Discordant patterns of genetic and phenotypic differentiation in five grasshopper species co-distributed across a microreserve network

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Running title: Comparative genetic and phenotypic structure

Abstract

Conservation plans can be greatly improved when information on the evolutionary and demographic consequences of habitat fragmentation is available for several co-distributed species. Here, we study spatial patterns of phenotypic and genetic variation among five grasshopper species that are co-distributed across a network of microreserves but show remarkable differences in dispersal-related morphology (body size and wing length), degree of habitat specialization and extent of fragmentation of their respective habitats in the study region. In particular, we tested the hypothesis that species with preferences for highly fragmented microhabitats show stronger genetic and phenotypic structure than co-distributed generalist taxa inhabiting a continuous matrix of suitable habitat. We also hypothesized a higher resemblance of spatial patterns of genetic and phenotypic variability among species that have experienced a higher degree of habitat fragmentation due to their more similar responses to the parallel large-scale destruction of their natural habitats. In partial agreement with our first hypothesis, we found that genetic structure, but not phenotypic differentiation, was higher in species linked to highly fragmented habitats. We did not find support for congruent patterns of phenotypic and genetic variability among any studied species, indicating that they show idiosyncratic evolutionary trajectories and distinctive demographic responses to habitat fragmentation across a common landscape. This suggests that conservation practices in networks of protected areas require detailed ecological and evolutionary information on target species in order to focus management efforts on those taxa that are more sensitive to the effects of habitat fragmentation.

Keywords: Phenotypic divergence, population fragmentation, population genetics, specialist species, generalist species, genetic diversity, genetic structure.

Introduction

Habitat destruction and fragmentation are major threats to global biodiversity (Noss & Csuti 1994; Lindenmayer & Fischer 2006). Extensive clearing of natural vegetation for agriculture and large-scale farming have dramatically modified landscapes over centuries (Blondel & Aronson 1999; Fahrig 2002). As a result of this process, many species have become extinct and others persist in highly fragmented or isolated habitat patches. These remnant populations often sustain small effective population sizes, which can increase vulnerability to demographic stochasticity and reduce genetic diversity and evolutionary potential to respond to environmental changes and diseases (Saunders *et al*. 1991; Willi *et al*. 2006). In the long-term, these processes can compromise population viability and lead to local extinctions, particularly when dispersal from other population sources is absent or limited (Saccheri *et al*. 1998; Spielman *et al*. 2004; Frankham 2005). For these reasons, understanding the ability of organisms to respond to habitat fragmentation and disperse among populations is a major concern for conservation biologists (Saunders *et al*. 1991). These fragmented populations also constitute an ideal "natural" laboratory to study the evolutionary consequences of population isolation, analyse spatial variation in selective regimes, and disentangle the relative role of gene flow and local evolutionary pressures on spatial patterns of adaptation (Richardson *et al*. 2014; e.g. Bonal *et al*. 2012; Pickup *et al*. 2012; Willi & Hoffman 2012; Phillipsen & Lytle 2013; Zhao *et al*. 2013).

Molecular markers able to resolve patterns of genetic variability at fine spatial and temporal scales, integrated with novel analytical approaches, have proven to be a powerful tool to infer species responses to habitat fragmentation, particularly in organisms for which dispersal movements are difficult to track for different technical

reasons (Lange *et al*. 2010; Quéméré *et al*. 2010). Most studies evaluating the effects of habitat fragmentation are focused on a single species, an approach that can certainly provide key information to guide management practices for the target species (e.g. Wang *et al*. 2009). However, reserve networks are generally intended to protect several organisms that are likely to be affected by habitat fragmentation in diverse and complex ways (Lange *et al*. 2010; Callens *et al*. 2011). For this reason, data on population genetic diversity and structure across multiple co-distributed species can inform whether at least some of them can be managed jointly or which one/s are more vulnerable to habitat fragmentation and require particular attention (Di Leo *et al*. 2010; Callens *et al*. 2011). Conservation plans can be greatly improved when information on the consequences of habitat fragmentation is available for several species, but so far only a relatively small number of studies on population and landscape genetics have employed a multi-species comparative approach (e.g. Lange *et al*. 2010; DiLeo *et al*. 2010; Callens *et al*. 2011; Aparicio *et al*. 2012; Habel *et al*. 2013; Phillipsen *et al*. 2015).

Combined with genetic information, data on phenotypic variation can help to infer patterns of local adaptation to divergent natural selection regimes (Merilä & Crnokrak 2001; McKay & Latta 2002; e.g. Leinonen *et al*. 2006; Oneal & Knowles 2013; García-Navas *et al*. 2014). Empirical and theoretical work suggests that local adaptation can evolve when the effect of selection is sufficiently strong to counter the homogenizing effect of gene flow, a phenomenon that can potentially occur at any spatiotemporal scale depending on the relative strength of both processes (Richardson *et al*. 2014). For these reasons, the opportunity for evolutionary change and local adaptation is likely to be higher in organisms with limited dispersal capacity and increased population fragmentation (Willi *et al*. 2007; Willi and Hoffman 2012). The study of phenotypic variation and local adaptation also has important implications from a conservation standpoint and can help to guide conservation agendas aimed to preserve

101 not only species but also the idiosyncratic evolutionary trajectories of their different
102 populations (Fraser & Bernatchez 2001; Moritz 2002). The study of patterns of
103 phenotypic divergence in species assemblages may reveal either the signature of
104 convergent evolutionary responses to shared environment (e.g. to predators or
105 microclimate) or reveal evidence of divergent sources of selection, which can inform on
106 whether co-occurring taxa are affected by similar evolutionary pressures (e.g. Ingley *et*
107 *al*. 2014) or if these are different or largely decoupled (e.g. Lowe *et al*. 2012).
108 Comparing phenotypic divergence across multiple species can also help to understand
109 whether the evolution of local adaptations is more frequent in taxa experiencing a
110 higher degree of habitat fragmentation than in those inhabiting more continuous habitats
111 and expected to be less prone to population subdivision. This has important implications
112 for the management of focal species of conservation concern: strong phenotypic
113 divergence indicative of local adaptation processes would call for actions aimed to
114 preserve the evolutionary particularities of individual populations, whereas management
115 practices intended to promote dispersal and population connectivity would be advisable
116 in the absence of local adaptation (Ouborg *et al*. 2010 and references therein). However,
117 with the exception of a study comparing phenotypic divergence between two species of
118 co-distributed salamanders (Lowe *et al*. 2012), no study has yet integrated phenotypic
119 and genotypic data across multiple co-occurring species to understand the evolutionary
120 consequences of habitat fragmentation and its implications for guiding conservation
121 actions.
122 Orthoptera have been often found to be highly sensitive to landscape alterations
123 in terms of genetic diversity and structure (Keller *et al*. 2013a; Gauffre *et al*. 2015;
124 Ortego *et al*. 2015), phenotypic variation (Heidinger *et al*. 2010; Gomez & Van Dyck
125 2012) and extinction risk (Reinhardt *et al*. 2005). Some studies have also shown that
126 certain species are more susceptible than others to suffer the negative effects of habitat

127 fragmentation (Reinhardt *et al*. 2005; Lange *et al*. 2010; Keller *et al*. 2013b), which
128 suggests that ecological assemblages of orthopterans are a good model system to study
129 the impacts of human-driven habitat alterations across multiple species with contrasting
130 life-history traits (Lange *et al*. 2010). In this study, we set out to analyse whether the
131 extent and spatial patterns of phenotypic and neutral genetic diversity and structure
132 differ among species that inhabit a common landscape but show contrasting life-
133 histories, particularly in terms of preferences for habitats that have experienced a
134 different degree of fragmentation (Fig. 1). To address this question, we used as a study
135 system an assemblage of five grasshopper species co-distributed across a singular
136 microreserve network located in Central Spain (Fig. 1-2). The study sites have been
137 protected in recent years due to their unique plant and animal communities associated
138 with their characteristic saline/hypersaline lagoons and lowlands (Cirujano-Bracamonte
139 & Medina-Domingo 2002; Cordero *et al*. 2007; Cordero & Llorente 2008). Although
140 the patchy distribution of these inland saline environments is mostly the result of natural
141 and historical processes, land clearing for agriculture has strongly contributed to their
142 increased fragmentation and the destruction of many other natural habitats of the region
143 such as esparto grass formations (Ortego *et al*. 2012a, 2015). The five focal study
144 species have important differences in dispersal-related morphology (body size and wing
145 length; e.g. Reinhardt *et al*. 2005; Heidinger *et al*. 2010; Butler 2012; Gomez & Van
146 Dyck 2012; Levy & Nufio 2015), degree of habitat specialization, and extent of
147 fragmentation of their respective habitats in the study region, factors that we expect to
148 have a significant impact on their patterns of genetic and phenotypic variability and
149 structure (Fig. 1a; see Methods for a detailed description of the study species). Even
150 though all of the studied taxa show some differences in at least one of the above
151 mentioned traits, they can be broadly classified into two main groups: small-medium
152 species with preferences for microhabitats that have experienced a considerable degree

153 of fragmentation and medium-large generalist species occupying both natural habitats
154 and agricultural lands (Fig. 1a). Using this system and genotypic and phenotypic data
155 for each species and population, we tested whether taxa that are highly host/habitat
156 specific and linked to highly fragmented habitats show stronger genetic and phenotypic
157 structure than co-distributed generalist species inhabiting a heterogeneous but
158 continuous matrix of suitable habitat.

159 We first analyse the patterns of genetic and phenotypic variability for each
160 studied species and test whether such patterns differ in magnitude and spatial
161 congruence among taxa. Second, we explore the underlying mechanism shaping
162 phenotypic divergence in order to determine whether it is primarily driven by selection
163 or random genetic drift (e.g. Palo *et al*. 2003; Saether *et al*. 2007; Lowe *et al*. 2012).
164 Specifically, we hypothesize (i) stronger genetic and phenotypic divergence in small-
165 medium species showing preferences for highly fragmented microhabitats due to their
166 limitations to disperse among distant suitable habitat patches, which ultimately can
167 increase the opportunity for the evolution of local adaptations. We also hypothesize that
168 medium-large body size generalist species inhabiting continuous habitats have (ii)
169 higher levels of genetic diversity and lower variance in genetic diversity across
170 populations as consequence of widespread gene flow and an ephemeral impact of local
171 demographic dynamics. According to the contrasting life-histories and degree of habitat
172 fragmentation among the studied taxa (Fig. 1), we hypothesize (iii) that spatial patterns
173 of genetic and phenotypic variability and structure are not congruent across most of the
174 studied species, but we expect higher resemblance in small-medium species with higher
175 degree of habitat fragmentation due to their more similar responses to the parallel large-
176 scale destruction of their natural habitats.

