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Physiological niche and geographical range in European diving beetles (Coleoptera: Dytiscidae)

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32 niche breadth hypothesis predicts that species with broad fundamental niches have larger range sizes [2,3],
with physiological traits which allow the exploitation of spatially and temporally variable environments
34 facilitating their expansion [4].

36 Previous work on diving beetles found that thermal physiology is strongly related to latitudinal range size and
position [8]. Since thermal tolerance relies on an organism's ability to maintain cellular function under stress,
38 more thermally tolerant species may also be expected to show higher metabolic plasticity [9]. For example, in
Antarctic fish it has been found that above their optimal temperature range, cellular processes switch from
40 mainly carbohydrate (glucose and glycogen) to lipid and finally protein metabolism [9]. Other studies on
marine gastropods into the effects of sub-lethal elevations in temperature reveal the accumulation of 'end-
42 product' metabolites (lactate and succinate) whilst ATP concentrations remain stable, indicating an increased
reliance on anaerobic metabolism with temperature, using carbohydrates as the energy source [10]. Plasticity
44 of such metabolic processes may be vital in widespread, more northerly distributed species, as organisms
encounter increasingly variable climates with latitude [6].

46
An organisms' ability to mount an immune response to parasites and pathogens can also be seen as a key
48 component of its physiological niche. Evolutionary ecology predicts that such responses are costly,
necessitating trade-offs with other nutrient-demanding metabolic processes [11]. In addition, the metabolic
50 requirements of immunity and the indirect effects of mounting induced immune responses are likely to have
life history consequences [11]. In insects, front-line defences include antimicrobial peptides (AMPs), targeting
52 mainly Gram-negative and Gram-positive bacteria but also fungi and viruses, and the phenoloxidase (PO)
pathway, which produces melanin for cuticle pigmentation, sclerotisation, wound healing and encapsulation,
54 and is an important part of an arthropod's armoury against parasite attack [11].

56 Despite the clear importance of metabolic plasticity in maintaining cellular processes under thermal stress and
the potential trade-offs with immunocompetence, no work to date has explored whether either differ between
58 widespread and restricted species. Here we compare metabolic plasticity and immunocompetence across five
closely-related species of European diving beetles (*Deronectes* Sharp; Coleoptera: Dytiscidae) which differ in

60 geographical range size and position, combining these data with thermal tolerance, dispersal ability and
61 phylogenetic history, to explore their relative importance in shaping distribution.

62

63 **Methods**

64 Post-teneral adult *Deronectes* were collected during summer and early autumn 2013-2014, minimising age
65 variation [12]. Individuals of each species were collected from as close as possible to their latitudinal range
66 central point (LRCP) to avoid possible confounding effects of local adaptation in range edge populations [13].
67 Specimens were transported to the laboratory and maintained in 5 L aquaria, containing aerated artificial pond
68 water (APW, pH 7.7, 15 °C) and submerged vegetation for 7 d [8]. Animals were kept at 20 individuals per
69 aquarium, with a 12:12 LD regime and fed chironomid larvae [8].

70

71 After acclimation, individuals used for metabolic assays were ramped to five environmentally-relevant sub-
72 lethal temperatures (15, 20, 25, 30 and 35 °C, at a rate of 0.25 °C min⁻¹) and held at temperature for 24 h,
73 before being frozen in liquid nitrogen. ATP, L-lactate, succinate, protein, glucose, glycogen and lipids were
74 measured in beetles held at each temperature (see supplementary material). Lowest values were subtracted
75 from highest values to calculate plasticities. Immunocompetence was assessed by measuring external parasite
76 load, encapsulation ability, PO activity (rates of dopachrome production and consumption), and AMP defence
77 against both Gram-positive and Gram-negative bacteria (see supplementary material). Acclimatory ability of
78 upper and lower thermal limits (Δ UTL and Δ LTL), absolute thermal tolerance range (TR), relative dispersal
79 ability (DA) and body mass (BM) were obtained from [8]. Phylogenetic relationships between taxa follow [14].

