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Interspecific interactions influence contrasting spatial genetic structures in two closely related damselfly species

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1 Title: **Interspecific interactions influence contrasting spatial genetic**
2 **structures in two closely related damselfly species**

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21 Running title: *Contrasting genetic structures in Calopteryx*

22 *ABSTRACT*

23 Spatial genetic structure (SGS) is largely determined by colonization history, landscape and
24 ecological characteristics of the species. Therefore, sympatric and ecologically similar species
25 are expected to exhibit similar SGSs, potentially enabling prediction of the SGS of one
26 species from that of another. On the other hand, due to interspecific interactions, ecologically
27 similar species could have different SGSs. We explored the SGSs of the closely related
28 *Calopteryx splendens* and *C. virgo* within Finland and related the genetic patterns to
29 characteristics of the sampling localities. We observed different SGSs for the two species.
30 Genetic differentiation even within short distances in *C. splendens* suggests genetic drift as an
31 important driver. However, we also observed indication of previous gene flow (revealed by a
32 negative relationship between genetic differentiation and increasing potential connectivity of
33 the landscape). Interestingly, genetic diversity of *C. splendens* was negatively related to
34 density of *C. virgo*, suggesting that interspecific interactions influence the SGS of *C.*
35 *splendens*. In contrast, genetic differentiation between *C. virgo* sub-populations was low and
36 only exhibited relationships with latitude, pointing to high gene flow, colonization history and
37 range margin effects as the drivers of SGS. The different SGSs of the two ecologically similar
38 species cautions indirect inferences of SGS based on ecologically similar surrogate species.

39 *INTRODUCTION*

40 The spatial distribution of genetic diversity is rarely homogenous, but exhibits pronounced
41 geographic variation (Eckert *et al.* 2008). The distribution of genetic diversity within and
42 between populations, i.e. spatial genetic structure (SGS), is driven by historic contingencies
43 such as range contractions and expansions due to glacial cycles (Hewitt 2000) and
44 contemporary processes of random genetic drift (Kimura & Crow 1964), gene flow between
45 populations (Slatkin 1985; Bohonak 1999), and selection (Ranta *et al.* 2009; Funk *et al.*
46 2011). Since these drivers of SGS are strongly influenced by the ecology of the species (e.g.
47 habitat preferences, dispersal abilities, mating system and life-history characteristics) and by
48 environmental characteristics of the landscape (e.g. the amount of habitat suitable for
49 population persistence and dispersal), sympatric and ecologically similar species can be
50 expected to exhibit similar SGSs (Whiteley *et al.* 2006; Fortuna *et al.* 2009; Dawson 2012;
51 Hughes *et al.* 2013).

52 On the other hand, increasing ecological similarity of species can lead to increasing
53 interspecific interactions, and thus affect their SGSs. For example, in a simulation, increasing
54 the strength of interspecific competition and the number of competing species decreased both
55 effective population sizes and dispersal between localities, leading to increased genetic drift
56 and differentiation between populations (Ranta *et al.* 2009). Similarly, increasing the number
57 of competitor species led to decreased population sizes and increased genetic drift of
58 experimental perennial ryegrass (*Lolium perenne*) populations (Nestmann *et al.* 2011).

59 Given that the SGS of species is important for determining management units and predicting
60 the outcomes of land use on the viability and evolutionary potential of populations (Frankham

61 2005; Whiteley *et al.* 2006; Fortuna *et al.* 2009) the generalizability of SGS across sympatric
62 and ecologically similar species is important also for applied conservation projects. If SGSs
63 can be generalized across species, SGSs can be predicted for other sympatric and ecologically
64 similar species based on that of only one (or a few) representative species. This implies that
65 conservation strategies designed for safeguarding the viability and evolutionary potential of a
66 single species could be effective in conserving other species as well (Whiteley *et al.* 2006).
67 On the other hand, if interspecific interactions between the focal species lead to differences in
68 their SGSs, conservation strategies targeted for single species systems might not be adequate.
69 In these cases the SGSs of each species of conservation concern would need to be determined
70 separately.

71 We studied the SGSs of *Calopteryx splendens* [HARRIS, 1782] and *C. virgo* [LINNAEUS,
72 1758] in Finland (Fig. 1). The two damselfly species provide a suitable system for studying
73 the generalizability of SGS across species with largely similar ecologies, since they are
74 considerably similar in their appearance, behavior, life-history and habitat choice (Askew
75 2004; Wellenreuther *et al.* 2012; Karjalainen & Hämäläinen 2013; but see Sternberg &
76 Buchwald 1999). To fully describe the SGSs of both species we determined how genetic
77 variation is distributed within (allelic richness and heterozygosity) and between sub-
78 populations (isolation-by-distance, population specific F_{ST} s, pairwise F_{ST} s and D_{est} s, and
79 number of genetic clusters). From each sub-population, we also recorded locality
80 characteristics (latitude and longitude, density of conspecific males and connectivity of the
81 landscape), which we considered to be associated with colonization history, random genetic
82 drift and dispersal between localities. To estimate the frequency of interspecific interactions
83 experienced, we recorded the density of heterospecific *Calopteryx* males at each sub-
84 population. Our aims were to see (1) if the SGSs of the two *Calopteryx* species are

85 concordant, (2) if the SGSs of the species have similar relationships with the characteristics of
86 the localities, and (3) if the SGSs of the two species are affected by interspecific interactions.

