 2003). In aquatic ecosystems, one of the most fundamental habitat constraints is the division between standing (lentic) and running (lotic) waters (Ribera, 2008). The contrasting geological permanence and ecological stability of lotic and lentic waters (Hutchinson, 1957) have been related to the evolutionary and biogeographical differences observed between species in the two habitat types. Taxa in the geologically shorter-lived and more ecologically unstable lentic habitats have less population genetic structure and larger geographical ranges than their lotic relatives, a pattern observed across a range of taxa, regions and systems (e.g. Ribera & Vogler, 2000; Hof *et al.*, 2006; Abellán *et al.*, 2009; Damm *et al.*, 2010). While the drivers of this pattern remain incompletely understood, the suite of traits making up the colonization capacity of a species will be instrumental in shaping such differences (Ribera, 2008). In this sense, colonization capacity has two components: dispersal to and establishment at a new site (Reaka, 1980; Gaston, 2003). Despite the assumed importance of colonization capacity, we have little understanding of the relative contribution of differences in dispersal ability and establishment success in shaping the observed differences between lentic and lotic lineages (Bilton *et al.*, 2001; Rundle *et al.*, 2002).

In order to understand species distributions, it is also necessary to consider the effects of scale (Hui *et al.*, 2010; Kriticos & Leriche, 2010). Of the three main factors determining species distributions (i.e. environment, biotic interactions and movement; Soberón, 2010), abiotic factors typically operate at large scales, whereas biotic interactions operate more locally (Soberón & Nakamura, 2009; Soberón, 2010). A similar difference of scale can be seen with movement-related factors, which at biogeographical scale have a significant effect on the large-scale processes that determine the size and shape of species distributional ranges, while occupancy dynamics are relevant at smaller scales within the landscape (Hortal *et al.*, 2010). To understand the processes shaping relative geographical range extent, we need to focus on factors such as fundamental niche breadth and dispersal ability, which constrain distribution patterns over large spatial scales.

In the case of aquatic organisms, the geological instability of lentic habitats selects for strongly dispersing individuals, while this restriction does not act on lotic taxa. As result of this asymmetric constraint, the dispersal ability of lentic species is higher than that of lotic species, resulting in the more dynamic and larger geographical ranges of lentic taxa compared with

their lotic relatives (Ribera, 2008). In aquatic insects, relative dispersal ability is driven largely by differences in flight capacity (Bilton *et al.*, 2001). While comparative flight performance and dispersal ability can be difficult to measure directly in insects (e.g. Jonsson, 2003), wing morphometrics are known to relate to flight performance (Brodsky, 1994; Berwaerts *et al.*, 2002; Gibb *et al.*, 2006; Larsen *et al.*, 2008); consequently, flight wing morphology can provide a proxy of relative dispersal ability amongst closely related, functionally similar species.

The ability of lentic species to establish in a wider range of localities results from broader fundamental niches (*sensu* Brown, 1984) in these taxa compared with their lotic relatives. Although niches are multidimensional, an organism's thermal physiology can be seen as a critical aspect of its fundamental niche (Spicer & Gaston, 1999), and, indeed, environmental temperatures have long been seen as critical in shaping species distributions (e.g. Andrewartha & Birch, 1954; Merriam, 1984; Pigot *et al.*, 2010). In biogeographical terms, many past studies have noted coincidences between geographical range boundaries and temperature isotherms (Salisbury, 1926; Caughley *et al.*, 1987; Iversen, 1994), and recent work has demonstrated that thermal tolerance ranges are the best predictors of geographical range size in a number of insect clades in running waters (Calosi *et al.*, 2008a,b, 2010). Relatively shallow, small- to medium-sized lentic water bodies, which support the majority of standing-water macroinvertebrate biodiversity world-wide (e.g. Oertli *et al.*, 2002; Scheffer *et al.*, 2006), are subjected to larger daily and seasonal temperature fluctuations than streams or rivers (Álvarez-Cobelas *et al.*, 2005; Florencio *et al.*, 2009), and selection to cope with such local fluctuations may enable species of small lentic waters to tolerate the thermal conditions present across a wider geographical area. Thus, the larger geographical ranges of lentic versus lotic macroinvertebrates (Ribera & Vogler, 2000; Monaghan *et al.*, 2005; Hof *et al.*, 2006; Ribera, 2008) could be caused by either higher dispersal ability or the higher establishment success provided by wider thermal tolerance.

We use a clade of aquatic beetles, the *Enochrus bicolor* complex (Schödl, 1998) (family Hydrophilidae), to explore evolutionary relationships between habitat (lotic versus lentic) and colonization capacity. Species of the *E. bicolor* complex are specialists of either small lentic or lotic saline waters, living in the margins of salt pans and saline streams. Lentic taxa have a much wider distributions than lotic, conforming to the general pattern in inland waters, discussed above. As a result, the group provides an ideal comparative framework within which to explore possible differences in dispersal and/or establishment ability associated with habitat type, and how these differences shape geographical distributions. We reconstruct the phylogeny of the western Mediterranean taxa of the group from mitochondrial and nuclear DNA sequence data, obtained from multiple populations of each species, to estimate the number and direction of habitat shifts between running and standing waters. We examine possible differences in dispersal and/or establishment ability in lentic and lotic sister taxa,

employing flight wing morphometrics and thermal physiology as proxies of these features, respectively. In this way we test whether habitat shifts, and associated changes in geographical range size, are linked to differences in dispersal ability, establishment ability, or a combination of these traits.