80

81 To test for phylogenetic effects, the phylogram was compared against species traits using Phylogenetic
82 Generalised Least Squares regression models (PGLS; 'caper' package). Maximum likelihood estimations of
83 Lambda, which measure the degree to which phylogeny predicts the pattern of covariance among species,
84 were not zero for all parameters indicating that phylogenetic correction was required [15]. Due to low degrees
85 of freedom affecting the number of variables included in the regression models, the principal component (PC)
86 scores from the first two PCs explaining most variation in mean immune (immPC1 and 2) and metabolic traits
87 (metPC1 and 2) were used from principal component analysis (prcomp; 'stats' package). Absolute thermal
88 tolerance range, Δ UTL, Δ LTL, DA and BM were also included as independent variables, and linear regressions

run on untransformed and phylogenetic independent contrasts (PIC; 'ape' package) to predict \log_{10}

90 transformed range extent and central position. In each analysis, models were constructed using all variable combinations and model selection was based on AIC and BIC values (AICcmodavg; 'AICcmodavg' package).

92 Permutation testing was also performed on the best model fits (Imorigin; 'ape' package). All analyses were run in R v. 3.5.

94

Results & Discussion

96 Previous studies of *Deronectes* [8,16] have pointed to the importance of physiology in shaping geographical range size and position. Studying a suite of metabolic and immunological parameters in both widespread and
98 restricted species, we show that whilst variation in latitudinal range extent and position is related to thermal physiology, metabolic plasticity and immunocompetence also appear to play a role. The best models for range
100 extent and central position contained a measure of metabolic plasticity or immunocompetence without phylogenetic correction, as did that for range extent using contrasts (Table 1), suggesting that a number of
102 immunological and metabolic markers are related to a species biogeography. For uncorrected data the best models for range extent and central position contained immPC2, metPC2 and DA or BM, respectively (Table 1;
104 Fig 1). ImmPC2 was most strongly influenced by AMP production against *Escherichia coli* (clear zone number) and metPC2 by the change in lipid concentration with temperature (Table S2, Fig S1). Model 2 (DA, Δ LTL and
106 TR) explained only 2% less variation in range extent than model 1 (Table 1). After phylogenetic correction the models explaining most variation in LRE and LRCP included immPC1 and Δ UTL, and TR and BM, respectively
108 (Table 1, Fig S1). Significant loadings for immPC1 corresponded to the presence of AMPs against *Arthrobacter globiformis* (number of clear zones - Table S2).

110

Physiological trade-offs in thermal adaptation can restrict the biogeography of aquatic ectotherms, with lipid
112 catabolism central to cold adaptation at high latitudes [9]. Our data suggest that more northerly distributed, wide-ranging species may use different energy reserves under thermal stress than their southerly endemic
114 counterparts (Fig 1). Maximum likelihood estimates suggest that more northerly-distributed, widespread species undergo more marked changes in lipid concentrations under thermal stress (Table 2). Organisms are
116 known to accumulate lipids during cold-acclimation as lipids are a more efficient energy store than others, such as glycogen [17]. However the fat body in insects, which controls the synthesis and utilisation of glycogen

118 and lipids, is not only important in energy storage but also for metabolic activity, embryogenesis, flight and
immune defence, producing AMPs [17].

120

Table 1. Model selection to estimate factors influencing latitudinal range extent and central position in

122 *Deronectes* showing the best supported models reported for each dependent variable ($\Delta AIC < 1$).