177

178 **Materials and methods**

179

180 *Study species*

181

182 We selected five grasshopper species that co-occur in most of the studied microreserves
183 and show contrasting life-history traits and degree of habitat fragmentation in the
184 region, factors that we hypothesize to impact their spatial patterns of genetic and
185 phenotypic variation (Fig. 1a). All the studied species belong to the family Acrididae
186 and are short-horned, winged grasshoppers with a one-year generation time. All the
187 studied species are native to the study area and distributed in many other adjacent areas
188 from the Iberian Peninsula and the western Mediterranean region (Llucià-Pomares 2002
189 and references therein). *Mioscirtus wagneri* (Kittary, 1859) (subfamily: Oedipodinae)
190 (hereafter, Mw) has a small body size (♂: 14-16 mm; ♀: 19-22 mm) and is a highly
191 specialized grasshopper (Fig. 1). In the Iberian Peninsula, this species exclusively
192 inhabits saline and hypersaline lowlands with patches of shrubby sea-blite (*Suaeda*
193 *vera*), the halophilic plant on which it depends for food (Ortego *et al*. 2012a). In the
194 study area, the habitat of this species is highly fragmented due to both its limited natural
195 extension and large-scale land clearing for agriculture in the region (Ortego *et al*. 2010).
196 As a result, the populations of this species only persist in small and highly isolated
197 patches of suitable habitat restricted to a few saline lowlands scattered across the
198 landscape (see Fig. 1 in Ortego *et al*. 2012 for a map showing available habitats of Mw
199 within the study area). In this sense, previous studies have revealed that this species
200 shows a very deep genetic structure at different spatiotemporal scales (Ortego *et al*.
201 2009, 2010, 2011, 2012a). *Ramburiella hispanica* (Rambur, 1838) (subfamily:
202 Gomphocerinae) (hereafter, Rh) is a specialized and medium-sized (♂: 17-23 mm; ♀:
203 25-30 mm) grasshopper that in the study area is restricted to semi-natural vegetation
204 areas covered with the esparto grasses *Lygeum spartum* and *Stipa tenacissima* (P. J.

205 Cordero & J. Ortego, pers. obs.) (Fig. 1a). Suitable habitats of this species are also
206 highly fragmented and have suffered a considerable reduction in parallel with the
207 contraction experienced by the habitats occupied by Mw due to extensive land clearing
208 for agriculture (Ortego *et al*. 2015). However, remnant habitats of Rh are more
209 connected than those of Mw given that Rh occupies all patches where Mw is present
210 plus many others not devoted to agriculture and covered with esparto grass formations
211 (see Fig. 1 in Ortego *et al*. 2015 for a map showing available habitats of Rh within the
212 study area). *Calliptamus barbarus* (Costa, 1836) (subfamily: Calliptaminae) (hereafter,
213 Cb) is a medium-sized (♂: 13-21 mm; ♀: 19-31 mm) and generalist grasshopper that
214 feeds on many grass species (Blanchet *et al*. 2012a, b and references therein) (Fig. 1a).
215 In the study area, this species is ubiquitous in any patch of semi-natural vegetation but
216 absent in agricultural areas (P. J. Cordero & J. Ortego, pers. obs.). The habitat of Cb is
217 highly fragmented, but in a lesser extent than in the two previous species as it occupies
218 many non-agricultural habitat patches where the specific plant formations required by
219 Mw and Rh are not present. Thus, the specific habitats of Mw are embedded within
220 those habitat patches occupied by Rh, which in turn are embedded within those larger
221 patches inhabited by Cb (Fig. 1a). *Calliptamus italicus* (L., 1758) (subfamily:
222 Calliptaminae) (hereafter, Ci) is a medium-sized (♂: 14-25 mm; ♀: 22-33 mm) and
223 generalist grasshopper species found in both semi-natural habitat patches and
224 agricultural systems (Fig. 1a). This species has been reported to be an occasional
225 agricultural pest (Blanchet *et al*. 2012a, b and references therein). *Oedaleus decorus*
226 (Germar, 1826) (subfamily: Oedipodinae) (hereafter, Od) is a large-size (♂: 18-24 mm;
227 ♀: 25-38 mm) generalist grasshopper (Fig. 1a) (all measurements according to Harz
228 1975). This species is declining or has become extinct in some European countries (see
229 Kindler *et al*. 2012 and references therein), but it is common in our study area and can

be found at high densities in most semi-natural habitat patches, field margins and agricultural systems (P. J. Cordero & J. Ortego, pers. obs.).

In order to illustrate the phylogenetic relationships among our study species, we built a phylogenetic tree in the program MEGA 6.06 using a maximum likelihood method and GTR + I + γ as substitution model (Tamura *et al*. 2013). We used sequences of a segment of the 16S rRNA mitochondrial gene (459-463 bp) obtained in our lab (for Mw, Rh and Od) as described in Ortego *et al*. 2009 or retrieved from the GenBank (for Cb and Ci) (Fig. 1b). New sequences were deposited in the GenBank with accession numbers KT380945-KT380946. Fig. 1b shows that the study species are not phylogenetically clustered according to the three main studied factors, indicating that similarities among species in body size and the degree of habitat specialization and susceptibility to fragmentation are independent of their phylogenetic relationships.

Study sites and sampling

The study was carried out in 12 localities from La Mancha region, Central Spain (~2500 km^2; Table 1; Fig. 2a). Population code descriptions and further information on sampling sites are given in Table 1. During 2006-13, we aimed to sample in each locality ~20 adult specimens of each studied species (Mw: $n = 242$; Rh: $n = 234$; Cb: $n = 204$; Ci: $n = 219$; Od: $n = 221$; Table 1). We intended to sample an equal number of males and females in each locality, but sample sizes are often male-biased due to the difficulties in capturing females at some sites for some species (Table 1). Identification of *Calliptamus* species based on morphological characters is challenging for females, so we only sampled males for the two studied species of this genus (Blanchet *et al*. 2012a, b). Two species (Ci and Od) were not present in OCA locality. Another species (Cb) was very scarce in HUE locality and we were only able to collect three specimens

256 despite intensive sampling effort in the area (Table 1). In ten localities all the species
257 could be collected in sufficient numbers (≥ 8 specimens) to perform population genetic
258 analyses (Table 1). Most comparisons across species reported in the Results section
259 refer to these ten populations. All specimens were preserved in 1500 µl of 96% ethanol
260 at -20 ºC until needed for genetic analyses.
261
262 *Microsatellite genotyping*
263
264 We used NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) kits or a salt
265 extraction protocol (Aljanabi & Martinez, 1997) to purify genomic DNA from a hind
266 leg of each individual. We used five to twelve microsatellite markers to genotype each
267 sampled individual from the different species (Mw: Aguirre *et al*. 2010; Rh: Aguirre *et*
268 *al*. 2014; Cb and Ci: Blanchet *et al*. 2010a; Od: Berthier *et al*. 2008; see Table S1,
269 Supporting information). Amplifications were conducted in 10-µL reaction volumes
270 containing approximately 5 ng of template DNA, 1× reaction buffer (67 mM Tris-HCL,
271 pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01 % Tween-20, EcoStart Reaction Buffer, Ecogen,
272 Madrid, Spain), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 µM of each dye-labeled
273 primer (FAM, PET, VIC or NED) and 0.1 U of *Taq* DNA EcoStart Polymerase
274 (Ecogen). The PCR cycling profile used was 9 min denaturing at 95 ºC followed by 40
275 cycles of 30 s at 94 ºC, 45 s at the annealing temperature (see Table S1, Supporting
276 information) and 45 s at 72 ºC, ending with a 10 min final elongation stage at 72 ºC.
277 Amplification products were electrophoresed using an ABI 310 Genetic Analyzer
278 (Applied Biosystems, Foster City, CA, USA) and genotypes were scored using
279 GENEMAPPER 3.7 (Applied Biosystems).
280 Microsatellite genotypes were tested for departure from Hardy-Weinberg
281 equilibrium at each locus within each sampling population and species using an exact

282 test (Guo & Thompson 1992) based on 900 000 Markov chain iterations as
283 implemented in the program ARLEQUIN 3.1 (Excoffier *et al*. 2005). We also used
284 ARLEQUIN 3.1 to test for linkage disequilibrium between each pair of loci for each
285 population and species sampled using a likelihood-ratio statistic, whose distribution was
286 obtained by a permutation procedure (Excoffier *et al*. 2005). We applied sequential
287 Bonferroni corrections to account for multiple comparisons (Rice 1989).
288
289 *Genetic diversity*
290
291 For each species and population, we calculated allelic richness (A_R) standardized for the
292 smallest sample size using the rarefaction method implemented in the program HP-
293 RARE (Kalinowski 2005) and observed heterozygosity (H_O) using FSTAT (Goudet 1995).
294 A_R and H_O were highly correlated across populations in all the studied species (Pearson
295 rank correlations, Mw: $r = 0.967$; Rh: $r = 0.924$; Cb: $r = 0.823$; Ci: $r = 0.852$; Od: $r =$
296 0.968; all Ps < 0.01) and for simplicity we only used A_R as an estimate of population
297 genetic diversity in subsequent analyses. We first compared genetic diversity among
298 species using a one-way ANOVA. Then, we analysed the correlation of genetic
299 diversity across populations between all pairs of species using Pearson rank
300 correlations. A significant positive correlation of population genetic diversity in two
301 species would suggest that their populations have similarly responded to the different
302 factors (e.g. habitat fragmentation, genetic bottlenecks, etc.) affecting local levels of
303 genetic diversity. Finally, we used Levene's tests to analyse if variance in population
304 genetic diversity is similar among the studied species. A high variance in genetic
305 diversity among populations of a given species would indicate that its populations are
306 differentially impacted by the demographic phenomena affecting local levels of genetic
307 diversity. In contrast, if a species shows levels of genetic diversity that are similar

across all its populations (i.e. low variance), this would imply that all of them are subjected to comparable demographic dynamics and/or that differences are ephemeral due to the homogenising effects of gene flow. All statistical analyses were performed in SPSS 19.0.

Genetic structure

We investigated population genetic structure among sample locations calculating pair-wise F_{ST}-values and testing their significance with Fisher's exact tests after 10 000 permutations as implemented in ARLEQUIN 3.1 (Excoffier *et al*. 2005). Critical *P*-values for pair-wise tests of allelic differentiation were determined using a sequential Bonferroni adjustment (Rice 1989). We calculated global F_{ST} values across all populations in FSTAT 2.9.3 and 95% confidence intervals (95% CI) were estimated by bootstrapping over loci (10 000 randomizations; Goudet 1995). Finally, we analysed patterns of genetic structure using the Bayesian Markov chain Monte Carlo clustering analysis implemented in the program STRUCTURE 2.3.3 (Pritchard *et al*. 2000; Falush *et al*. 2003; Hubisz *et al*. 2009). STRUCTURE assigns individuals to *K* populations based on their multilocus genotypes. We ran STRUCTURE assuming correlated allele frequencies and admixture and using prior population information (Hubisz *et al*. 2009). We conducted ten independent runs for each value of K = 1-10 to estimate the "true" number of clusters with 200000 MCMC cycles, following a burn-in step of 100000 iterations. The number of populations best fitting the data set was defined both using log probabilities [Pr($X|K$)] (Pritchard *et al*. 2000) and the ΔK method (Evanno *et al*. 2005), as implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). We used CLUMPP to align multiple runs of STRUCTURE for the optimum *K* value using the Greedy algorithm (Jakobsson & Rosenberg, 2007).