MATERIALS AND METHODS

Study group

Water beetles are one of the most diverse types of animal inhabiting small inland waters world-wide (Velasco *et al.*, 2006; Millán *et al.*, 2011). Amongst these, the genus *Enochrus* Thomson (Coleoptera, Hydrophilidae) includes a number of saline and hyper-saline habitat specialists, such as the *Enochrus bicolor* complex, which comprises five recognized Palearctic species: *E. falcarius* (Hebauer), *E. bicolor* (Fabricius), *E. segmentinotatus* (Kuwert), *E. sinuatus* D'Orchymont and *E. turanicus* Schödl. Of these, three (*E. falcarius*, *E. bicolor* and *E. segmentinotatus*) are found in the central and western Mediterranean region, and form a closely related complex of species recognized by Schödl (1998). The three species show striking differences in ecology and distribution. *Enochrus falcarius* is restricted to saline streams in the south of the Iberian Peninsula, Tunisia, Sicily and Morocco (Hansen, 2004; A. Millán *et al.*, pers. obs.). In contrast, *E. bicolor* and *E. segmentinotatus* inhabit lentic saline systems (saline ponds and salt-pans), and are both distributed widely across much of Europe, North Africa and Asia, east to Mongolia (Hansen, 2004).

Phylogeny of the *Enochrus bicolor* complex

In total, 73 specimens from the *E. bicolor* complex were collected from 44 localities in the western Mediterranean (see Tables S1.1 and S1.2 in Appendix S1 in Supporting Information). We sampled across the entire known range of *E. falcarius*, including populations from the Iberian Peninsula, Sicily, Tunisia and Morocco (Fig. 1, Table S1.1). Sampling localities for *E. bicolor* extended from North Africa to Ireland and east to Algeria, Sicily, southern France and the Balearic Islands. Finally, *E. segmentinotatus* was sampled from northern Morocco, southern Iberia, southern France and the Balearic Islands (Fig. 1, Table S1.1). In addition, several species of *Enochrus* in the same subgenus (*Lumetus* Zaitzev) were included in the analyses: *E. testaceus* (Fabricius), *E. ater* (Kuwert) and *E. salomonis* (J. Sahlberg). As an outgroup we used *E. natalensis* (Gemminger & Harold) from the subgenus *Methydrys* Rey.

Samples were collected into absolute ethanol in the field and stored at -20°C in the laboratory until required. DNA was extracted using an Invisorb Spin Tissue Mini Kit (Invitex, Berlin, Germany), following the manufacturer's recommendations. We sequenced three mitochondrial (3' end of cytochrome *c* oxidase subunit I, *COI*; an internal fragment of cytochrome *b*, *cyt b*; 3' end of the large ribosomal unit plus

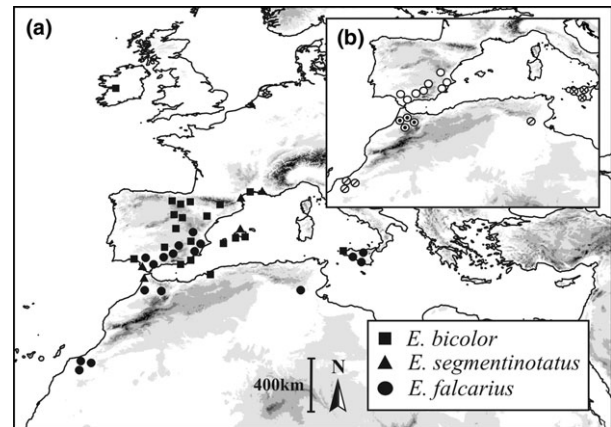


Figure 1 Sampling sites in the western Mediterranean for (a) *Enochrus falcarius*, *E. bicolor* and *E. segmentinotatus*; (b) *E. falcarius* localities grouped according to clades identified in phylogenetic analyses. The grey shadings represent 500-m isoclines.

Leucine transferase and the 5' end of NADH dehydrogenase subunit 1, *rrnL+trnL+ND1*) and one nuclear (an internal fragment of the large ribosomal unit, 28S rRNA, *LSU*) gene (see Table S1.3 for the primers used). Sequencing was conducted using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA), and sequenced products were electrophoresed on ABI 310 and 3700 automated sequencers (Applied Biosystems). Sequences were assembled and edited with SEQUENCHER 4.7 (GeneCodes Corporation, Ann Arbor, MI, USA) and submitted to GenBank (see Table S1.2 for accession numbers).

Evolutionary models were estimated prior to the analysis with jMODELTEST (Posada, 2008). Bayesian analyses (BA) were conducted on a combined data matrix with MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003), using four partitions corresponding to the four sequenced fragments. MRBAYES ran for 10 million generations using default values, saving trees every 100 generations. The half compact consensus tree was calculated with the 'sumt' option of MRBAYES. Posterior probabilities of nodes were interpreted as Bayesian support. MRBAYES searches were carried out on the freely available Bioportal at the University of Oslo (available at: <http://www.bioportal.uio.no>). A maximum likelihood (ML) analysis was also conducted in RAXML 7.0.3 (Stamatakis, 2006), with a GTR + gamma model for each gene fragment partition and 100 alternative runs to select the best tree. Node support was calculated with 1000 bootstrap replicates. RAXML analyses were computed on CIPRES (Miller *et al.*, 2010).

