1 Diet diversification shapes broad-scale distribution

2 patterns in European bats

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6

7 Abstract

8 Large-scale species' distributions have been traditionally attributed to physiological

9 traits related to abiotic factors, while behavioural features linked to biotic interactions

10 have received little attention. We tested the relationship between trophic and spatial

11 niche breadths through combining species distribution modelling with dietary DNA

12 metabarcoding of over 400 bats sampled across Europe belonging to seven species. Our

13 results point to a causality cascade between hunting plasticity, trophic niche breadth and

14 spatial niche breadth, and thus indicate that behavioral plasticity and dietary

15 diversification can contribute to shaping broad-scale species distributions.

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Introduction

29 The characterisation and comparison of trophic niches have been core topics in animal ecology

30 since Hutchinson's conceptualisation of the ecological niche (1). While traditionally considered

31 important only for local-scale species interactions (2), dietary features have also proven relevant

32 for broader scale species distributions (3). Nevertheless, the link between species' trophic

33 niches and large-scale distribution patterns is still inconclusive (4). This could be because diet

34 analyses have traditionally relied on particular diversity metrics that overlook some components

35 of dietary diversity, and because until recently, methodological constraints have limited the

36 possibility of performing broad-scale high-resolution diet studies (5).

37

38 Following the advent of high throuput DNA sequencing-based tools, it is now possible to

39 characterise dietary niches across much larger sample sizes, and at levels of detail never seen

40 before (6). DNA-based diversity assessment also enables comprehensive analysis of trophic

41 variation through considering different components of dietary diversity, such as richness (how

42 many prey are consumed), evenness (the balance of the relative consumption of each prey) and

43 regularity (the degree of similarity across consumed prey) (7,8). Richness, evenness and

44 regularity metrics are positively associated with performance in several ecological systems (9).

45 Hence, we hypothesised that these metrics applied to trophic niches also impact the capacity of

46 animals to thrive in a wider range of environmental conditions, and hence trophic niche breadth

47 could potentially contribute to the shaping of species' distributions.

48

49 To test this, we contrasted broad-scale dietary and spatial niches of a vertebrate system,

50 namely the European bat community. Bats provide an excellent opportunity for understanding

51 dietary and spatial diversity patterns due to their spatial variability and well-studied behavioural

52 traits (10). We collected faecal samples from over 400 bats representing seven species

2

53 captured at 40 locations scattered across the European continent. Faeces of each individual bat

54 were independently analysed through DNA metabarcoding and high throughput sequencing by

55 using two complementary primer sets and three replicates per primer. We used the statistical

56 framework recently developed around Hill numbers (11) to contrast trophic niche measures

57 based on richness (dR), richness+evenness (dRE) and richness+evenness+regularity (dRER).

58 The different species-level trophic niche measures were then statistically related to spatial niche

59 breadth metrics as measured by species distribution modelling, as well as to a range of

60 behavioural traits to assess the causal directionality between dietary diversification and spatial

61 niche expansion.

62 # Results

63 ***The trophic niche of European bats is dominated by Lepidoptera and Diptera***

64 After applying all quality filters, the dataset included dietary information of 355 individual bats

65 belonging to seven species (DNA sequencing details in Table S4). Using two primer sets, we

66 detected over 3000 different prey taxa belonging to 29 arthropod orders (Fig. 1A), though the

67 pandiet of European bats was dominated by Lepidoptera and Diptera (Fig. 1B). Our results

68 complement the existing broad-scale molecular dietary data of *Miniopterus schreibersii* (12),

69 and provide the first geographically widespread molecular insights into the dietary ecology of

70 *Myotis daubentonii, M. myotis*, *M. emarginatus*, *M. capaccinii*, *Rhinolophus euryale* and *R.*

71 *ferrumequinum*, which had only been studied at local scales previously (13–15).