334

335 *Concordance of spatial patterns of genetic structure*

336

337 We analysed congruent patterns of genetic structure in two different ways. First, we
338 assessed the correlation between genetic distance matrices ($F_{ST,}$ calculated as described
339 above) of all species pairs using classical Mantel tests. We also used partial Mantel tests
340 to remove any confounding effects of geographical distance (i.e. isolation-by-distance,
341 IBD) (e.g. Morgan *et al*. 2011; Widmer *et al*. 2012). All Mantel tests were performed
342 using ZT software with 10 000 permutations (Bonnet & Van de Peer 2002). Second, we
343 performed Procrustes rotation tests to analyse the degree of congruence between
344 multivariate population allele frequency data of all species pairs (Jackson 1995; Peres-
345 Neto & Jackson 2001; e.g. Widmer *et al*. 2012). In a first step, we summarized variation
346 in population allele frequencies for each species using mean-centred principal
347 component analyses (PCAs) as implemented in R 3.0.3 (R Core Team, 2012) package
348 ADEGENET 1.4.1 (Jombart 2008). Next, we performed a Procrustes rotation to rotate the
349 raw principal component (PC) matrices of the first three axes for each pair of species
350 using the 'procuste' function in R 3.0.3 package ADE4. Procustean rotations were scaled
351 to unit variance to get the more scale-independent and symmetric descriptive statistic
352 "Procrustes sum of squares" (m^2). Finally, we performed a PROTEST analysis (Jackson
353 1995) to test the significance of the similarity between the genetic matrices of each pair
354 of species using the 'procuste.rtest' function with 9999 iterations in ADE4.

355

356 *Phenotypic divergence*

357

358 We studied the underlying factors shaping phenotypic variation examining the levels of
359 quantitative divergence based on phenotypes (P_{ST}). P_{ST} (or "phenotypic" Q_{ST}) is

analogous to Q_{ST}, a measure of differentiation in quantitative genetic traits and the equivalent of F_{ST} for morphological characters (Spitze 1993). P_{ST} is used as a proxy for Q_{ST} when the required quantitative genetic information cannot be estimated and it is not possible to disentangle genetic variation among populations from environmental variation (e.g. in field studies; Raeymaekers *et al*. 2007; Brommer 2011). Here, we focused on body size, a morphological trait that typically exhibits a substantial additive genetic basis (Mousseau & Roff 1987; Merilä & Crnokrak 2001). The calculation of P_{ST} values allowed us to study patterns of phenotypic divergence across the different studied species that differ considerably in body size (Fig. 1) and for which simple Euclidean distance between population mean values of body size (e.g. Ortego *et al*. 2012b) are not directly comparable. Phenotypic differentiation was only studied in adult males, as females were not available for some species (Cb and Ci) as described above. For all individuals, we measured femur length to the nearest 0.1 mm using a stereoscopic microscope Leica S8 APO and the software LAS version 2.8.1. This morphological trait provides a good estimate of overall body size in grasshoppers and is highly correlated with estimates of body size based on other morphological traits (Ortego *et al*. 2012b). Global and pairwise P_{ST} values for all population pairs were estimated as

$$P_{ST} = [(c/ h^2)\, \sigma^2_{GB}\,] /[(c/ h^2)\, \sigma^2_{GB} + 2\sigma^2_{GW}],$$

where the scalar c expresses the additive genetic proportion of differences between populations (i.e. the proportion of the total variance that is presumed to be due to additive genetic effects across populations), h^2 is the assumed additive genetic proportion of differences between individuals within populations (narrow sense "heritability"), σ^2_{GB} is the observed between-population variance component and σ^2_{GW}

386 is the observed within-population variance component. Given the unknown magnitude
387 of c and h^2 (whose ratio determines the accuracy of the approximation of Q_{ST} by P_{ST}),
388 we computed P_{ST} values by varying the c and h^2 parameters (c/h^2 range: 0.1-2.0). The
389 reported P_{ST} values are those obtained assuming $c = h^2 = 0.5$. These values were chosen
390 given that the heritability estimate of male body size in the grasshopper *Chorthippus*
391 *brunneus* has been previously reported to be 0.48 (Butlin & Hewitt 1986), which means
392 that environmental and non-additive genetic effects account for about half of the
393 observed phenotypic variation. We assumed the proportion of variation due to additive
394 genetic effects across populations c equals the proportion within population h^2 (i.e. c/h^2
395 = 1), which is a biologically realistic assumption (Brommer 2011). P_{ST} estimates did not
396 change much when considering other more conservative scenarios ($c < h^2$) and provided
397 analogous results (data not shown). Confidence intervals (CI) were estimated from 1000
398 bootstrap replicates using the "boot" package (Ripley 2015) in R (R Development Core
399 Team 2012).
400 The relationship between genetic (F_{ST}) and morphological (P_{ST}) differentiation
401 across populations was analysed using Mantel tests. If genetic and phenotypic
402 population divergence are positively correlated, this would imply that genetic drift has
403 played an important role on phenotypic divergence. In contrast, if genetic and
404 phenotypic divergence are decoupled this would suggest that phenotype is plastic or, in
405 the case of highly heritable traits such as body size (Mousseau & Roff 1987), controlled
406 by local selection (e.g. Leinonen *et al*. 2006; Lehtonen *et al*. 2009). These comparisons
407 therefore serve as a gauge of the likely overall importance of genetic drift *vs.* local
408 adaptation in body size variation. We analyse congruent patterns of phenotypic
409 differentiation across the studied species assessing the correlation between phenotypic
410 distance matrices (P_{ST}) of all species pairs using Mantel tests. We also used partial

411 Mantel tests to remove any confounding effects of geographical distance (see previous
412 section for details on Mantel and partial Mantel tests).
413
414 ## Results
415
416 *Microsatellite data*
417
418 All microsatellite markers were highly polymorphic across most populations and
419 species, with 8-56 alleles per locus (Table S1, Supporting information). After applying
420 sequential Bonferroni corrections to compensate for multiple statistical tests, only two
421 loci (RhA113 and RhC1) from Rh consistently deviated from HWE across all the
422 studied populations and were excluded from further analyses (Table S1, Supporting
423 information). We did not find any evidence of genotypic linkage disequilibrium at any
424 pair of loci in any population and species (exact tests; all *P*s > 0.05).
425
426 *Genetic diversity*
427
428 A_R for each species and population is indicated in Table 1. Considering only the 10
429 localities where all the five species were collected, we found that A_R differed
430 significantly among taxa (one-way ANOVA: $F_{4, 45} = 223.99$, $P < 0.001$; Fig. 3). Post-
431 hoc Tukey tests showed that A_R was significantly different between all species pairs (all
432 *P*s < 0.003) with the exception of the comparison involving Cb and Od ($P = 0.223$; Fig.
433 3). A_R increased in the order Mw < Rh < Ci < Cb < Od and was not significantly
434 correlated across populations between any pair of species after sequential Bonferroni
435 correction for multiple testing (all *P*s > 0.05). Finally, variance in population A_R

436 significantly differed among species (Levene's test: $F_{4, 45} = 2.78$, $P = 0.038$) and post-
437 hoc analyses indicated that only pair-wise comparisons involving Mw were significant
438 (Mw-Rh: $P = 0.025$; Mw-Ci = $P = 0.013$; Mw-Od = $P = 0.029$) (Fig. 3). In all
439 comparisons, Mw had higher variance in A_R than the other species (Fig. 3). Analyses
440 including all populations (Fig. 3) or using H_O as an estimate of population genetic
441 diversity provided analogous results (data not shown).
442
443 *Genetic structure*
444
445 Global F_{ST} values were significantly higher in specialist Mw than in all the other
446 studied species (non-overlapping 95% CI), but did not differ among Rh, Cb, Ci and Od
447 (Fig. 4a). Considering only the 10 localities where all the taxa were collected, global
448 F_{ST} values decreased in the order Mw ($F_{ST} = 0.055$, 95% CI: 0.044-0.066) > Rh ($F_{ST} =$
449 0.017, 95% CI: 0.012-0.023) > Od ($F_{ST} = 0.015$, 95% CI: 0.011-0.018) > Ci ($F_{ST} =$
450 0.011, 95% CI: 0.004-0.019) > Cb ($F_{ST} = 0.011$, 95% CI: 0.004-0.017) (Fig. 4a; see also
451 Table S2, Supporting information for pair-wise F_{ST} values). Pair-wise population
452 comparisons provided analogous results (Fig. 4a; Table S2, Supporting information).
453 STRUCTURE analyses considering all populations indicated a maximum value of Pr(X|*K*)
454 for $K = 5$ in Mw, $K = 3$ in Rh and Cb, and $K = 1$ in Ci and Od (Fig. 2 and 1S). The
455 Evanno *et al*. (2005) method indicated an optimal value of $K = 2$ for Mw and $K = 3$ for
456 Rh and Cb (Fig. 2 and 1S). STRUCTURE analyses considering only the 10 localities
457 where all the taxa were collected, indicated an optimal value of $K = 3$ for Mw, $K = 2$ for
458 Rh and Cb and $K = 1$ for Ci and Od.
459
460 *Concordance of spatial genetic structure across species*
461

462 Genetic and geographical distances were positively correlated in Mw ($r = 0.674$, $P <$
463 0.001), Rh ($r = 0.320$, $P = 0.017$) and Ci ($r = 0.303$, $P = 0.022$), but not in Cb ($r =$
464 0.138, $P = 0.177$) or Od ($r = 0.166$, $P = 0.128$). Mantel tests showed that genetic
465 distance matrices were correlated between Mw and Ci ($P = 0.0048$), Rh and Ci ($P =$
466 0.0026), and Cb and Od ($P = 0.0017$) after controlling for multiple testing. However,
467 only the correlation between genetic distance matrices of Mw and Ci remained
468 significant after controlling for geographical distances in partial Mantel tests ($P = 0.004$;
469 Table 2a). Procrustes rotations on the PCA matrices and PROTEST analyses showed no
470 significant correlation of population allele frequencies in any species pair (all Ps > 0.1
471 and all $m^2 > 0.6$; Table 2c).
472
473 *Phenotypic divergence*
474
475 All species showed very high levels of phenotypic differentiation (Fig. 4b; see Table S2,
476 Supporting information). Global P_{ST} values did not differ among species (overlapping
477 95% CI; Fig. 4b). P_{ST} values were not correlated with genetic (F_{ST}) or geographical
478 distance matrices in any species (all $r < 0.20$, all Ps > 0.11). Similarly, P_{ST} and F_{ST}
479 distance matrices were not correlated in any species after controlling for geographical
480 distance in partial Mantel tests (all $r < 0.07$, all Ps > 0.32). Considering only the 10
481 localities where all the taxa were collected, we found that P_{ST} values were not correlated
482 between any species pair after sequential Bonferroni correction. No comparison was
483 significant after controlling for geographical distances in partial Mantel tests (all Ps >
484 0.05; Table 2b). After controlling for multiple testing, average population femur length
485 was correlated only between Mw and Cb ($r = 0.805$, $P = 0.005$). Finally, variance in
486 population femur length differed significantly among species (Levene's test: $F_{4, 45} =$
487 7.87, $P < 0.001$) and post-hoc analyses indicated that significant pair-wise comparisons