87 *METHODS*

88 ***Calopteryx* species**

89 The Eurasian *C. splendens* and *C. virgo* have largely overlapping biogeographic ranges with
90 both species inhabiting small rivers and streams across most of Europe (Askew 2004;
91 Karjalainen & Hämäläinen 2013). Within their sympatric range in southern Fennoscandia, the
92 environmental niches of the *Calopteryx* species also overlap, with the niche of *C. splendens*
93 being nested within that of *C. virgo* (Wellenreuther *et al.* 2012). However, some habitat
94 partitioning between the two species has been reported in Central Europe, where *C. splendens*
95 is most often found in slightly warmer (18-24°C) lowland rivers and *C. virgo* is found in
96 cooler and more shaded rivers (13-18°C) at higher elevations (Sternberg & Buchwald 1999;
97 Schütte & Schrimpf 2002). The different temperature affinities of the two species are
98 reflected in differences in their range limits in the north: the range of *C. splendens* extends to
99 roughly 64° Lat., whereas the range of *C. virgo* extends to 68° Lat. (Valle 1952; Karjalainen
100 2010; Karjalainen & Hämäläinen 2013). However, although *C. virgo* seems to occupy a wider
101 range of water temperatures, we found no habitat partitioning with respect to water
102 temperature in the two *Calopteryx* species at our study sites (Supplementary material A).
103 Also, since the wings of *C. virgo* are more melanised than those of *C. splendens*, it has been
104 suggested that *C. virgo* has better thermoregulation abilities allowing them to adapt to colder
105 environments (Outomuro & Ocharan 2011). However, recent work concluded that differences

106 in wing melanisation between the *Calopteryx* species is primarily driven by sexual selection,
107 and that it is less important for adaptation to colder environments (Svensson & Waller 2013).

108 Capture-mark-recapture studies indicate that sexually mature individuals of both *Calopteryx*
109 species rarely disperse more than 300 meters from the site of first observation (Stettmer 1996;
110 Schutte *et al.* 1997; Ward & Mill 2007). The longest observed dispersal distances of adult
111 *Calopteryx* individuals have been 1.7 km and 4 km for *C. splendens* and *C. virgo*, respectively
112 (Stettmer 1996). We are not aware of studies explicitly focusing on dispersal prior to sexual
113 maturation (i.e. natal dispersal), but we expect it to be limited since significant population
114 differentiation has been reported for *C. splendens* (overall F_{ST} s range from 0.05 to 0.14 in
115 different studies; Svensson *et al.* 2004; Chaput-Bardy *et al.* 2008; Viitaniemi 2009). However,
116 we are not aware of any study on the SGS of *C. virgo*.

117 In Finland, *C. splendens* sub-populations are nearly exclusively sympatric with *C. virgo*,
118 although *C. virgo* sub-populations can be locally allopatric within the distribution range of *C.*
119 *splendens* (Tynkkynen *et al.* 2004; but see Ilvonen *et al.* 2011). The species frequently
120 interact in locally sympatric sub-populations and occasionally hybridize (Svensson *et al.*
121 2007; Keränen *et al.* 2013). Both species are territorial, and the males exhibit interspecific
122 aggression when competing for breeding territories (De Marchi 1990; Tynkkynen *et al.* 2004,
123 2006). *C. virgo* seems to be the stronger competitor (Tynkkynen *et al.* 2004, 2006), and
124 interspecific interactions with *C. virgo* are known to influence the secondary sexual characters
125 of *C. splendens* (Tynkkynen *et al.* 2005; Honkavaara *et al.* 2011; Kuitunen *et al.* 2011). Since
126 the mating success of *Calopteryx* males is related to their territory holding ability (Plaistow &
127 Siva-Jothy 1996), interspecific competition could reduce the effective population size of the
128 weaker competitor. Indeed, a previous study suggested that the genetic diversity of *C.*

129 *splendens* declines as the proportion of *C. virgo* individuals in sympatric sub-populations
130 increases (Viitaniemi 2009).