Model	K	AIC	ΔAIC	AIC weight	BIC
<i>Latitudinal range extent</i>					
1. immPC2 + metPC2 + DA	5	-21.18	0.00	0.48	-23.13
2. DA + ΔLTL + TR	5	-21.09	0.08	0.46	-23.04
3. DA + BM	4	-1.14	0.00	0.87	-2.70
4. ΔUTL	3	6.56	0.00	0.52	5.39
<i>Latitudinal range central position</i>					
5. immPC2 + metPC2 + BM	5	-79.98	0.00	0.99	-81.93
6. TR + BM	4	-34.39	0.00	0.98	-35.95
7. TR	3	-15.03	0.00	0.44	-16.20
<i>Latitudinal range extent (independent contrasts)</i>					
8. immPC1 + ΔUTL	3	-14.85	0.00	0.56	-16.69
9. BM	2	-3.88	0.00	0.36	-5.10
<i>Latitudinal range central position (independent contrasts)</i>					
10. TR + BM	3	-35.39	0.00	0.94	-37.23
11. metPC1	2	-23.47	0.00	0.54	-24.70

In each case absolute thermal tolerance range (TR), acclimatory ability of upper and lower thermal tolerance (ΔUTL & ΔLTL), body mass (BM), dispersal ability (DA), immPC1, immPC2, metPC1 and metPC2 were included as independent variables (for principal component loadings see Table S2). Latitudinal range position is the latitude of the range centre. K = number of parameters; AIC = Akaike's Information Criteria set at 95% confidence; $\Delta AIC < 2$ reported; AIC weight represents the likelihood of the model given the data; BIC = Bayesian Information Criteria, which unlike AIC takes into account n and K. Best fitting models highlighted in bold.

126

Figure 1. Biplots for the most significant variables predicting latitudinal range extent and position. Codes:

128 *Deronectes bicostatus* (BIC), *D. depressicollis* (DEP), *D. hispanicus* (HIS), *D. moestus* (MOE) and *D. latus* (LAT).

130 In terms of immunity, maximum likelihood estimates suggest that more southerly, range-restricted species
have stronger antibacterial activity against Gram-positive and Gram-negative bacteria than their more wide-
132 ranging counterparts (Table 2; Fig 1; Fig S1). Lower-latitude freshwaters may support a greater number of
bacterial taxa [18], meaning that southerly, range-restricted species may require more, diverse AMPs.

134 Alternately it could be that high latitude species exhibit seasonal immunocompetence, with lower immunity in
summer and higher in winter than their southerly range-restricted counterparts [19], or that faster growth in

136 higher latitude species compromises adult immunocompetence [20]. The higher AMP levels in range-restricted
southerly species may trade-off against other immune functions, such as melanin production (measured as PO
138 activity), which increased with latitude [21].

140 Table 2. Summary statistics for the best models predicting \log_{10} -transformed latitudinal range extent and
central position on untransformed data and phylogenetic independent contrasts including Maximum
142 Likelihood Estimates (MLE) for untransformed data. Model numbers refer to those in Table 1.

Source	MLE	SE	<i>t</i> -value	<i>p</i> -value	DF	<i>F</i> -statistic	Adjusted R^2	<i>p</i> -value
<i>Latitudinal range extent (model 1)</i>								
immPC2	-1.020	0.028	-36.57	0.017	4	599.9	0.998	0.030
metPC2	-1.000	0.027	-36.40	0.018				
DA	14.452	0.514	28.12	0.023				
<i>Latitudinal range central position (model 5)</i>								
immPC2	-0.034	<0.001	-1244.8	<0.001	4	1065000	0.999	<0.001
metPC2	-0.055	<0.001	-1720.2	<0.001				
BM	0.006	<0.001	288.3	<0.001				
Source	Coefficient		<i>p</i> -value	DF	<i>F</i> -statistic	Adjusted R^2	<i>p</i> -value	
<i>Latitudinal range extent (independent contrasts) (model 8)</i>								
immPC1	0.067		0.029	4	78.4	0.975	0.020	
Δ UTL	0.401		0.010					
<i>Latitudinal range central position (independent contrasts) (model 10)</i>								
TR	0.029		0.004	4	122.6	0.984	0.023	
BM	0.024		0.023					