Evaluation of dispersal ability: flight wing morphometrics

Morphometric measurements of flight wings were taken for two pairs of lotic–lentic sister taxa: Iberian *E. falcarius* (*E. falcarius* IP, see Results) and *E. bicolor*, and Sicilian *E. falcarius* (*E. falcarius* SI) and *E. segmentinotatus*, respectively,

in order to evaluate comparative dispersal ability in both lotic and lentic species. Adults of Iberian *E. falcarius* and *E. bicolor* were collected from the same two localities as those used for thermal tolerance experiments (see Appendix S2: Table S2.1), and adults of Sicilian *E. falcarius* and *E. segmentinotatus* were collected from Nicosia, Sicily (Italy) and Puerto de Santa María, Cádiz (Spain), respectively. Beetles were maintained for 24 h in filtered water from their respective localities to allow them to empty their gut contents. Subsequently, beetles were killed in 100% ethanol and dried at 56 °C in an oven (Universal Precision Digitronic oven, JP Selecta, Barcelona, Spain) for 16 h. Body mass was measured using a digital balance (1204 MP2, Sartorius, Epsom, UK) to ± 0.001 g precision. Measured individuals were digested in 2% proteinase K (Invitex, Berlin, Germany) for 8 h, in order to facilitate manipulation of the wings.

Length, width and area of the membranous wings and elytral length were measured for each individual. The left wing was removed, teased open and mounted in a 50% dimethyl hydantoin formaldehyde (DMHF) solution on a microscope slide. Wings and individuals were photographed under a Zeiss Stemi 2000C Trinocular Zoom Stereomicroscope (Zeiss, Thornwood, NY, USA), and subsequently measured using a Spot Insight Firewire digital camera (Spot Imaging Solutions, Sterling Heights, MI, USA) and associated software. Left elytral length was used as a proxy of body length to avoid measurement error owing to contraction of the head and pronotum. Elytra length and total body length are strongly correlated in these beetles (correlation coefficient $r = 0.974$, $P < 0.001$, $n = 18$).

Between 11 (*E. segmentinotatus*) and 16 (Iberian *E. falcarius*) individuals were measured for each clade. Raw measures of body and wing were used to derive wing loading (body mass/wing area) and wing aspect ratio (wing length/wing width) (Gibb *et al.*, 2006). Preliminary results showed that body mass was highly variable between individuals within clades, probably resulting from differences in age and sexual development, and cubed elytral length was therefore used as surrogate of body mass to avoid such confounding influences. Such an approach is justified here because maximum body mass and body length will scale in a very similar manner across such morphologically similar species (Benke *et al.*, 1999). Dispersal ability estimates were compared between four species using ANOVAs and post-hoc tests with Bonferroni correction. Neither raw data nor general linear model (GLM) residuals showed significant departure from normality and homoscedasticity, so untransformed values were used in all analyses, which were conducted using SPSS 15.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

Evaluation of establishment ability: thermal physiology

We used thermal tolerance as a surrogate of the establishment abilities of *E. bicolor* and Iberian *E. falcarius* (IP *E. falcarius*), determining their lethal thermal limits after acclimation at different salinities and temperatures in the laboratory. Previous

work on the Coleoptera of inland saline waters has demonstrated that exposure to different salinities can influence the thermal tolerance of adult beetles (Sánchez-Fernández *et al.*, 2010), and we employed multiple salinity and temperature treatments here in order to compare species thermal niches across a range of salinities.

Adult beetles were collected from two populations at similar latitudes (see Table S2.1). Specimens were maintained for 7 days under constant conditions (20 °C, salinity 30 g L⁻¹) to minimize the effects of recent thermal history on measures of temperature tolerance (Sokolova & Portner, 2003). After this, specimens of each species were randomly divided into three equal-sized groups, corresponding to three acclimation temperatures (15, 20 and 25 °C), and within each of these sets individuals were re-assigned to five salinity treatments (1, 6, 12, 35 and 60 g L⁻¹), with a total of 16 individuals per treatment. Acclimation temperatures and salinities were chosen to represent the range of environmental conditions under which these species are usually found in Iberia (Table S2.1; Velasco *et al.*, 2006). Beetles were maintained under their corresponding thermal-osmotic acclimation treatments for 6 days (Terblanche & Chown, 2006). Following this period, individuals from each treatment were assigned randomly to two equal-sized subgroups – one used to measure upper thermal limits (UTLs) and the other one to measure lower thermal limits (LTLs; Chown & Nicolson, 2004).