131 **Sampling localities and collecting *Calopteryx* individuals**

132 Sampling was conducted between 27th of June and 29th of July, and between 23rd of June and
133 28th of July, in 2008 and 2009, respectively. Nineteen of 40 sampling localities contained both
134 *C. splendens* and *C. virgo* (i.e. sympatric sub-populations) and the others contained only *C.*
135 *virgo* (i.e. allopatric sub-populations), nine of which were located in Northern Finland,
136 outside the range of *C. splendens* (Fig. 1). Considering the relatively poor dispersal
137 capabilities of *Calopteryx* damselflies (see above), we decided to sample sub-populations
138 from different rivers separated by at least one lake and a minimum distance of ten kilometers,
139 an exception being *C. virgo* sub-populations AJ and AK (Fig. 1; Supplementary material B,
140 Table B1), which were only 4.9 km apart.

141 *Calopteryx* individuals were collected using butterfly nets. Either whole individuals or a
142 single leg per individual (if population sizes seemed low) were stored in 95% ethanol (EtOH)
143 at 6-8°C until DNA extraction. Removing a leg from damselflies does not impact fitness
144 (Thompson *et al.* 2011). Since only two *C. splendens* individuals were collected from sub-
145 population BG, these individuals were not genotyped or included in the analyses. Therefore,
146 the sample size for *C. splendens* sub-populations was 18. For most sub-populations,
147 genotyped individuals were collected in either 2008 or 2009, but for populations AA, AC, AD
148 and AK (Supplementary material B, Table B1.) we genotyped individuals collected in both
149 years to test for temporal population structure (Supplementary material C).

150 **Recording locality characteristics**

151 Geographical coordinates were recorded on site with a hand-held GPS device (Garmin eTrex
152 Legend HCx or Magellan Triton 300). Latitude and longitude were used in our analyses as
153 UTM coordinates according to grid ETRS-TM35FIN. *Calopteryx* densities were estimated by
154 counting all individuals within a 300-meter section of river, which was defined as 150 meters
155 both up- and downstream from the site of the perceived highest density of *Calopteryx*
156 individuals. Although both male and female density were estimated, only male densities were
157 included in the analyses, since females frequently move away from rivers in response to
158 changing weather, making estimates of their density less reliable (K. Kuitunen, pers. obs.).
159 All counts were recorded between 9 AM and 4 PM, when the damselflies are most active
160 (Corbet 1999; Karjalainen & Hämäläinen 2013). Densities at most sites were estimated at
161 least twice during the season and some were also estimated in both years (Supplementary
162 material B, Table B1). Repeatability of the density estimates was high (*C. splendens*:
163 repeatability=0.64, $F=5.745$, $df_1=16$, $df_2=29$, $n_0=2.63$; *C. virgo*: repeatability=0.62, $F=5.477$,
164 $df_1=26$, $df_2=47$, $n_0=2.77$; Lessells & Boag 1987). We used logarithm-transformed (\log_{10})
165 averages of the multiple estimates in our analyses. In the case of *C. splendens*, we added one
166 to each density estimate before transformation, since several sites did not contain a single *C.*
167 *splendens* individual. For simplicity, the logarithm-transformed density estimates are
168 hereafter referred to simply as density estimates.

169 To obtain a locality specific measure for potential connectivity we quantified the total length
170 of river habitat within a five-kilometer radius buffer zone from each sampling site. Width of
171 river habitat was not included because breeding sites of *Calopteryx* are mainly found along
172 the shoreline. Measurements were made from a combination of two map layers (“River

173 Network” & “Water formations, EU Water Framework Directive”, provided by The Finnish
174 Environment Institute & Centres for Economic Development, Transport and the
175 Environment) using ArcGIS 10.1 (ESRI 2012. ArcGIS Desktop: Release 10. Redlands, CA:
176 Environmental Systems Research Institute). Our measure reflects potential connectivity
177 because it does not consider whether the habitat contains *Calopteryx* individuals (Calabrese &
178 Fagan 2004; Hughes *et al.* 2013). Our measure neglects the potentially important branching
179 structure of the rivers (Chaput-Bardy *et al.* 2008), however, it more accurately reflects our
180 sampling scheme, given that we sampled sub-populations residing in different rivers rather
181 than different tributaries of the same river. The five kilometer radius buffer should sufficiently
182 represent potential connectivity since this distance is at the higher range of dispersal for both
183 *C. virgo* and *C. splendens* (Stettmer 1996; Schutte *et al.* 1997).