488 involved Mw-Rh (P = 0.025), Mw-Od (P = 0.001), Rh-Od (P = 0.016), Cb-Od (P =
489 0.013), and Ci-Od (P = 0.003). In all comparisons, Od had higher variance in femur
490 length than the other species and Rh had higher variance than Mw.
491
492 ## Discussion
493
494 Our analyses supported the hypothesis predicting that species with preferences for
495 highly fragmented microhabitats show stronger patterns of genetic structure, harbour
496 lower levels of within-population genetic diversity and have higher variance of among-
497 population genetic diversity than co-distributed generalist taxa inhabiting a continuous
498 matrix of suitable habitat. This pattern was particularly marked for the small and highly
499 specialist Mw, which inhabits extremely fragmented habitats and probably has a scarce
500 capacity to disperse among isolated patches of suitable habitat (Fig. 1a). However, we
501 did not find support for the hypothesis predicting that phenotypic divergence is more
502 marked among species linked to highly fragmented microhabitats. We neither found
503 support for congruent patterns of phenotypic and genetic variability among any studied
504 species, indicating that the studied taxa show idiosyncratic evolutionary (i.e. distinct
505 patterns of phenotypic divergence) and demographic (i.e. contrasting levels of genetic
506 diversity and structure) trajectories even though they share a common landscape.
507 Data on genetic structure indicate strong differences among taxa, with the
508 specialist Mw showing a much higher genetic differentiation than the other species
509 studied (Fig. 4a). Mw is a small and highly specialist grasshopper that in the study area
510 exclusively inhabits patches with shrubby sea-blite formations, the plant on which it
511 depends exclusively for food (Cordero *et al*. 2007). These life-history traits and the high
512 fragmentation of its particular habitats are likely to have strongly limited inter-

513 population gene flow in this species and lead to strong genetic subdivision (King &
514 Lawson 2001; DiLeo *et al*. 2010; Lange *et al*. 2010; Blanchet *et al*. 2010b; Keller *et al*.
515 2013b). The remarkable population genetic differentiation of Mw in contrast to the
516 other species studied puts into a comparative context the deep genetic structure at
517 landscape (Ortego *et al*. 2012a) and phylogeographic scales (Ortego *et al*. 2009)
518 previously reported in this specialist grasshopper and highlights the extraordinary
519 isolation of most of its populations. The other species studied here inhabit continuous
520 habitats (Ci, Od), show a much lower degree of fragmentation of their specific habitats
521 in the region (Rh, Cb) or have larger body/wing sizes (Rh, Cb, Ci, Od), factors that can
522 explain their increased population connectivity and weak genetic differentiation (DiLeo
523 *et al*. 2010; Lange *et al*. 2010). In the study area, the specialist grasshopper Rh inhabits
524 semi-natural habitat patches occupied by two different host plant species (Ortego *et al*.
525 2015). These habitats also show a high fragmentation but are more widespread and
526 connected than those occupied by Mw, which is exclusively restricted to small patches
527 of saline and hypersaline lowlands (Ortego *et al*. 2012a; Ortego *et al*. 2015). A higher
528 habitat connectivity, together with the larger body size of Rh, can result in the actual
529 level of habitat fragmentation being insufficient to strongly limit gene flow among
530 populations (Lange *et al*. 2010; Keller *et al*. 2013b). This can explain why, contrary to
531 our predictions, Rh shows a shallow genetic structure that is comparable to that reported
532 in the generalist and more widespread studied species (Fig. 4a).

533 Explicit analyses to test congruent patterns of genetic structure have been
534 employed in comparative phylogeography (e.g. Widmer *et al*. 2012; Borer *et al*. 2012),
535 but such approaches have only rarely been used to compare the spatial distribution of
536 genetic variation among co-distributed species at the landscape scale (Fortuna *et al*.
537 2009). Our analyses of spatial congruence of genetic structure indicate that, not only the
538 degree of genetic differentiation, but also the spatial distribution of genetic variation

539 strongly differs among the studied species. This incongruence between taxa may reflect
540 differences in the spatial location of species-specific barriers to dispersal (Goldberg &
541 Waits 2010; Richardson 2012; Frantz *et al*. 2012). However, the subtle genetic structure
542 observed in most studied species is also likely to have strongly reduced the power to
543 detect any concordance between population genetic distances or multivariate allele
544 frequencies across the studied species. Contrary to our predictions, the species
545 inhabiting highly fragmented natural habitats (Mw, Rh and Ci) did not show a
546 significant spatial congruence in the distribution of genetic variation. Despite these three
547 species having suffered a parallel drastic reduction of their suitable natural habitats,
548 remnant non-agricultural lands and esparto grass formations occupied by Cb and Rh,
549 respectively, are more common than the highly restricted habitats of Mw, which can
550 explain the lack of congruence in the patterns of genetic differentiation among these co-
551 distributed species that *a priori* were expected to be severely impacted by habitat
552 fragmentation (Ortego *et al*. 2012a, 2015).
553 Comparative analyses of genetic diversity indicate that the studied species also
554 show contrasting responses to the different factors shaping within-population levels of
555 genetic variability (Lange *et al*. 2010; Aparicio *et al*. 2012). In absolute terms, genetic
556 diversity was lower in specialist than in generalist species (Fig. 3), which suggests that
557 population fragmentation in the former (particularly in Mw) has resulted in higher
558 genetic drift due to low local effective population sizes and more frequent population
559 bottlenecks (Lange *et al*. 2010; Habel & Schmitt 2012; Keller *et al*. 2013b). In relative
560 terms, we found that within-population levels of genetic diversity were not correlated
561 across populations in any species pair. This suggests that gene flow, habitat
562 fragmentation and local demographic dynamics affect each species in very different
563 ways despite the fact that they share a common landscape (Aparicio *et al*. 2012; Lange
564 *et al*. 2010). Mw also had higher variance in genetic diversity among populations than

565 most of the other studied taxa, which indicates that the different populations of this
566 species experience more contrasting demographic dynamics (Ortego *et al*. 2012a). The
567 higher population connectivity in the other species (Fig. 2 and 4a) may result in
568 demographic changes (e.g. bottlenecks, arrival of immigrants, etc.) that are only
569 ephemerally reflected in local levels of variability due to the homogenising effects of
570 gene flow and this leads to similar patterns of genetic diversity across all their
571 populations (Lange *et al*. 2010).

572 Phenotypic divergence was comparably strong across all the studied taxa (global
573 $P_{ST} > 0.7$; Fig. 4b), but was not correlated with population genetic divergence or
574 geographical distances in any species. This implies that body size is not merely
575 controlled by gene flow and drift and points to an important role of local adaptation in
576 determining inter-population differences in the studied trait (Leinonen *et al*. 2006;
577 Lehtonen *et al*. 2009; Lowe *et al*. 2012). Phenotypic divergence was not correlated
578 between any pair of species, indicating that they do not show convergent evolutionary
579 responses to their common environment (Lowe *et al*. 2012; Ingley *et al*. 2014). The
580 contrasting body sizes and life-histories of the studied species may be the result of
581 different selective pressures brought about by contrasting communities of predators and
582 inter-specific interactions (Basolo & Wagner 2004; Berger *et al*. 2006; Ingley *et al*.
583 2014). Thus, different ecological pressures causing selection are likely to have
584 decoupled the evolutionary responses of the different studied species (Lowe *et al*. 2012;
585 Richardson *et al*. 2014).

586

587 *Conclusions and implications for conservation*

588

589 Our study highlights that habitat fragmentation can have very different demographic
590 and evolutionary consequences even among closely related organisms (Short &

591 Caterino 2009; Olsen *et al*. 2011). The studied generalist species inhabiting more
592 continuous habitats (Ci and Od) present a low degree of genetic differentiation and,
593 contrary to our hypothesis, these patterns are similar in absolute terms to those found in
594 some taxa experiencing a high degree of habitat fragmentation in the study area (Rh and
595 Cb; Fig. 4a). Only Mw shows a much higher degree of genetic differentiation than the
596 other taxa (Fig. 4a), which suggests that only the extreme habitat fragmentation
597 experienced by this species is above the threshold that remarkably disrupts inter-
598 population gene flow and considerably reduces local levels of genetic diversity. Our
599 results support previous studies suggesting that basic data on life-history traits and
600 habitat specialization and fragmentation can help to anticipate species demographic
601 responses and patterns of genetic divergence (DiLeo *et al*. 2010; Lange *et al*. 2010;
602 Keller *et al*. 2013b; Phillipsen *et al*. 2015), but they also indicate that it is complicated
603 to get accurate predictions about the degree of habitat fragmentation beyond of which
604 population genetic structure and diversity are affected due to complex interactions
605 among multiple influential factors (Lange *et al*. 2010; Callens *et al*. 2011; Keller *et al*.
606 2013b).

607 Our multispecies comparative approach can help to (i) determine baseline levels
608 of genetic and phenotypic variation for taxa that are expected to maintain well
609 connected populations (e.g. high-mobility and generalist species with a low degree of
610 habitat fragmentation), (ii) identify the most (e.g. Mw) and least vulnerable (e.g. Rh and
611 Cb) species among those that have experienced a considerable fragmentation of their
612 respective habitats, and (iii) focus future research efforts on other taxa that may be
613 affected by similar threats to those species with which they share similar habitats and
614 life-history traits and that have been identified to be more vulnerable (e.g. low-mobility
615 species linked to hypersaline lowlands; Cordero & Llorente 2008). In view of our
616 results, we suggest that biodiversity conservation in networks of protected areas

617 requires detailed ecological and evolutionary information on several taxa with different
618 habitat requirements and life-history traits in order to identify target species that are
619 more sensitive to the effects of habitat fragmentation and would gain more benefits
620 from management practices aimed to improve population connectivity, increase the size
621 and quality of appropriate habitat within each fragment, and maintain the idiosyncratic
622 evolutionary trajectories of those populations presenting strong local adaptations
623 (Rouget *et al*. 2006; Ouborg *et al*. 2010; Habel & Schmitt 2012; Habel *et al*. 2013). In
624 more general terms, our multispecies comparative study offers a useful approach to
625 identify the proximate causes of genetic and phenotypic variation in natural populations
626 and guide future research aimed to assess the impacts of habitat fragmentation across
627 multiple co-distributed species for which little information is available and that may
628 show very different responses to the alterations affecting their common landscape.
629 Overall, our study highlights the importance of inferring the evolutionary and
630 demographic processes behind genetic and phenotypic patterns and offers a
631 comprehensive framework to identify the causal factors that may be compromising the
632 long-term viability of natural populations and, ultimately, develop conservation agendas
633 targeting specific problems and putting into practice the most efficient management
634 solutions.
635
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637
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649

650 **References**

651

652 Aguirre MP, Bloor P, Ramirez-Escobar U, Ortego J, Cordero PJ (2010) Isolation and
653 characterization of polymorphic microsatellite markers in the grasshopper
654 *Mioscirtus wagneri* (Orthoptera: Acrididae). *Conservation Genetics*, **11**, 1119-
655 1121.
656 Aguirre MP, Noguerales V, Cordero PJ, Ortego J (2014) Isolation and characterization
657 of polymorphic microsatellites in the specialist grasshopper *Ramburiella*
658 *hispanica* (Orthoptera: Acrididae). *Conservation Genetics Resources*, **6**, 723-
659 724.
660 Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality
661 genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692-
662 4693.
663 Aparicio A, Hampe A, Fernandez-Carrillo L, Albaladejo RG (2012) Fragmentation and
664 comparative genetic structure of four mediterranean woody species: complex
665 interactions between life history traits and the landscape context. *Diversity and*
666 *Distributions*, **18**, 226-235.