Thermal tolerance tests were carried out in air, using a dynamic method (Lutterschmidt & Hutchison, 1997), ramping temperature at ± 1 °C min⁻¹ in a computer-controlled water bath (Grant LTC 6–30, using the Grant COOLWISE software, Grant Instruments, Cambridge, UK). Heating or cooling commenced at the temperature at which a particular treatment group had previously been acclimated. UTL and LTL were estimated as lethal points following the approach of other recent studies of aquatic beetles (see Calosi *et al.*, 2008a,b, 2010; Sánchez-Fernández *et al.*, 2010).

Multifactorial ANCOVAs were used with untransformed data to investigate the effects of temperature and salinity acclimation and species on both UTLs and LTLs. Body mass was included as a covariable, because species differed significantly [ANOVA d.f. = 1, $F = 411.5$, $P < 0.001$; Iberian *E. falcarius* average body mass (bm) = 6.63 mg, *E. bicolor* bm = 11.31 mg]. Similar analyses were implemented separately for each species. Homoscedasticity of raw data was met, but for some treatments the assumption of normality was not. Despite this, we used raw data because residuals were normally distributed, thus ensuring a lack of bias or spurious effects in the GLM results (see Rutherford, 2001). To employ a more conservative approach, post-hoc tests with Bonferroni correction were used to identify significant differences amongst mean UTLs or LTLs for different acclimation treatments. All statistical analyses were conducted using SPSS 15.0.1 for Windows.

Thermal windows were also constructed for each species as an integrative and synthetic index of thermal tolerance that takes into consideration both LTLs and UTLs measured under different acclimation conditions (see Spicer & Gaston, 1999;

Calosi *et al.*, 2008b). Mean UTLs and LTLs measured at the three acclimation temperatures (15, 20 and 25 °C) and salinity 35 g L⁻¹ were used, since this is the modal salinity at which *E. bicolor* complex species are found in the field. The width and position of these thermal windows were then qualitatively compared between Iberian *E. falcarius* and *E. bicolor*.

RESULTS

Phylogeny of the *Enochrus bicolor* complex

The aligned sequence data matrix had 2487 characters, of which 494 were variable. There was no length variation in the protein-coding genes, and the main variation in the ribosomal genes was concentrated in the fragment *rrnL+trnL*. For MRBAYES analysis, standard deviation of the split frequencies between the two runs reached a value of *c.* 0.005 at 10 million generations, and the half compact consensus tree was calculated removing 15% of initial trees as a 'burn-in'.

The topologies of the tree were similar for the two reconstruction methods (Bayesian probabilities and ML), with very similar support values for the main nodes (Fig. 2). Species of the *E. bicolor* complex (*E. falcarius*, *E. bicolor* and *E. segmentinotatus*) formed a clade with strong support, to the exclusion of the remaining *Enochrus* species (Fig. 2, node A). Within the *E. bicolor* complex, the recognized species *E. bicolor* and *E. segmentinotatus* are each monophyletic, while *E. falcarius* is paraphyletic. Thus, within the complex, two main, well-supported clades can be recognized. One clade (Fig. 2, node B) encompasses all populations of *E. bicolor* and Iberian and northern Moroccan populations of *E. falcarius* [this itself containing two independent well-supported clades comprising Iberian (IP) and northern Moroccan (NM) populations, respectively; Fig. 2, node C]. The other clade (Fig. 2, node D) includes all populations of *E. segmentinotatus*, and the Sicilian, Tunisian and southern Moroccan *E. falcarius*. Within this second main clade, *E. segmentinotatus* is sister to Sicilian *E. falcarius* (SI clade) (Fig. 2, node E), and these two together are sister to a clade including populations of *E. falcarius* from southern Morocco and Tunisia (SM-TU clade) (Fig. 2, node F). Clades correlate with clear differences in range size between the lotic and lentic species of the western Mediterranean region. Within this area, while lotic clades were restricted to small–medium continental areas (i.e. Sicily, south Iberian Peninsula, north Morocco and south Morocco–Tunisia), the two lentic clades included specimens from different continental entities and extended across larger geographical areas (from Ireland to Algeria including Sicily for *E. bicolor*, and from France to north Morocco for *E. segmentinotatus*).

Evaluation of dispersal ability: flight wing morphometrics

The lotic *E. falcarius* IP and *E. falcarius* SI had higher wing loadings than the lentic *E. bicolor* and *E. segmentinotatus* (ANOVA species, d.f. = 3, $F = 26.9$, $P < 0.001$; Fig. 3a and

Table S3.1 in Appendix S3). No differences were found within lentic species, but lotic *E. falcarius* SI showed a slightly higher wing loading than lotic *E. falcarius* IP (see Fig. 3a).

Wing aspect ratio was also lower in the lentic *E. bicolor* and *E. segmentinotatus* (ANOVA species, d.f. = 3, $F = 10.8$, $P < 0.001$; Fig. 3b and Table S3.1) than in the two lotic *E. falcarius* clades, pointing to a broader, more rounded wing shape in lentic taxa.

Evaluation of establishment ability: thermal physiology

Overall tolerance of high temperatures was significantly higher in *E. falcarius* IP than in *E. bicolor* (ANCOVA species, d.f. = 1, $F = 48.1$, $P < 0.001$; *E. falcarius* IP UTL = 53.06 °C, *E. bicolor* UTL = 52.45 °C) (see Table S3.2). In contrast, tolerance of low temperatures did not differ between the two species (*E. falcarius* IP LTL = -11.57 °C, *E. bicolor* LTL = -11.58 °C) (see Table S3.2).