72

73 ***Trophic niche differences depend on the components of diversity accounted for***

74 Trophic niche breadth measures (Fig. 1C), and the species ranks derived from them (Fig. 1D),

75 were different depending on the components of diversity considered. Similar contrasting results

76 have also been reported in other systems (16), due to the fact that each diversity component

77   might be driven by different ecological forces (17). For instance, the trophic niches of *M.*

78   *schreibersii* and *R. euryale* showed similar dRE values, yet the contribution of richness and

79   relative evenness components differed. The dietary richness of *M. schreibersii* was almost 40%

80   larger than *R. euryale*'s, while the evenness factor of *R. euryale* was almost 30% higher than

81   that of *M. schreibersii.* These differences could be explained by i) the larger home range of *M.*

82   *schreibersii* compared to *R. euryale* (18,19), which might expose the former to more prey

83   species —thus increasing dietary richness, and ii) the higher incidence of a few locally abundant

84   pest moth species in the diet of *M. schreibersii* than in that of *R. euryale* (12,14) —yielding lower

85   relative evenness*. As reported for other systems (20), trophic niche differences would be

86   overlooked if the niche breadth analyses were limited to a single diversity metric.
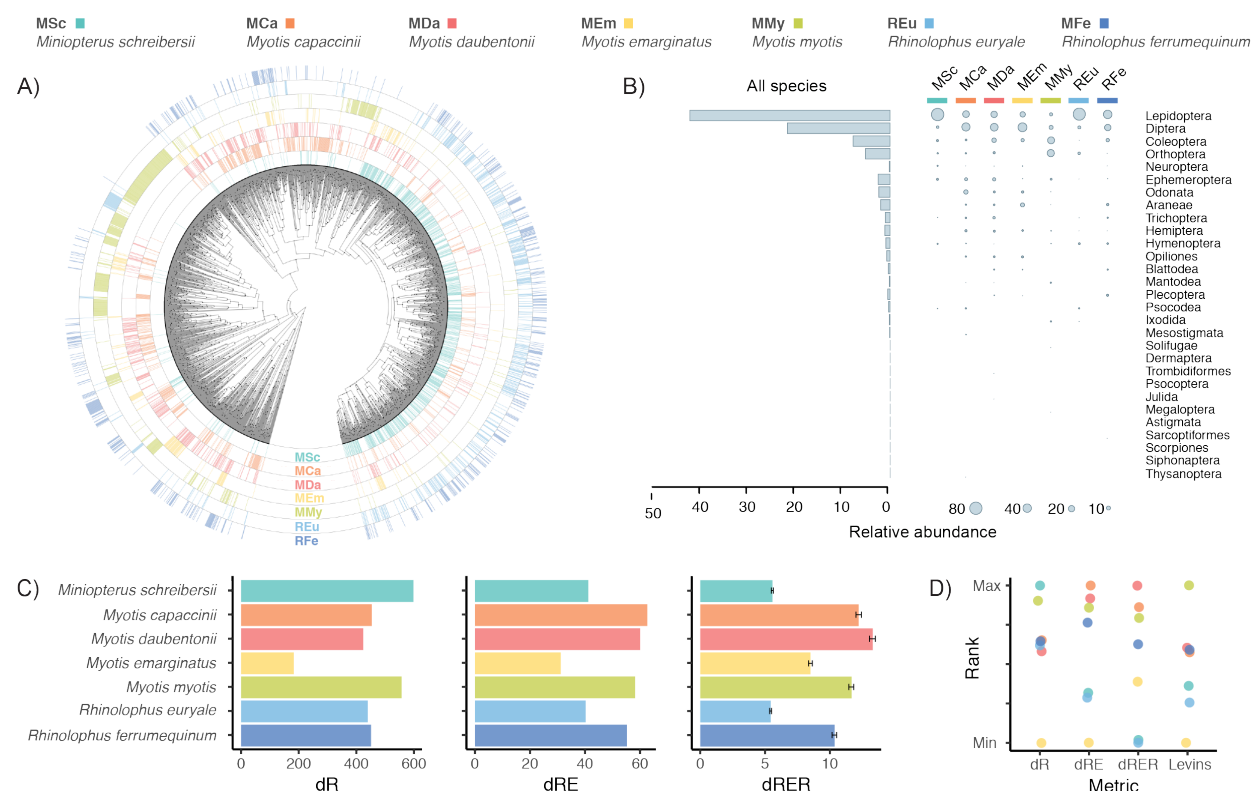
87