667 Basolo AL, Wagner WE (2004) Covariation between predation risk, body size and fin
668 elaboration in the green swordtail, *Xiphophorus helleri*. *Biological Journal of*
669 *the Linnean Society*, **83**, 87-100.
670 Berger D, Walters R, Gotthard K (2006) What keeps insects small? - Size dependent
671 predation on two species of butterfly larvae. *Evolutionary Ecology*, **20**, 575-589.
672 Berthier K, Loiseau A, Streiff R, Arlettaz R (2008) Eleven polymorphic microsatellite
673 markers for *Oedaleus decorus* (Orthoptera, Acrididae), an endangered
674 grasshopper in Central Europe. *Molecular Ecology Resources*, **8**, 1363-1366.
675 Blanchet E, Lecoq M, Sword GA, *et al.* (2012a) A comparative analysis of fine-scale
676 genetic structure in three closely related syntopic species of the grasshopper
677 genus *Calliptamus*. *Canadian Journal of Zoology-Revue Canadienne de*
678 *Zoologie*, **90**, 31-41.
679 Blanchet E, Lecoq M, Sword GA, *et al.* (2012b) Population structures of three
680 *Calliptamus* spp. (Orthoptera: Acrididae) across the Western Mediterranean
681 Basin. *European Journal of Entomology*, **109**, 445-455.
682 Blanchet E, Pages C, Blondin L, *et al.* (2010a) Isolation of microsatellite markers in the
683 *Calliptamus* genus (Orthoptera, Acrididae). *Journal of Insect Science*, **10**, 133.
684 Blanchet S, Rey O, Etienne R, Lek S, Loot G (2010b) Species-specific responses to
685 landscape fragmentation: implications for management strategies. *Evolutionary*
686 *Applications*, **3**, 291-304.
687 Blondel J, Aronson J (1999) *Biology and Wildlife of the Mediterranean Region*. Oxford
688 University Press, Oxford.
689 Bonal R, Hernández M, Ortego J, Munoz A, Espelta JM (2012) Positive cascade effects
690 of forest fragmentation on acorn weevils mediated by seed size enlargement.
691 *Insect Conservation and Diversity*, **5**, 381-388.

692 Bonnet E, Van de Peer Y (2002) ZT: a software tool for simple and partial Mantel tests.
693 *Journal of Statistical Software*, **10**, 1-12.
694 Borer M, Arrigo N, Buerki S, Naisbit RE, Alvarez N (2012) Climate oscillations and
695 species interactions: large-scale congruence but regional differences in the
696 phylogeographic structures of an alpine plant and its monophagous insect.
697 *Journal of Biogeography*, **39**, 1487-1498.
698 Brommer JE (2011) Whither P_{ST}? The approximation of Q_{ST} by P_{ST} in evolutionary and
699 conservation biology. *Journal of Evolutionary Biology*, **24**, 1160-1168.
700 Butler EM (2012) *Habitat selection and anti-predator responses of acridid*
701 *grasshoppers*. PhD Thesis, North Carolina State University, Raleigh.
702 Butlin RK, Hewitt GM (1986) Heritability estimates for characters under sexual
703 selection in the grasshopper, *Chorthippus brunneus*. *Animal Behaviour*, **34**,
704 1256-1261.
705 Callens T, Galbusera P, Matthysen E*, et al.* (2011) Genetic signature of population
706 fragmentation varies with mobility in seven bird species of a fragmented Kenyan
707 cloud forest. *Molecular Ecology*, **20**, 1829-1844.
708 Cirujano-Bracamonte S, Medina-Domingo L (2002) *Plantas acuáticas de las lagunas y*
709 *humedales de Castilla-La Mancha*. CSIC-JCCM, Madrid.
710 Cordero PJ, Llorente V (2008) New data on the 'silver-bell cricket' (Orthoptera,
711 Gryllidae), a forgotten and overlooked cricket subject to a high risk of extinction
712 in western Europe. *Graellsia*, **64**, 171-180.
713 Cordero PJ, Llorente V, Aparicio JM (2007) New data on morphometrics, distribution
714 and ecology of *Mioscirtus wagneri* (Kittary, 1859) (Orthoptera, Acrididae) in
715 Spain: is maghrebi a well defined subspecies? *Graellsia*, **63**, 3-16.

716 DiLeo MF, Row JR, Lougheed SC (2010) Discordant patterns of population structure
717 for two co-distributed snake species across a fragmented Ontario landscape.
718 *Diversity and Distributions*, **16**, 571-581.
719 Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for
720 visualizing STRUCTURE output and implementing the Evanno method.
721 *Conservation Genetics Resources*, **4**, 359-361.
722 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals
723 using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**,
724 2611-2620.
725 Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software
726 package for population genetics data analysis. *Evolutionary Bioinformatics*
727 *Online*, **1**, 47-50.
728 Fahrig L (2002) Effect of habitat fragmentation on the extinction threshold: A synthesis.
729 *Ecological Applications*, **12**, 346-353.
730 Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using
731 multilocus genotype data: Linked loci and correlated allele frequencies.
732 *Genetics*, **164**, 1567-1587.
733 Fortuna MA, Albaladejo RG, Fernandez L, Aparicio A, Bascompte J (2009) Networks
734 of spatial genetic variation across species. *Proceedings of the National Academy*
735 *of Sciences of the United States of America*, **106**, 19044-19049.
736 Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131-140.
737 Frantz AC, Bertouille S, Eloy MC*, et al.* (2012) Comparative landscape genetic
738 analyses show a Belgian motorway to be a gene flow barrier for red deer
739 (*Cervus elaphus*), but not wild boars (*Sus scrofa*). *Molecular Ecology*, **21**, 3445-
740 3457.

741 Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified
742 concept for defining conservation units. *Molecular Ecology*, **10**, 2741-2752.
743 García-Navas V, Ferrer ES, Sanz JJ, Ortego J (2014) The role of immigration and local
744 adaptation on fine-scale genotypic and phenotypic population divergence in a
745 less mobile passerine. *Journal of Evolutionary Biology*, **27**, 1590-1603.
746 Gauffre B, Mallez S, Chapuis MP, *et al*. (2015) Spatial heterogeneity in landscape
747 structure influences dispersal and genetic structure: empirical evidence from a
748 grasshopper in an agricultural landscape. *Molecular Ecology*, **24**, 1713-1728.
749 Goldberg CS, Waits LP (2010) Comparative landscape genetics of two pond-breeding
750 amphibian species in a highly modified agricultural landscape. *Molecular*
751 *Ecology*, **19**, 3650-3663.
752 Gomez GSY, Van Dyck H (2012) Ecotypic differentiation between urban and rural
753 populations of the grasshopper *Chorthippus brunneus* relative to climate and
754 habitat fragmentation. *Oecologia*, **169**, 125-133.
755 Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate *F*-statistics.
756 *Journal of Heredity*, **86**, 485-486.
757 Guo SW, Thompson EA (1992) A monte-carlo method for combined segregation and
758 linkage analysis. *American Journal of Human Genetics*, **51**, 1111-1126.
759 Habel JC, Rodder D, Lens L, Schmitt T (2013) The genetic signature of ecologically
760 different grassland Lepidopterans. *Biodiversity and Conservation*, **22**, 2401-
761 2411.
762 Habel JC, Schmitt T (2012) The burden of genetic diversity. *Biological Conservation*,
763 **147**, 270-274.
764 Harz K (1975) *The Orthoptera of Europe II*. W. Junk Publishers, The Hague.
765 Heidinger IMM, Hein S, Bonte D (2010) Patch connectivity and sand dynamics affect
766 dispersal-related morphology of the blue-winged grasshopper *Oedipoda*

767 *caerulescens* in coastal grey dunes. *Insect Conservation and Diversity*, **3**, 205-
768 212.
769 Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population
770 structure with the assistance of sample group information. *Molecular Ecology*
771 *Resources*, **9**, 1322-1332.
772 Ingley SJ, Billman EJ, Belk MC, Johnson JB (2014) Morphological divergence driven
773 by predation environment within and between species of *Brachyrhaphis* fishes.
774 *Plos One*, **9**, e90274.
775 Jackson DA (1995) PROTEST - A procrustean randomization test of community
776 environment concordance. *Ecoscience*, **2**, 297-303.
777 Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation
778 program for dealing with label switching and multimodality in analysis of
779 population structure. *Bioinformatics*, **23**, 1801-1806.
780 Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic
781 markers. *Bioinformatics*, **24**, 1403-1405.
782 Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction
783 on measures of allelic richness. *Molecular Ecology Notes*, **5**, 187-189.
784 Keller D, Holderegger R, van Strien MJ (2013a) Spatial scale affects landscape genetic
785 analysis of a wetland grasshopper. *Molecular Ecology*, **22**, 2467-2482.
786 Keller D, van Strien MJ, Herrmann M*, et al.* (2013b) Is functional connectivity in
787 common grasshopper species affected by fragmentation in an agricultural
788 landscape? *Agriculture Ecosystems & Environment*, **175**, 39-46.
789 Kindler E, Arlettaz R, Heckel G (2012) Deep phylogeographic divergence and
790 cytonuclear discordance in the grasshopper *Oedaleus decorus*. *Molecular*
791 *Phylogenetics and Evolution*, **65**, 695-704.

King RB, Lawson R (2001) Patterns of population subdivision and gene flow in three sympatric natricine snakes. *Copeia*, **3**, 602-614.

Lange R, Durka W, Holzhauer SIJ, Wolters V, Diekotter T (2010) Differential threshold effects of habitat fragmentation on gene flow in two widespread species of bush crickets. *Molecular Ecology*, **19**, 4936-4948.

Lehtonen PK, Laaksonen T, Artemyev AV*, et al.* (2009) Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Molecular Ecology*, **18**, 4463-4476.

Leinonen T, Cano JM, Makinen H, Merila J (2006) Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, **19**, 1803-1812.

Levy RA, Nufio CR (2015) Dispersal potential impacts size clines of grasshoppers across an elevation gradient. *Oikos*, **124**, 610-619.

Lindenmayer DB, Fischer J (2006) *Habitat Fragmentation and Landscape Change: An Ecological and Conservation Synthesis*. Island Press, Washington.

Llucià-Pomares D (2002) Revisión de los ortópteros (Insecta: Orthoptera) de Cataluña (España). Monografías de la Sociedad Entomológica Aragonesa, **7**. Zaragoza, 226 p.

Lowe WH, McPeek MA, Likens GE, Cosentino BJ (2012) Decoupling of genetic and phenotypic divergence in a headwater landscape. *Molecular Ecology*, **21**, 2399-2409.

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, **17**, 285-291.

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892-903.