Enochrus falcarius IP showed an acclimation response for both UTL and LTL. Mean UTLs increased with both temperature and salinity of acclimation, UTLs in general being higher following 20 or 25 °C acclimation than following 15 °C (see Fig. 4b). Following acclimation at 20 or 25 °C, UTLs also tended to be higher in individuals acclimated at higher salinities (ANCOVA temperature × salinity interaction, d.f. = 8, $F = 19.2$, $P < 0.001$; Fig. 4b and Table S3.3). In contrast, in *E. falcarius* IP, mean LTLs increased with increasing acclimation temperature only (ANCOVA temperature, d.f. = 2, $F = 6.0$, $P = 0.003$; Fig. 4d and Table S3.3).

In *E. bicolor*, mean UTL was significantly affected by an interaction of temperature and salinity of acclimation (ANCOVA temperature × salinity interaction, d.f. = 8, $F = 2.5$, $P = 0.016$; Fig. 4a and Table S3.3) and also by both individual factors (see Table S3.3), but no clear acclimation response was observed. LTLs did not show any significant differences following acclimation in this species (Fig. 4c and Table S3.3).

The thermal window of *E. falcarius* IP (652.43 °C²) was greater than that of *E. bicolor* (642.82 °C²): although the two thermal windows largely overlapped, the *E. bicolor* thermal polygon lay within that of *E. falcarius* IP (see Fig. S2.1).

DISCUSSION

The differences in geographical range size and genetic structure found between lotic and lentic macro-invertebrates have been interpreted to result from selection for higher colonization capacity in lentic species, owing to the fact that small–medium standing waters are relatively short-lived on evolutionary time-scales (Ribera, 2008). Despite the robustness of these patterns, the sequence of ecological, biogeographical and evolutionary changes associated with lotic–lentic habitat shifts has never been described, and the degree to which contrasting colonization capacities of species are explained by dispersal or establishment abilities had not been tested until now.