144 Despite the inclusion of additional variables representing metabolic plasticity and immunocompetence,
thermal physiology was still clearly related to biogeography in our analyses (Table 1). Indeed the second best
146 model predicting range extent on untransformed data and the best model for range extent and central
position using contrasts both include measures of thermal tolerance, supporting the findings of [8]. Our study
148 demonstrates, however, that in addition to temperature, other aspects of an organism's physiological niche
may be related to its geographical range, including the plasticity of metabolic pathways and antimicrobial
150 defences. Although widespread species have broader thermal tolerances to cope with more variable climates,
they also appear to have different energetic strategies and immune trade-offs to endemic species.

152

As species distributions respond to climate change, it is vital we understand the mechanisms shaping
154 geographical ranges. It is suggested that further studies of the parameters measured here, on a range of
arthropod groups, would improve understanding of the drivers of relative range size and position. In all cases,
156 investigations should be conducted within a phylogenetically controlled framework and in a manner which
controls for other possible drivers, such as relative dispersal ability. Future work should also incorporate these
158 traits into predictive models, to aid the development of more accurate adaptation and mitigation strategies for
conservation.

160

Data accessibility

162 The datasets supporting this article have been uploaded in supplementary material.

Competing interests

We have no competing interests.

166

Ethical approval

168 Ethical approval was not required.

Authors' contributions

DTB, AJM and RAB conceived the study, RC, DTB, AJM and RAB designed it, RC conducted laboratory assays,
172 analyses and drafted the manuscript, AM and DTB co-ordinated fieldwork; all authors contributed to and
approved the final draft, and agree to be held accountable for the content therein