817 Morgan K, O'Loughlin SM, Chen B*, et al.* (2011) Comparative phylogeography reveals
818 a shared impact of pleistocene environmental change in shaping genetic
819 diversity within nine Anopheles mosquito species across the Indo-Burma
820 biodiversity hotspot. *Molecular Ecology*, **20**, 4533-4549.
821 Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes
822 that sustain it. *Systematic Biology*, **51**, 238-254.
823 Mousseau TA, Roff DA (1987) Natural selection and the heritability of fitness
824 components. *Heredity*, **59**, 181-197.
825 Noss RF, Csuti B (1994) Habitat fragmentation. *Principles of Conservation Biology* (ed.
826 by G. K. Meffe and C. R. Carroll), pp. 237-264. Sinauer Associates, Sunderland.
827 Olsen JB, Crane PA, Flannery BG*, et al.* (2011) Comparative landscape genetic analysis
828 of three Pacific salmon species from subarctic North America. *Conservation*
829 *Genetics*, **12**, 223-241.
830 Oneal E, Knowles LL (2013) Ecological selection as the cause and sexual
831 differentiation as the consequence of species divergence? *Proceedings of the*
832 *Royal Society B-Biological Sciences*, **280**, 20122236.
833 Ortego J, Aguirre MP, Cordero PJ (2010) Population genetics of *Mioscirtus wagneri*, a
834 grasshopper showing a highly fragmented distribution. *Molecular Ecology,* **19**,
835 472-483.
836 Ortego J, Aguirre MP, Cordero PJ (2011) Fine-scale spatial genetic structure and within
837 population male-biased gene-flow in the grasshopper *Mioscirtus wagneri*.
838 *Evolutionary Ecology*, **25**, 1127-1144.
839 Ortego J, Aguirre MP, Cordero PJ (2012a) Landscape genetics of a specialized
840 grasshopper inhabiting highly fragmented habitats: a role for spatial scale.
841 *Diversity and Distributions*, **18**, 481-492.

842 Ortego J, Aguirre MP, Cordero PJ (2012b) Genetic and morphological divergence at
843 different spatiotemporal scales in the grasshopper *Mioscirtus wagneri*
844 (Orthoptera: Acrididae). *Journal of Insect Conservation*, **16**, 103-110.
845 Ortego J, Bonal R, Cordero PJ, Aparicio JM (2009) Phylogeography of the Iberian
846 populations of *Mioscirtus wagneri* (Orthoptera: Acrididae), a specialized
847 grasshopper inhabiting highly fragmented hypersaline environments. *Biological*
848 *Journal of the Linnean Society*, **97**, 623-633.
849 Ortego J, Aguirre MP, Noguerales V Cordero PJ (2015) Consequences of extensive
850 habitat fragmentation in landscape-level patterns of genetic diversity and
851 structure in the Mediterranean esparto grasshopper. *Evolutionary Applications*,
852 **8**, 621-632.
853 Ouborg NJ, Pertoldi C, Loeschcke V, Bijlsma R, Hedrick PW (2010) Conservation
854 genetics in transition to conservation genomics. *Trends in Genetics*, **26**, 177-
855 187.
856 Palo JU, O'Hara RB, Laugen AT*, et al.* (2003) Latitudinal divergence of common frog
857 (*Rana temporaria*) life history traits by natural selection: evidence from a
858 comparison of molecular and quantitative genetic data. *Molecular Ecology*, **12**,
859 1963-1978.
860 Peres-Neto PR, Jackson DA (2001) How well do multivariate data sets match? The
861 advantages of a Procrustean superimposition approach over the Mantel test.
862 *Oecologia*, **129**, 169-178.
863 Phillipsen IC, Kirk EH, Bogan MT*, et al.* (2015) Dispersal ability and habitat
864 requirements determine landscape-level genetic patterns in desert aquatic
865 insects. *Molecular Ecology*, **24**, 54-69.

866 Phillipsen IC, Lytle DA (2013) Aquatic insects in a sea of desert: population genetic
867 structure is shaped by limited dispersal in a naturally fragmented landscape.
868 *Ecography*, **36**, 731-743.
869 Pickup M, Field DL, Rowell DM, Young AG (2012) Predicting local adaptation in
870 fragmented plant populations: implications for restoration genetics. *Evolutionary*
871 *Applications*, **5**, 913-924.
872 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
873 multilocus genotype data. *Genetics*, **155**, 945-959.
874 Quéméré E, Crouau-Roy B, Rabarivola C, Louis EE, Chikhi L (2010) Landscape
875 genetics of an endangered lemur (*Propithecus tattersalli*) within its entire
876 fragmented range. *Molecular Ecology*, **19**, 1606-1621.
877 R Development Core Team (2012) *R: a language and environment for statistical*
878 *computing. Version 3.0.2*. R Foundation for Statistical Computing, Vienna.
879 Available at: http://www.r-project.org.
880 Raeymaekers JAM, Van Houdt JKJ, Larmuseau MHD, Geldof S, Volckaert FAM
881 (2007) Divergent selection as revealed by P-ST and QTL-based F-ST in three-
882 spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland
883 gradient. *Molecular Ecology*, **16**, 891-905.
884 Reinhardt K, Kohler G, Maas S, Detzel P (2005) Low dispersal ability and habitat
885 specificity promote extinctions in rare but not in widespread species: the
886 Orthoptera of Germany. *Ecography*, **28**, 593-602.
887 Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.
888 Richardson JL (2012) Divergent landscape effects on population connectivity in two co-
889 occurring amphibian species. *Molecular Ecology*, **21**, 4437-4451.
890 Richardson JL, Urban MC, Bolnick DI, Skelly DK (2014) Microgeographic adaptation
891 and the spatial scale of evolution. *Trends in Ecology & Evolution*, **29**, 165-176.

892 Ripley B (2015) BOOT: Bootstrap Functions. R Package Version
893 1.3-17. Available at https://cran.r-project.org/web/packages/boot/
894 Rouget M, Cowling RM, Lombard AT, Knight AT, Graham IHK (2006) Designing
895 large-scale conservation corridors for pattern and process. *Conservation*
896 *Biology*, **20**, 549-561.
897 Saccheri I, Kuussaari M, Kankare M, *et al.* (1998) Inbreeding and extinction in a
898 butterfly metapopulation. *Nature*, **392**, 491-494.
899 Saether SA, Fiske P, Kalas JA, *et al.* (2007) Inferring local adaptation from Q(ST)-F-ST
900 comparisons: neutral genetic and quantitative trait variation in European
901 populations of great snipe. *Journal of Evolutionary Biology*, **20**, 1563-1576.
902 Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem
903 fragmentation - A review. *Conservation Biology*, **5**, 18-32.
904 Short AEZ, Caterino MS (2009) On the validity of habitat as a predictor of genetic
905 structure in aquatic systems: a comparative study using California water beetles.
906 *Molecular Ecology*, **18**, 403-414.
907 Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction
908 before genetic factors impact them. *Proceedings of the National Academy of*
909 *Sciences of the United States of America*, **101**, 15261-15264.
910 Spitze K (1993) Population-structure in *Daphnia obtusa* - Quantitative genetic and
911 allozymic variation. *Genetics*, **135**, 367-374.
912 Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013) MEGA6: Molecular
913 Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*,
914 **30**, 2725-2729.
915 Wang IJ (2009) Fine-scale population structure in a desert amphibian: landscape
916 genetics of the black toad (*Bufo exsul*). *Molecular Ecology*, **18**, 3847-3856.

Widmer I, Dal Grande F, Excoffier L, *et al.* (2012) European phylogeography of the epiphytic lichen fungus Lobaria pulmonaria and its green algal symbiont. *Molecular Ecology*, **21**, 5827-5844.

Willi Y, Hoffmann AA (2012) Microgeographic adaptation linked to forest fragmentation and habitat quality in the tropical fruit fly *Drosophila birchii*. *Oikos*, **121**, 1627-1637.

Willi Y, Van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 433-458.

Willi Y, Van Buskirk J, Schmid B, Fischer M (2007) Genetic isolation of fragmented populations is exacerbated by drift and selection. *Journal of Evolutionary Biology*, **20**, 534-542.

Zhao Y, Vrieling K, Liao H, *et al.* (2013) Are habitat fragmentation, local adaptation and isolation-by-distance driving population divergence in wild rice *Oryza rufipogon*? *Molecular Ecology*, **22**, 5531-5547.

Author contributions: J.O. and P.J.C. conceived the study. J.O. and V.G-N. designed the study and analysed the data. J. O. wrote the paper. J.O., V.N. and P.J.C. collected the samples.

Data accessibility

DNA sequences: GenBank Accession nos. KT380945-KT380946.

Phenotypic and genotypic data; DNA sequence alignments; phylogenetic tree file; input files for STRUCTURE analyses; input files for Mantel tests in ZT software: Dryad doi:10.5061/dryad.3nr2f

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci used to genotype each studied species.

Table S2 Pair-wise population F_{ST} and P_{ST} values for each studied species.

Table 1 Geographical location of the 12 micro-reserves from La Mancha region considered in this study and sample sizes (number of males/females in parentheses; only males were collected for Cb and Ci) and genetic variability (A_R) for each studied species.

Locality	Code	Latitude	Longitude	Sample size					Allelic richness (A_R)				
				Mw	Rh	Cb	Ci	Od	Mw	Rh	Cb	Ci	Od
Saladar de Ocaña	OCA	-3.630508	39.985445	20 (9/11)	20 (12/8)	20	-	-	5.68	8.02	9.96	-	-
Saladar de Huerta	HUE	-3.617103	39.838697	20 (10/10)	18 (15/3)	3	20	20 (11/9)	5.18	8.15	-	8.83	9.96
Laguna de Longar	LON	-3.321046	39.700548	20 (10/10)	20 (10/10)	20	20	18 (8/10)	5.02	7.91	9.78	8.79	9.39
Laguna de La Albardiosa	ALB	-3.288700	39.658024	20 (12/8)	20 (10/10)	17	20	19 (10/9)	5.42	7.51	9.69	8.40	10.60
Laguna Larga	LAR	-3.317164	39.609088	20 (16/4)	19 (11/8)	20	20	19 (15/4)	4.67	8.06	10.01	8.53	10.44
Laguna de Tírez	TIR	-3.354411	39.546603	20 (10/10)	19 (9/10)	8	17	22 (10/12)	5.38	7.93	8.48	9.00	10.27
Laguna de Palomares	PAL	-3.172344	39.535906	20 (10/10)	20 (10/10)	19	22	22 (13/9)	4.30	7.98	9.73	8.51	10.01
Laguna de Los Carros	CAR	-3.262528	39.472016	20 (10/10)	19 (9/10)	18	19	20 (10/10)	4.43	7.95	9.13	8.99	9.78
Laguna de Las Yeguas	YEG	-3.281576	39.418396	20 (10/10)	20 (10/10)	19	21	20 (10/10)	5.07	8.08	10.18	8.94	10.01
Laguna de Salicor	SCO	-3.173809	39.470083	22 (15/7)	20 (14/6)	20	20	22 (11/11)	4.03	8.18	10.07	9.22	10.01
Laguna de Alcahozo	ALC	-2.875947	39.391585	20 (10/10)	19 (15/4)	20	20	20 (10/10)	3.63	7.14	9.82	8.34	9.92
Saladar de El Pedernoso	PED	-2.767518	39.491164	20 (10/10)	20 (17/3)	20	20	19 (9/10)	3.45	8.43	9.44	8.85	10.34

Mw = *Mioscirtus wagneri*; Rh = *Ramburiella hispanica*; Cb = *Calliptamus barbarus*; Ci = *Calliptamus italicus*; Od = *Oedaleus decorus*

A_R, standardized allelic richness.