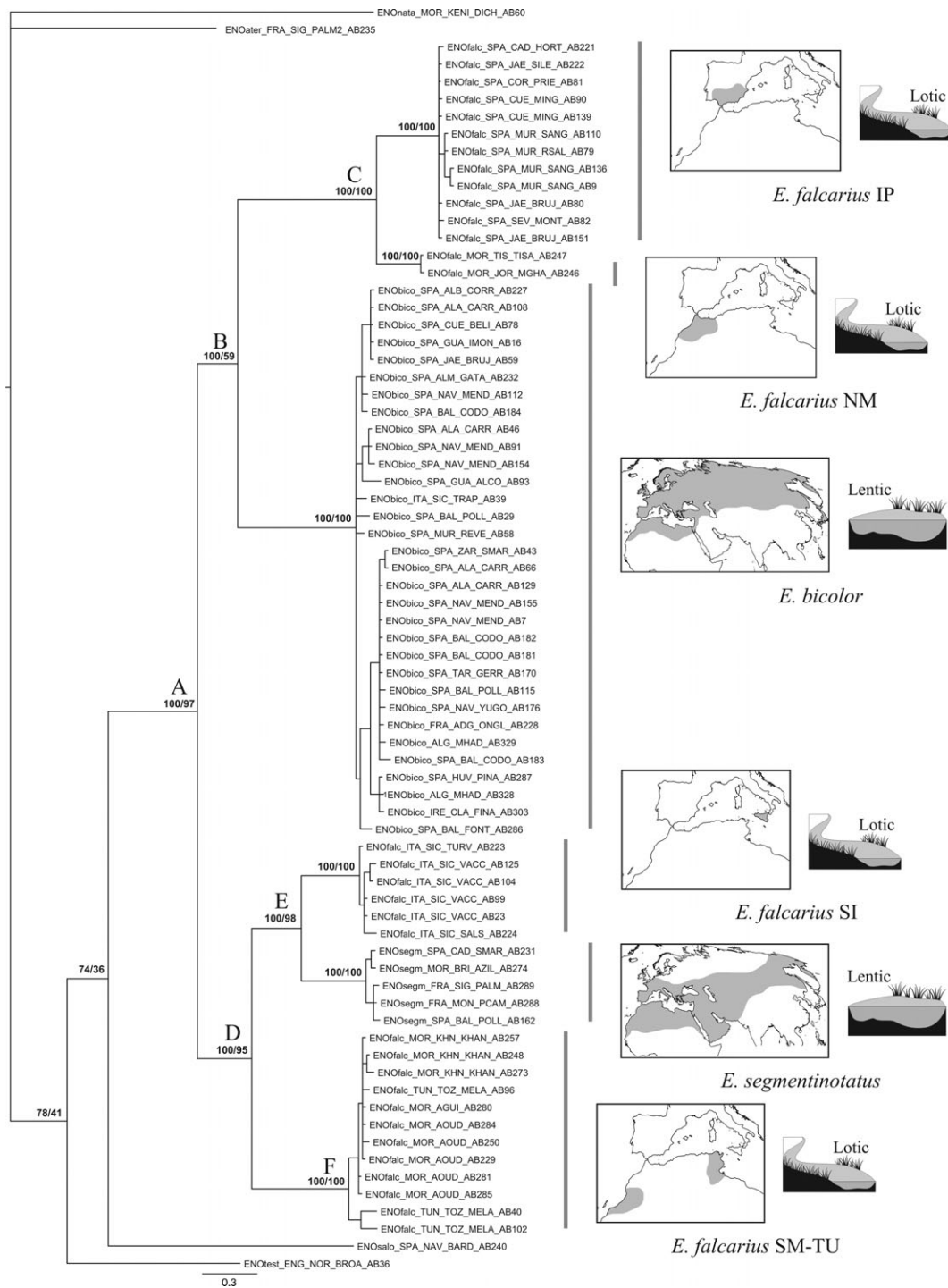


Figure 2 Phylogenetic reconstruction of the *COI*, *cyt b*, *rrnL+trnL+ND1* and *LSU* haplotypes of western Mediterranean species of the *Enochrus bicolor* complex, obtained with Bayesian analysis. Numbers above branches are Bayesian posterior probabilities ($\times 100$)/maximum likelihood bootstrap values. Only support values of main nodes are shown. Codes correspond to morphological species, voucher and localities (see Tables S1.1 and S1.2 in Appendix S1). Letters over the main nodes are included to aid reference within the text. Clade abbreviations: IP, Iberian Peninsula; NM, northern Morocco; SI, Sicily; SM–TU, southern Morocco and Tunisia.

The phylogeny of the *E. bicolor* complex points to two shifts in habitat utilization (lotic/lentic) within this clade. *Enochrus falcarius*, as currently understood, actually consists of a complex of lotic clades, which may represent morphologically

cryptic species, each with restricted, disjunct distributions across the Mediterranean area. Within this group of narrow-range lotic taxa, two more widely distributed, morphologically distinct, lentic species have evolved independently: *E. bicolor*

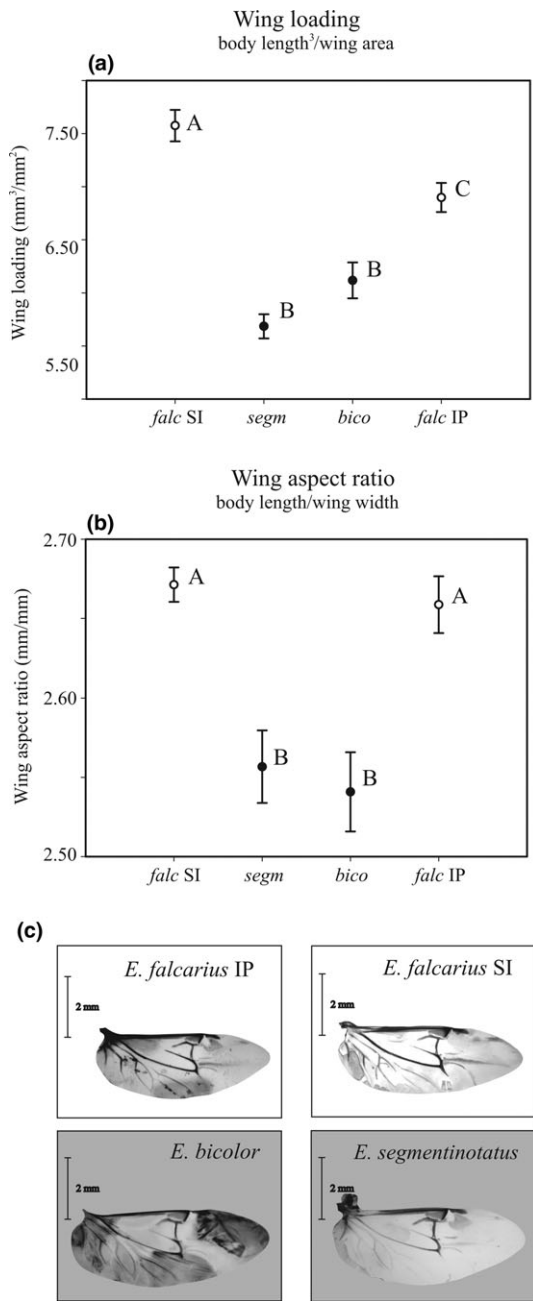


Figure 3 Wing characteristics of *Enochus bicolor* complex species: (a) wing loading, (b) wing aspect ratio and (c) representative examples of wings of four clades. Data points represent mean values \pm SE for each species (black circles, lentic species; white circles, lotic species). Between 11 (*E. segmentinotatus*) and 16 (Iberian *E. falcarius*) individuals were measured for each clade. Significantly different means ($P < 0.05$) between species are indicated by different letters (according to Bonferroni correction). Clade abbreviations: IP, Iberian Peninsula; SI, Sicily.