89   Figure 1. **Dietary diversity statistics of the analysed bat species**. (A) Radial phylogenetic tree of prey

90   detected using the Zeale primers and their occurrence patterns in each of the studied bats. A higher

91   resolution image (Fig. S1) and the homologous figure built from the Epp data (Fig. S2) are available in the

4

92   Supplementary Information. (B) Overall and predator species-specific representation of the arthropod

93   taxonomic orders. (C) Dietary niche breadth measures accounting for richness (dR), richness+evenness

94   (dRE) and richness+evenness+regularity (dRER). The error bars (±SE) of dRER indicate the dispersion

95   of the trophic niche breadths yielded when using different prey phylogenetic trees (N=50) sampled from

96   the Bayesian MCMC. (D) Species ranks according to the dietary niche breadth measure type. Levin's

97   index is also included for being the most common metric employed in the literature.

98

99   ***Trophic niche breadth explains spatial niche breadth***

100   The distribution models generated (Table S5) to test whether trophic niche breadth correlates

101   with spatial niche breadth yielded different spatial projections (Fig. S3) and niche breadth

102   measures for each species. We found that the trophic niche breadth measures accounting for all

103   diversity components (dRER) were positively correlated with the two spatial niche breadth

104   metrics computed, both for each primer-specific dataset (Table S8, Fig. S4) and the overall

105   averaged dataset (Levins' B1: Pearson's r = 0.85; t = 29.57, df = 348, p-value < 0.001; Levins'

106   B2 (Fig. 2A): Pearson's r = 0.80; t = 24.58, df = 348, p-value < 0.001). The species that

107   consume a wider variety of distinct prey are the ones that exhibit broader spatial niches.

108

109   We performed a range of analyses to further assess the plausibility of the causal relationship

110   between dietary diversification and spatial niche breadth. We first assessed whether dietary

111   breadths could be broadened passively as a result of spatial niche expansion (21). If the ability

112   to thrive in more distinct environments was driving dietary expansion, we would expect species

113   dietary breadth to be driven by beta diversity, i.e. dietary differences across individuals within

114   species. However, we observed that the correlation between trophic and spatial niche breadth

115   remained significant (Pearson's r = 0.20, t = 3.78, df = 353, p-value < 0.001; Fig. 2B), which

116   highlights the relevance of alpha (individual) dietary diversity. Additionally, if dietary breadths

117   were passively broadened due to spatial niche expansion, we would expect species dietary

118   richness to also increase, as predators are exposed to a larger variety of potential prey.

119   Nevertheless, we observed that the correlation between trophic and spatial niche breadths

120   disappeared when relative evenness and regularity components were removed (Levins' B1:

121   Pearson's r = -0.09; t = -0.20, df = 5, p-value = 0.846; Fig. 2C). Consequently, these two

122   observations rule out the possibility that dietary breadths are passively broadened as a result of

123   spatial niche expansion.

124

125   We then investigated the relation between different behavioural traits (hunting plasticity, habitat

126   use diversity and roosting plasticity) and spatial niche breadths to assess whether a third factor

127   could be shaping both dietary and spatial niches. We found no significant correlation between

128   any of the analysed traits and spatial niche breadth (Table S9), yet we found that hunting

129   plasticity is positively related with the trophic niche breadth of predator species (Pearson's r =

130   0.79, t = 24.347, df = 348, p-value < 0.001; Fig. 2D). This suggests that the ability to use a more

131   diverse range of hunting strategies, such as capturing prey from the ground, foliage or water