Table 2 Correlation coefficients (r) for Mantel test between (a) genetic (F_{ST}) and (b) phenotypic distance (P_{ST}) matrices of each species pair (below the diagonal) and for partial Mantel test controlling for geographical distance (above the diagonal); (c) Procrustes sum of squares (m^2) from PROTEST analyses. Values in bold are statistically significant after sequential Bonferroni correction ($\alpha = 0.05$).

(a) Mantel and partial Mantel tests (r) for F_{ST}

	Mw	Rh	Cb	Ci	Od
Mw	-	-0.083	0.125	**0.268**	0.040
Rh	0.158	-	-0.126	0.329	0.265
Cb	0.184	-0.075	-	0.004	0.422
Ci	**0.394**	**0.395**	0.046	-	0.281
Od	0.141	0.301	**0.435**	0.315	-

(b) Mantel and partial Mantel tests (r) for P_{ST}

	Mw	Rh	Cb	Ci	Od
Mw	-	0.142	0.320	0.036	0.001
Rh	0.126	-	0.146	-0.078	0.112
Cb	0.284	0.162	-	0.017	0.121
Ci	0.025	-0.070	0.031	-	0.234
Od	0.026	0.091	0.080	0.216	-

(c) PROTEST analyses (m^2)

	Mw	Rh	Cb	Ci	Od
Mw	-				
Rh	0.854	-			
Cb	0.894	0.704	-		
Ci	0.920	0.639	0.731	-	
Od	0.608	0.942	0.874	0.833	-

974 **Figure legends**

975

976 **Fig. 1** (a) Characteristics of the five studied species in terms of body size and wing
977 length, habitat specialization, and degree of fragmentation of their respective habitats in
978 the study area (photos by Pedro J. Cordero). The five species are co-distributed and
979 were sampled across a microreserve network located in La Mancha region, Central
980 Spain. The last column indicates the predicted patterns of genetic differentiation (F_{ST}),
981 genetic diversity (A_R) and phenotypic differentiation (P_{ST}) for each studied species; (b)
982 Maximum likelihood tree based on partial sequences of the 16S mitochondrial gene
983 showing the phylogenetic relationships among the five studied species. GenBank
984 accession numbers (in parentheses) and subfamilies for each species are also indicated.

985

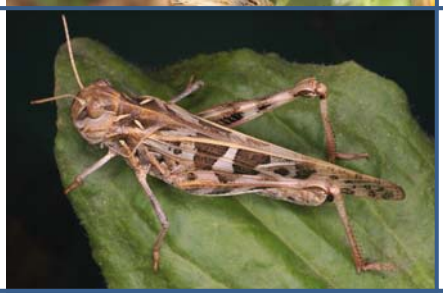
986 **Fig. 2** (a) Geographic location of sampling sites and (b-f) genetic assignment of
987 populations for each species based on the Bayesian method implemented in the program
988 STRUCTURE. The admixture proportions generated by STRUCTURE for each species were
989 represented using pie charts, with each colour indicating a different genotypic cluster.
990 Insets show the mean (± S.D.) log probability of the data (ln Pr(X|*K*)) over 10 runs (left
991 axis, black dots and error bars) for each value of *K* and the magnitude of Δ*K* as a
992 function of *K* (right axis, open dots). Population codes are described in Table 1.

993

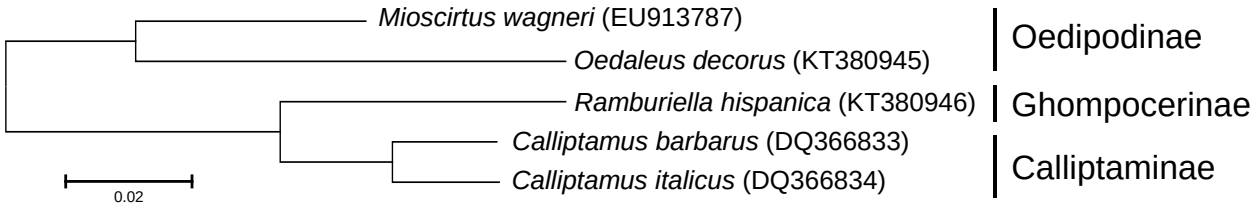
994 **Fig. 3** Allelic richness (A_R) (mean ± SE) for each studied species (Mw = *Mioscirtus*
995 *wagneri*; Rh = *Ramburiella hispanica*; Cb = *Calliptamus barbarus*; Ci = *Calliptamus*
996 *italicus*; Od = *Oedaleus decorus*), including all populations (black bars) or only the ten
997 populations where all the taxa were sampled (white bars).

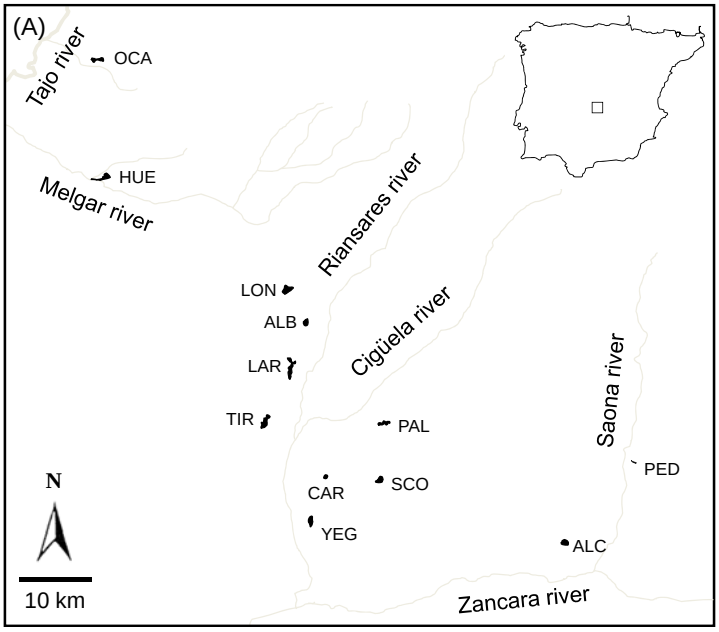
998

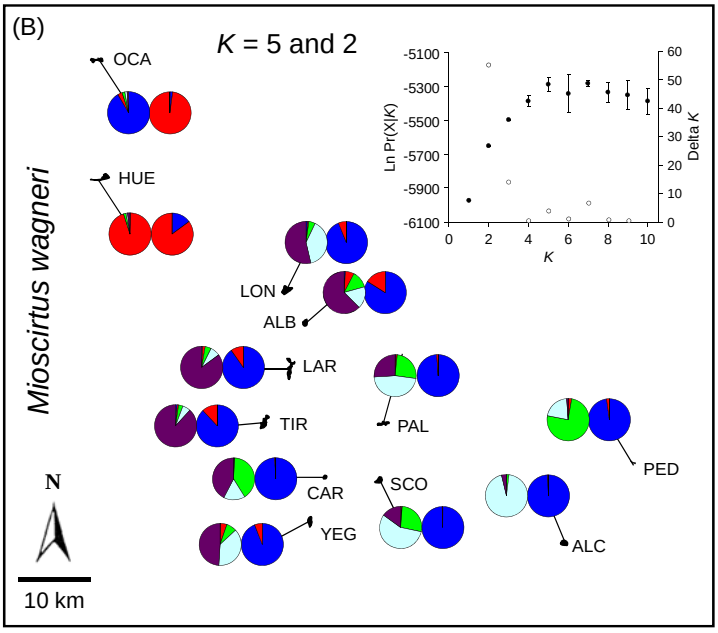
999 **Fig. 4** Global (a) F_{ST} and (b) P_{ST} values and 95% confidence intervals for each studied
1000 species (Mw = *Mioscirtus wagneri*; Rh = *Ramburiella hispanica*; Cb = *Calliptamus*
1001 *barbarus*; Ci = *Calliptamus italicus*; Od = *Oedaleus decorus*), including all populations
1002 (black squares) or only the ten populations where all the taxa were sampled (white
1003 squares).

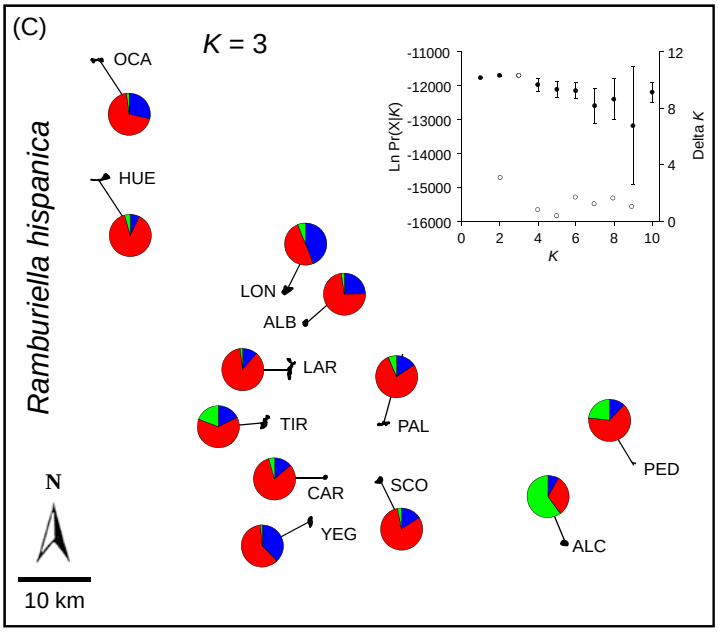
(a) Species		Body size and wing length	Habitat specialization	Habitat fragmentation	Predictions F_{ST} A_R P_{ST}
Mioscirtus wagneri		Small size	One plant species	Highly fragmented (shrubby sea-blite formations)	↑ ↓ ↑
Ramburiella hipanica		Medium size	Two plant species	Highly fragmented (esparto grass formations)	↑ ↓ ↑
Calliptamus barbarus		Medium size	Multiple plant species	Highly fragmented (non-agricultural lands)	↑ ↓ ↑
Calliptamus italicus		Medium size	Multiple plant species	Continuous (agricultural lands+ natural habitats)	↓ ↑ ↓
Oedaleus decorus		Large size	Multiple plant species	Continuous (agricultural lands + natural habitats)	↓ ↑ ↓