and *E. segmentinotatus*. The nesting of the lentic species within the paraphyletic lotic group suggests that the use of lotic habitats is ancestral within the *E. bicolor* complex, a finding that is consistent with the high morphological similarity of

lotic clades, until now considered to belong to a single species. Given such lotic ancestry in the *E. bicolor* group, it is concluded that the two transitions to a lentic habitat were accompanied by increases in colonization capacity, which could drive geographical range expansions. The habitat shift associated with the divergence of *E. bicolor* from its lotic sister taxon is likely to have occurred in the Iberian Peninsula–northern Morocco, from where this lentic species expanded its range across much of the northern Palaearctic. A similar process is evident in the eastern lotic clade (Sicily–Tunisia–southern Morocco), resulting in the appearance of the widespread *E. segmentinotatus*. While we have not been able to sample throughout the wide Palaearctic ranges of *E. bicolor* and *E. segmentinotatus*, the addition of populations from outside the western Palaearctic is unlikely to alter our general conclusions. For both widespread and restricted taxa we have sampled widely in the western Mediterranean, including almost all known populations of restricted lotic clades. No lotic taxa are known further east in the range of the complex, and even if *E. bicolor* and *E. segmentinotatus* are shown to be species complexes through further sampling in the central and eastern Palaearctic in the future, our conclusions hold for the west of the region: lentic taxa have larger geographical ranges, and are apparently derived from lotic ancestors.

It has been suggested that the higher persistence of local populations, together with their smaller geographical ranges and reduced gene flow, may favour the evolutionary specialization of lotic species (Ribera, 2008). If true, such a process could bias the probability of habitat shifts, since once lotic specialization is established the possibility of transition to lentic habitats (and relative ecological generalism) may be impaired; that is, lotic specialization acts as an evolutionary trap (Ribera, 2008). For western Mediterranean species of the *E. bicolor* complex, however, the sequence of habitat transitions that we argue is from lotic to lentic and suggests that the degree of evolutionary specialization of lotic species does not prevent sporadic colonization of lentic habitats. A similar pattern of lotic–lentic transitions is suggested by the phylogeny of an unrelated clade of water beetles inhabiting saline waters in the western Mediterranean (the *Ochthebius notabilis* group; Abellán et al., 2009), and it seems that, in saline inland waters at least, lotic habitats do not always act as evolutionary traps in the sense suggested by Ribera (2008). The extent to which this is a general pattern, in particular in systems with cold headwater streams that may favour the evolution of specialists, should be explored through future comparative studies.

Although the relatively short-lived nature of lentic habitats is believed to result in higher colonization capacities in standing-water species compared with their lotic relatives, the relative contribution of each component (i.e. dispersal and establishment abilities) of colonization capacity in individual clades has not been evaluated previously, and indeed aspects of both traits have been independently correlated with species range sizes. Dispersal ability is one of the more commonly cited potential determinants of the geographical range size of a species (Brown, 1995; Gaston, 2003), and there are many

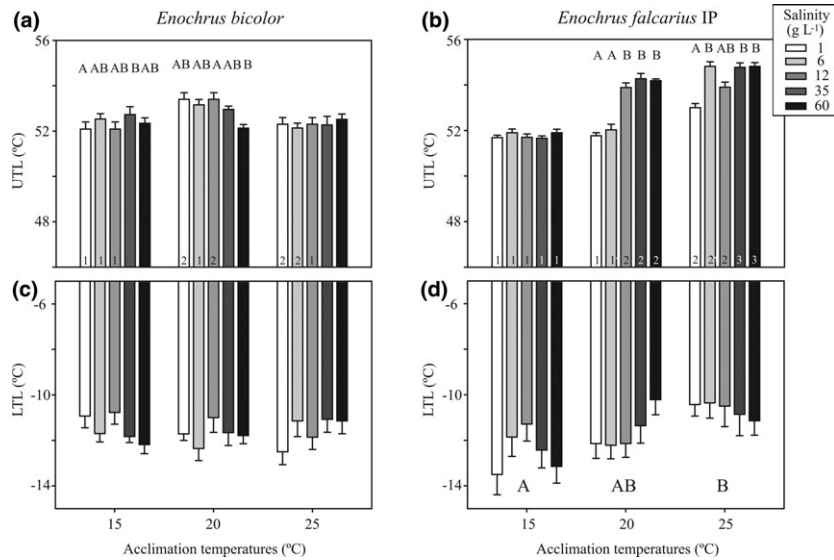


Figure 4 The effect of temperature and salinity of acclimation on the upper thermal limits (UTLs) and lower thermal limits (LTLs) of (a,c) *Enochrus bicolor* and (b,d) *E. falcarius* IP (Iberian Peninsula). Histograms represent means + SE. A total of eight individuals were used per treatment and measure. Significantly different means ($P < 0.05$) measured at different acclimation temperatures within the same salinity treatment are indicated by different numbers inside the histograms, whereas significantly different means ($P < 0.05$) measured at different acclimation salinities within the same temperature treatment are indicated by different letters below the histograms (all according to Bonferroni correction).

examples of positive relationships between dispersal ability and range size in both terrestrial and aquatic systems across a wide range of taxa (e.g. Brown, 1984, 1995; Malmqvist, 2000; Lester *et al.*, 2007; Rundle *et al.*, 2007). Similarly, establishment ability, as determined by breadth of the fundamental niche, has been proposed to be one of the main factors shaping the geographical ranges of species (Brown, 1984), and, of the many possible niche dimensions, thermal tolerance shows a strong correlation with distributional range in many lineages (e.g. Gaston & Chown, 1999; Stillman, 2002; Somero, 2005). Although the contrasting patterns in the genetic structure of lotic versus lentic species at the same geographical scales (Marten *et al.*, 2006; Abellán *et al.*, 2009) suggest that dispersal ability is the main factor driving the differences between lotic and lentic species, changes in both dispersal and establishment ability could potentially generate the observed association between habitat and geographical range size in lotic and lentic taxa. If the primary factor driving these differences was the short persistence time of lentic systems relative to the evolutionary life span of individual taxa, then it would be expected that lentic and lotic taxa would differ mostly in their dispersal abilities, whereas if differences result primarily from the greater ecological instability of lentic waters one would expect this to be reflected instead in differences in establishment ability (Ribera, 2008).