174

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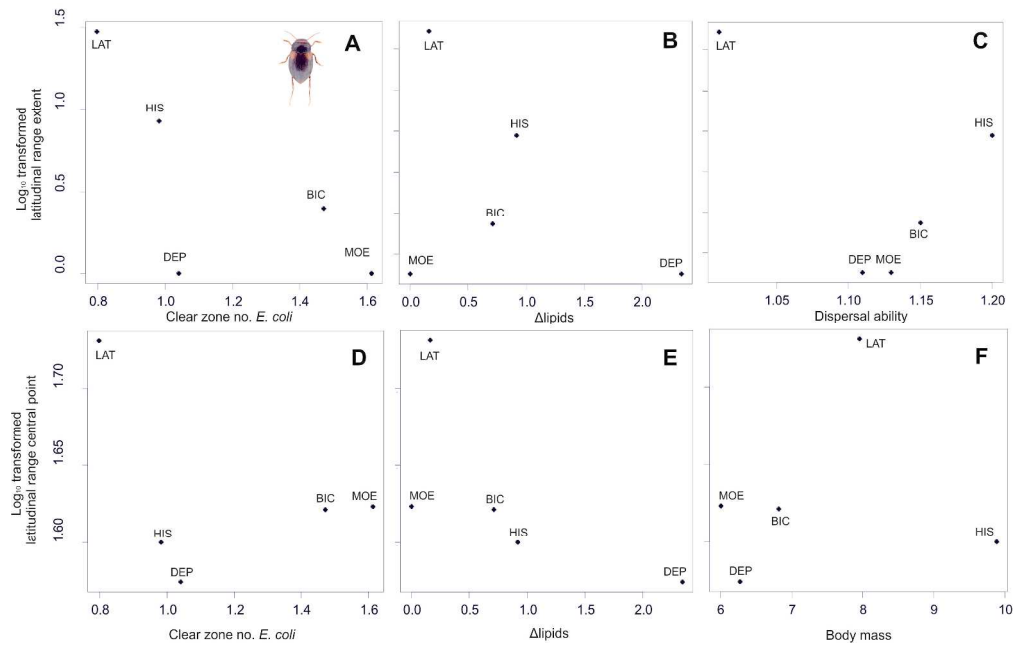
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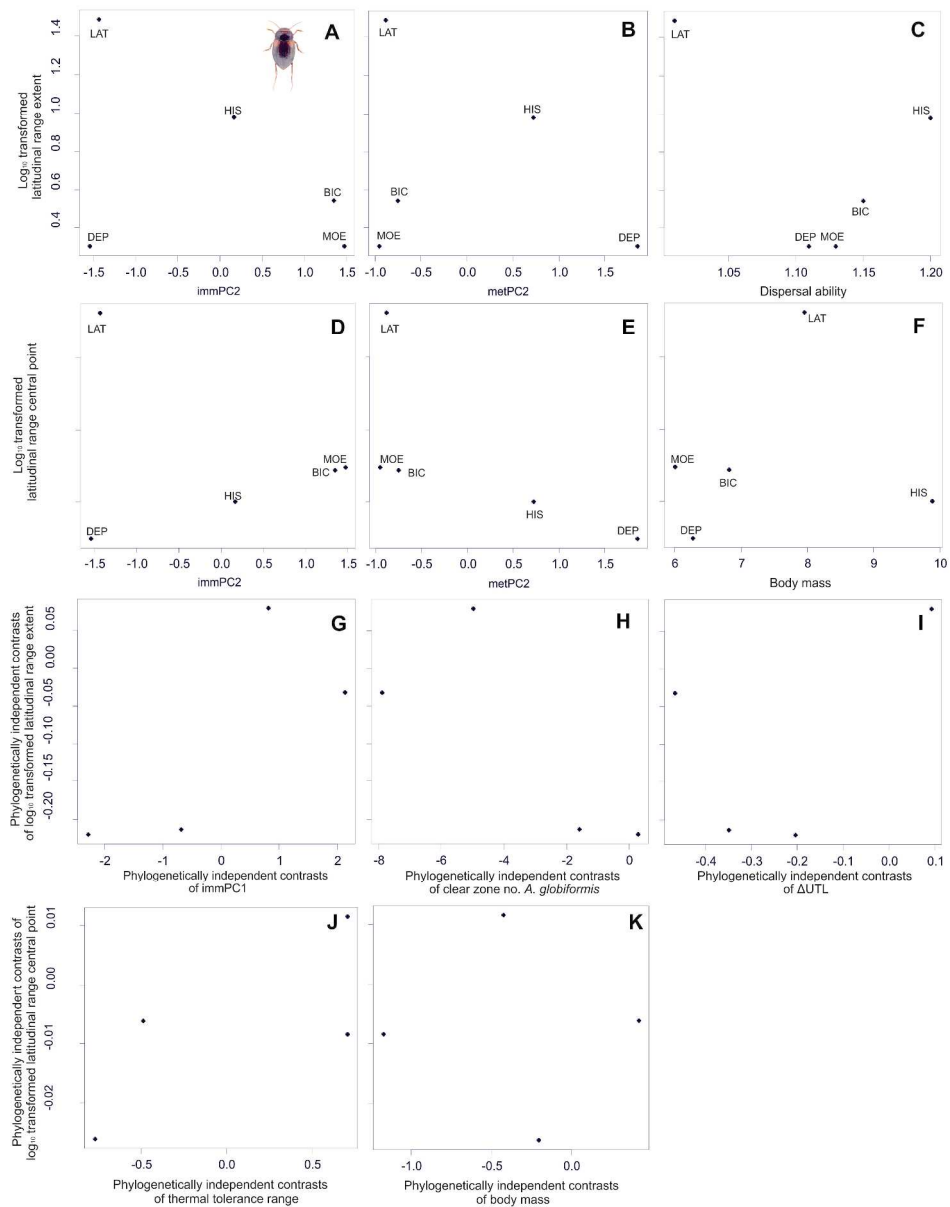
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