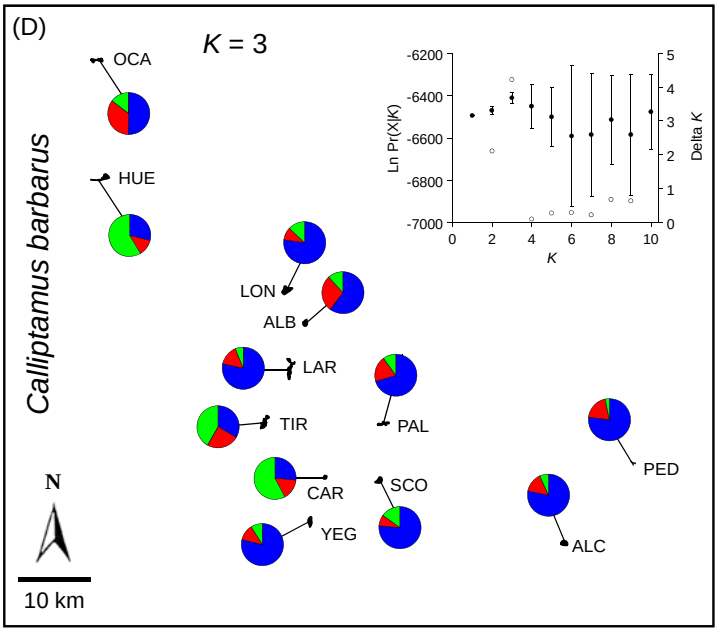
(b)

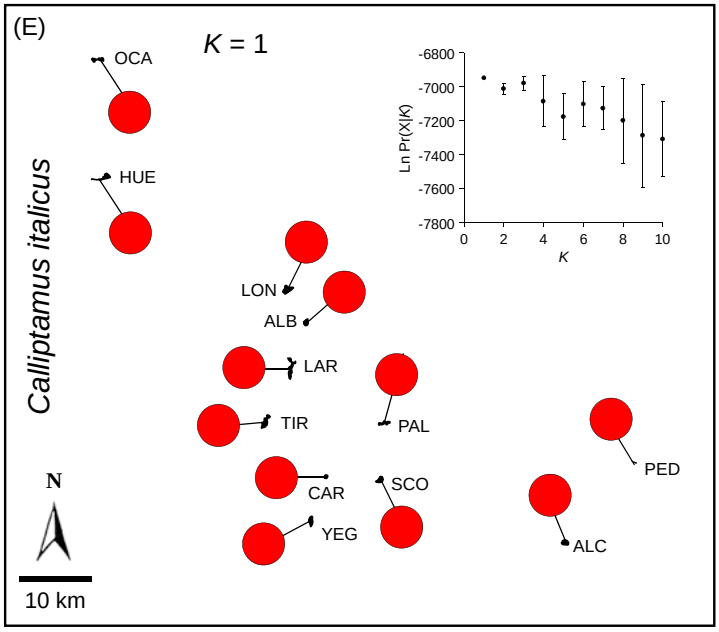


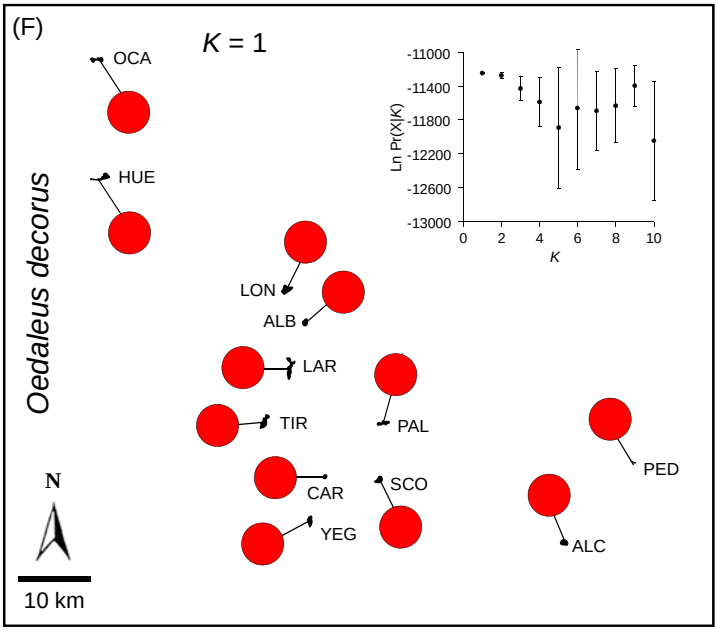

(A)
Tajo river
OCA
HUE
Melgar river
Riansares river
LON
ALB
Cigüela river
LAR
TIR
PAL
Saona river
PED
CAR
SCO
YEG
ALC
Zancara river
N
10 km

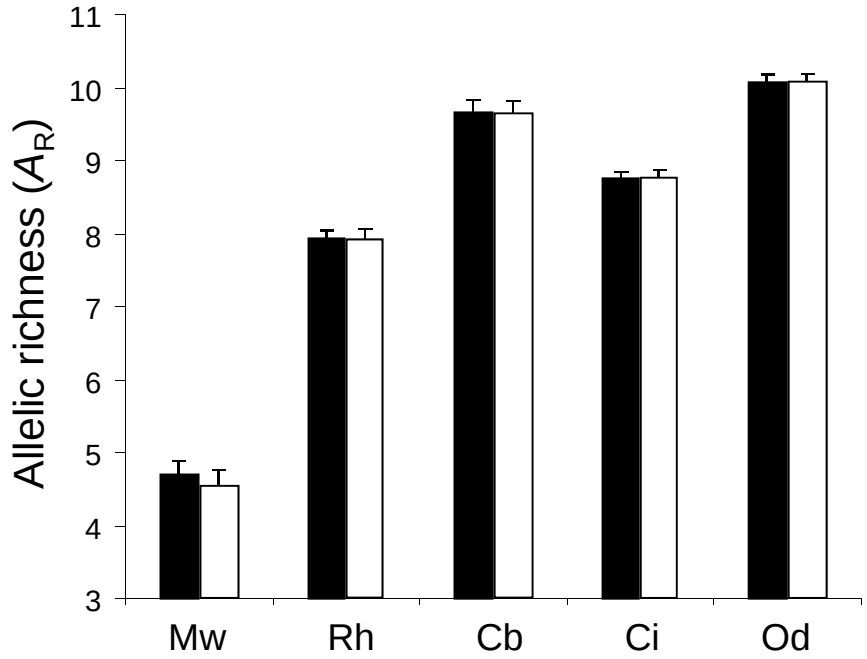

(B)
K = 5 and 2
Miosciritus wagneri
OCA
HUE
LON
ALB
LAR
TIR
PAL
PED
CAR
SCO
YEG
ALC
Ln Pr(X|K)
Delta K
K
N
10 km

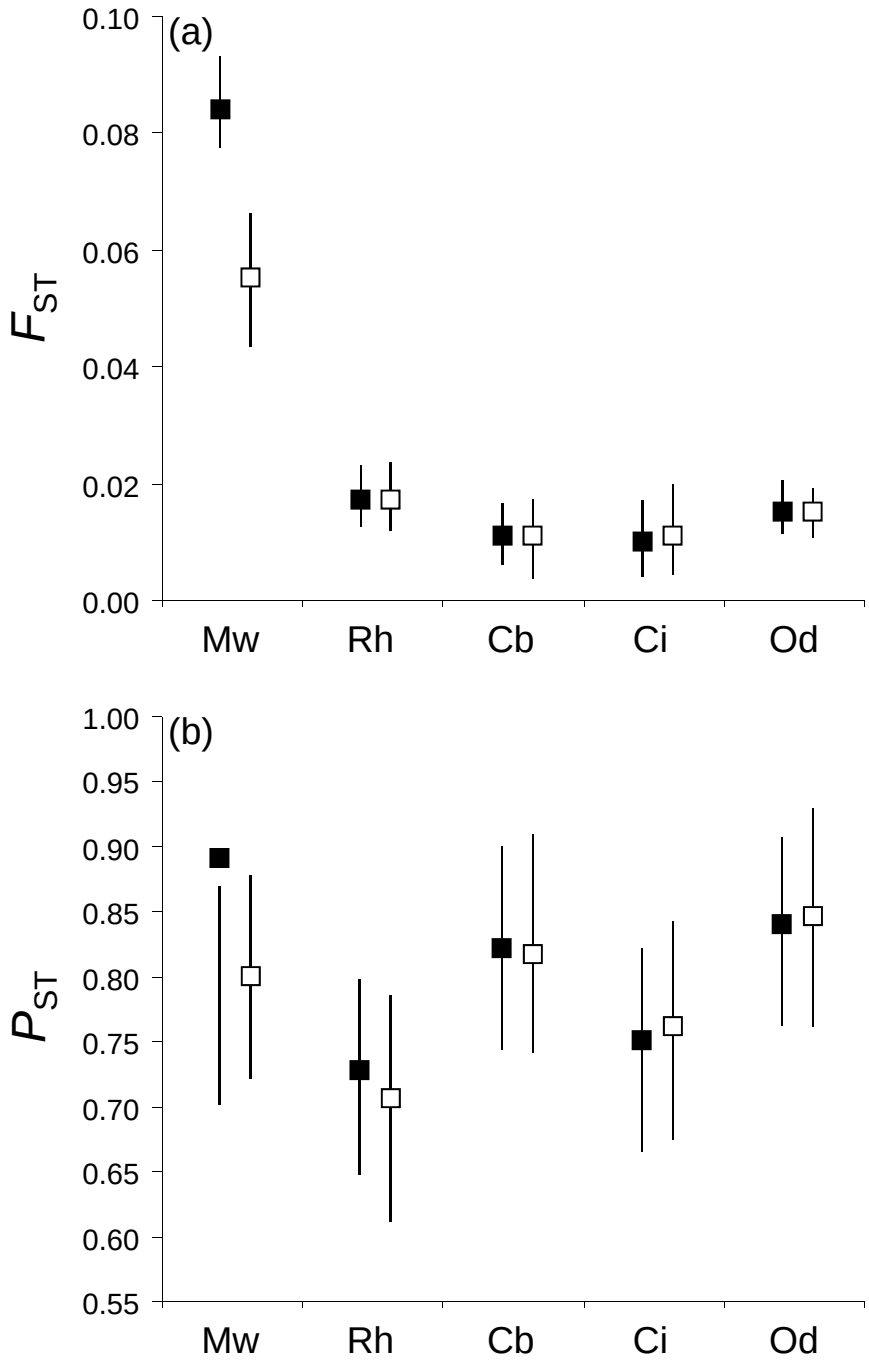

(C)
K = 3
Ramburiella hispanica
OCA
HUE
LON
ALB
LAR
TIR
PAL
PED
CAR
SCO
YEG
ALC
Ln Pr(X|K)
Delta K
K
N
10 km


(D)
K = 3
Calliptamus barbarus
OCA
HUE
LON
ALB
LAR
TIR
PAL
PED
CAR
SCO
YEG
ALC
Ln Pr(X|K)
Delta K
K
N
10 km


(E)
K = 1
Calliptamus italicus
OCA
HUE
LON
ALB
LAR
TIR
PAL
PED
CAR
SCO
YEG
ALC
Ln Pr(X|K)
K
N
10 km


(F)
K = 1
Oedaleus decorus
OCA
HUE
LON
ALB
LAR
TIR
PAL
PED
CAR
SCO
YEG
ALC
Ln Pr(X|K)
K
N
10 km


Allelic richness (A_R)
11
10
9
8
7
6
5
4
3
Mw
Rh
Cb
Ci
Od


(a)
0.10
0.08
0.06
0.04
0.02
0.00
F_{ST}
Mw
Rh
Cb
Ci
Od
(b)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
P_{ST}
Mw
Rh
Cb
Ci
Od