132   surface, in addition to hunting flying prey (10), broadens the functional spectrum of captured
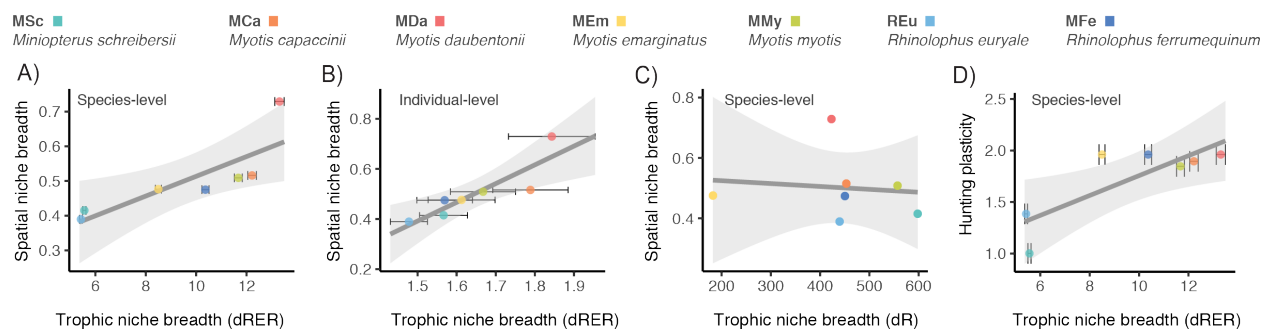
133   prey, which in turn widens the spatial niche.

134

135

136   Figure 2. **Relation of species trophic niche breadth measures with spatial niche breadth and**

137   **hunting plasticity**. (A) Species-level relation between niche breadth measures accounting for the three

138   components of diversity (dRER: richness+evenness+regularity) and spatial niche breadths of the

139   respective species. (B) Individual-level relation between dRER niche breadth measures and spatial niche

140   breadths of the respective species. (C) Species-level relation between niche breadth measures

141   accounting for only one component of diversity (dR: richness) and spatial niche breadths of the respective

142   species. (D) Species-level relation between dRER niche breadth measures and hunting plasticity of the

143   respective species. Dots indicate mean values per bat species. Note that error bars (±SE) at the species-

144   level charts indicate the dispersion of the different trophic niche breadth values yielded from the 50

145   iterations run with different prey phylogenetic trees to account for phylogenetic uncertainty. In contrast,

146   the error bars at the individual-level chart indicates the dispersion of the individual bats' trophic niche

147   breadth values. Chart C does not contain error bars as for dR a single niche breadth measure was

148   computed for each species.

Discussion

150   This is the first time that high-resolution trophic (DNA metabarcoding) and spatial (Species

151   Distribution Modelling) niche characterisation identify diet as a driving factor of broad-scale

152   spatial patterns. While ecological niche breadth has been previously shown to predict

153   geographical range sizes, trophic niche breadth has so far only been associated with broad-

154   scale spatial patterns in arthropods (4). The causality cascade between hunting plasticity,

155   dietary niche breadth and spatial niche breadth seems plausible and is ecologically meaningful.

156   Hunting plasticity has previously been linked to adaptability (22), and could directly affect the

157   fitness of bats, for instance by enabling shifting diets when specific prey types become scarce.

158   Trophic niche breadth could also indirectly affect the fitness of bats by, for example, fostering

159   gut microbiome diversification and dynamism, which have been associated with adaptation

160   capacity in vertebrates (23).

161

162   Our results contradict the Eltonian noise hypothesis, which proposes that biotic interactions do

163   not affect species distributions at large geographical scales (2). However, in this case spatial

164   patterns would not primarily depend on the availability of resources as previously shown (3), but

165 on the inherent behavioural properties of predators. It is noteworthy that all the patterns we

166 found in this study were recovered from trophic niche measures that considered all three

167 components of diversity. No diversity component alone was correlated with either hunting

168 plasticity or spatial niche breadth, which highlights the importance of accounting for relative

169 evenness and regularity of prey when measuring trophic niches. It is also remarkable that a

170 single snapshot of the diet of individual bats was enough to recover a clear link between trophic

171 and spatial niche breadth, as niche patterns are not always coupled at individual and population

172 levels (24). Overall, our study demonstrates the potential of combining environmental DNA with

173 species distribution modelling and behavioural ecology, to unveil broad-scale ecological

174 patterns and links between different components of the ecological niche of species. Finally, our

175 results also highlight the relevance of diet in shaping broad-scale animal distributions, which

176 supports the use of behavioural plasticity as a relevant feature to predict species' range shifts in

177 response to climate change (25).