The small differences observed in temperature tolerance and acclimation ability between the two species studied here reveal a wider thermal window, and a more developed thermal plasticity, in *E. falcarius* IP, the geographically restricted lotic species. *Enochrus falcarius* IP and *E. bicolor*

do not differ significantly in their tolerance of low temperatures, but *E. falcarius* IP has a higher tolerance to heat following acclimation across a range of temperatures and salinities. In addition, *E. falcarius* IP displays some ability to acclimate its temperature tolerance in response to environmental temperature and salinity, whereas *E. bicolor*, despite being the geographically widespread lentic sister, does not show any clear (directional) acclimation responses to either temperature or salinity. Such a finding suggests that differences in geographical range size between these sister taxa are not driven by thermal physiology as, if anything, *E. falcarius* IP has the broader fundamental niche. While we cannot rule out the possibility that other organismal traits lead to differences in establishment ability, our data suggest that thermal physiology is not fundamental here. The observed relative conservatism of thermal tolerance, despite a habitat shift, is in marked contrast to recent work on wholly lotic clades of water beetles, where thermal tolerance ranges were the best predictors of geographical range sizes (Calosi *et al.*, 2008b, 2010). While data on thermal tolerance in *Enochrus* are limited to a single species pair, our finding of parallel differences in wing morphology between lentic and lotic species (see below) is consistent with differences in dispersal ability playing a major role in shaping the differences in range size associated with habitat shifts in the genus.

Wing morphometrics reveal clear, consistent differences between lotic and lentic *Enochrus* species pairs, the latter having lower wing loading and lower wing aspect ratios. Low wing loading is clearly related to superior flying ability, since flight becomes more energetically efficient when wings are

larger relative to body size (Angelo & Slansky, 1984; Berwaerts *et al.*, 2002). In *Enochrus*, the shift from lotic to lentic habitats has been twice accompanied by the evolution of relatively large wings, probably as a result of selection for increased dispersal ability in taxa occupying geologically short-lived habitats (Ribera, 2008). Interpreting the significance of wing aspect ratio for dispersal ability in these beetles is more difficult, as long, narrow wings with high aspect ratios (as in our two clades of *E. falcarius*) are generally associated with fast-flapping flight, while broad wings with low aspect ratios (as in *E. bicolor* and *E. segmentinotatus*) are associated with gliding flight (Wootton, 1992). Although high aspect ratios are often linked to greater flying ability (e.g. Gibb *et al.*, 2006), the degree to which this is the case will depend on the flight mode of individual taxa, particularly the degree to which this is active or reliant on air currents (Bilton *et al.*, 2001). Moreover, wing aspect ratios are known to influence flight performance in a temperature-dependent manner in some insects, high aspect ratios being advantageous at low temperatures in *Drosophila* (Azevedo *et al.*, 1998).

In short, we conclude that in the western Mediterranean *E. bicolor* complex there have been two habitat shifts from lotic to lentic waters, which have been accompanied by marked expansions in geographical range size. Range expansion has not, in the case of *E. bicolor* at least, been driven by an increase in fundamental niche breadth, as estimated from the species thermal physiology. Instead, in this species and in the equally widespread lentic *E. segmentinotatus*, the colonization of standing waters has been accompanied by changes in wing morphology that confer greater dispersal ability. In this lineage of water beetles, selection for dispersal in geologically short-lived lentic systems has probably driven the evolution of larger range sizes compared with those of their lotic relatives.

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SUPPORTING INFORMATION

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

Appendix S1 Sampling localities, specimens and primers used in phylogenetic analysis of the *Enochrus bicolor* complex.

Appendix S2 Thermal windows and information about sampling localities used in dispersal and physiological measurements of *Enochrus falcarius* IP and *E. bicolor*.

Appendix S3 ANCOVA results for dispersal and thermal physiology measurements of the *Enochrus bicolor* complex species.

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BIOSKETCH

Paula Arribas is an entomologist with a particular interest in the biogeography, ecology, evolution and conservation of beetles. Her research is currently focused on the fauna of saline waters in the Mediterranean region.

Author contributions: A.M., D.T.B., I.R., J.V., P.Ab. and P.Ar. conceived the ideas; A.M., J.V. and I.R. led the specimen collection; C.A., I.R. and P.Ar. collected and analysed the molecular data; C.A. and P.Ar. collected and analysed the morphometric data; D.T.B., D.S.-F., P.C. and P.Ar. collected and analysed the physiological data; all authors contributed to the writing.

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