178 # Methods

179 ***Data collection and generation***

180 We collected droppings from 402 individual bats captured in 40 locations distributed across

181 Europe (Table S1), in June-October of 2015-2017. The droppings belonged to seven species:

182 *Miniopterus schreibersii* (MSc), *Myotis capaccinii* (MCa), *Myotis daubentonii* (MDa), *Myotis*

183 *emarginatus* (MEm), *Myotis myotis* (MMy), *Rhinolophus euryale* (REu) and *Rhinolophus*

184 *ferrumequinum* (RFe). Using a randomised setup, DNA was extracted from all individual

185 samples and amplified in three replicates using two primer pairs, referred to as Zeale (26) and

186 Epp (27). Amplicons were purified, pooled and built into libraries before Illumina MiSeq

187 sequencing. To ensure maximum DNA sequence reliability, only high quality sequences that

188 appeared in at least two of the three PCR replicates were retained, and sequences identical to

8

189   those detected in the extraction and library blanks of the corresponding processing batch of

190   each sample were removed. Appropriate sampling depth per sample was ensured by discarding

191   samples with insufficient sequencing depth as assessed by rarefaction curves and curvature

192   indexes. DNA sequences were clustered into operational taxonomic units (OTUs) based on

193   98% identity following Alberdi et al. (28) and taxonomy was assigned by aligning the OTU

194   representative sequences to the Genbank nt (29) —and in the case of Zeale also BOLD (30)—

195   databases. Full details of the field, laboratory and bioinformatics methodologies are reported in

196   the Supplementary Information and Supplementary Code 1.

197

198   ***Data analysis***

199   Diversity analyses were carried out using the R package hilldiv (31) based on abundance-based

200   Hill numbers (32,33). The Hill numbers framework enables i) the relative weight given to

201   abundant and rare OTUs to be modulated through a single parameter, namely the order of

202   diversity q (32), and ii) the similarity level across OTUs to be overlooked or accounted for when

203   computing diversity. Although functional diversities can be computed using Hill numbers (34),

204   given the infeasibility of gathering ecological trait information of thousands of prey items, OTU

205   phylogenies were employed as proxies of ecological resemblance across OTU. Hence, dR

206   (richness) was computed as the neutral Hill number of order of diversity q=0; dRE

207   (richness+evenness) was computed as the neutral Hill number of order of diversity q=1 —i.e.

208   Shannon diversity— and dRER (richness+evenness+regularity) was computed as the

209   phylogenetic Hill number of order of diversity q=1. Phylogenetic Hill numbers were computed

210   based on Bayesian phylogenies generated from metabarcoding DNA sequences, and the

211   analyses accounted for the phylogenetic uncertainty of generated trees, as detailed in

212   Supplementary Information. Sample size appropriateness was assessed by comparing

213   observed vs. estimated trophic niche breadth values (Table S14).

214

215   Predator species' hunting strategy, habitat-use and roosting data were gathered from 45 articles

216   available in the literature (Tables S12-14). Plasticity indices were computed by means of

217   Shannon diversity of ecological traits. The species distributions models that characterised the

218   spatial niche of predator species were generated using BIOMOD (35), and niche breadths were

219   measured by means of Levins' B1 and B2 metrics (36) based on the spatial projections using

220   the R version ENMTools (37). For all statistical tests, significance threshold was set at p=0.05.

221   All statistical analyses were performed in R (38) after averaging the results yielded by both

222   primers unless otherwise stated.

Data availability

224   The datasets generated during and/or analysed during the current study are available in the

225   Dryad repository (ref. [to be included in the reference list when a DOI is available]).

Code availability

227   The bash, python and R scripts used for analysing the data during the current study are

228   available in the Supplementary Files as Supplementary Code 1 (DNA metabarcoding),

229   Supplementary Code 2 (Species Distribution Modelling) and Supplementary Code 3 (ecological

230   niche statistical analyses).

Author contributions

232   A.A., O.A. and M.T.P.G designed the study. All authors participated in the sample and data

233   collection. A.A. and O.A. performed the laboratory procedures. O.R. and R.N.F. carried out the

234   species distribution modelling. O.A. performed the ecological trait analyses. A.A. performed the

10

235   DNA metabarcoding and statistical analyses. A.A. wrote the manuscript. All authors contributed

236   to and approved the final version.

Competing interests

238   The authors declare no competing interests.

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