184 Prior to incorporating any of the variables into the analyses we checked the data for
185 collinearity and spatial autocorrelation (Moran’s I). Collinearity was tested by calculating
186 variance inflation factors (VIF) for all variables in R (R Core Team 2012), and spatial
187 autocorrelation was tested across the entire 40 site dataset using SAM 4.0 (Rangel *et al.*
188 2010). VIFs were low for all variables (all below 2), suggesting no collinearity. However, a
189 negative spatial autocorrelation (Moran’s I = -0.321; P=0.005) was observed in the density of
190 *C. splendens* males in the longest distance class (center at c.a. 607 km). This occurred because
191 nine of the sampled localities (*C. virgo* sub-populations) were outside the distribution range of
192 *C. splendens*, resulting in spatial aggregation of localities with zero density of *C. splendens*
193 (Fig. 1). Thus, we analyzed a separate subset of the *C. virgo* data containing only the
194 sympatric populations (see below). There was no spatial autocorrelation in any of the other
195 variables.

196 **Population Genetic Methods**

197 DNA was extracted from the legs of *Calopteryx* individuals and used for genotyping of
198 **microsatellite loci** as described in Supplementary material C and Molecular Ecology
199 Resources Primer Development Consortium et al. (2011). The microsatellite loci were
200 assessed for reliable scoring and potential deviations from equilibrium and neutrality
201 assumptions (Supplementary material C). Finally, the *C. splendens* dataset included 567
202 individuals genotyped at 12 loci and the *C. virgo* dataset included 1401 individuals genotyped
203 at six loci (Supplementary material C). For each locus and subpopulation, unbiased expected
204 heterozygosity (uH_E) estimated using GenAlEx 6.5 (Peakall & Smouse 2012) and allelic
205 richness (AR) adjusted by rarefaction was estimated using FSTAT 2.9.3 (updated from
206 Goudet 1995; See Supplementary material C, Tables C3 and C4).

207 **Statistical Methods**

208 We studied how environmental characteristics (see above) and density of heterospecific males
209 are related to intrapopulation genetic diversity as measured by uH_E and AR , using a beta
210 regression and a linear multiple regression, respectively. Analyses were conducted using R
211 and the package “betareg” (Cribari-Neto & Zeileis 2010) was used for the beta regression. All
212 independent variables were initially entered into the model and then removed on a stepwise
213 basis by comparing nested models with a log-likelihood ratio test.

214 To study how genetic diversity was distributed among populations (i.e. population
215 differentiation), we tested for isolation-by-distance (IBD), pairwise genetic differentiation
216 between sub-populations (both F_{ST} and D_{est} , Weir & Cockerham 1984; Jost 2008), and

217 conducted individual-based spatial Bayesian clustering analyses. IBD was tested with a
218 permutation test of the relationship between the Euclidean geographic distance matrix (both
219 untransformed distances in kilometers and logarithm transformed distances) and the pairwise
220 relatedness matrix (F_{ij} ; Ritland 1996) with 5000 randomizations using SPAGeDi 1.4 (Hardy
221 & Vekemans 2002). To visualize the relationship, the pairwise F_{ij} s were divided into distance
222 classes with approximately equal numbers of comparisons and averaged to acquire a distance
223 class specific $F_{(d)}$. We estimated the pairwise differentiation coefficients and their 95%
224 confidence intervals using the R package “diveRsity” (Keenan *et al.* 2013) with 5000
225 bootstraps for both estimates and for both species.

226 The individual based Bayesian clustering was conducted in the program TESS 2.3.1 (Chen *et*
227 *al.* 2007; Durand *et al.* 2009). Because all individuals from the same sub-population shared
228 the same coordinates, we randomized geographic coordinates for each individual in TESS
229 2.3.1. Specifically, latitude and longitude were allowed to vary a maximum of one kilometer
230 from the sampling site coordinates, with a standard deviation of 300 meters. Given what is
231 known about the dispersal of *Calopteryx* individuals (Stettmer 1996; Schutte *et al.* 1997;
232 Ward & Mill 2007), we considered these parameters for the randomization to be biologically
233 realistic. Assuming a conditional autoregressive Gaussian admixture model with a quadratic
234 trend surface degree, we ran TESS 2.3.1 for 50 000 iterations (preceded by a 50 000 iteration
235 burn-in) 20 times for each maximum number of clusters (K_{max}), which ranged from two to the
236 number of sampling localities ($K_{max}=2\dots18$, and $K_{max}=2\dots40$, for *C. splendens* and *C. virgo*,
237 respectively). We used deviance information criterion (*DIC*) values and stabilization of the *Q*-
238 matrix of posterior probabilities (i.e. when the number of observed clusters did not increase
239 with an increase in K_{max}) as metrics for choosing the most appropriate K_{max} for the data. An

240 admixture model was assumed since such models are expected to be more robust against
241 overestimation of K_{max} when there are genetic clines (Guillot 2009; François & Durand 2010).
242 Once K_{max} was deduced, 80 additional replicate runs were conducted to yield a total of 100
243 replicate runs for K_{max} . From these, 20 runs with the lowest DIC values were used to calculate
244 average individual admixture proportions with CLUMPP 1.1.2 (Jakobsson & Rosenberg
245 2007). These were visualized as a bar plot using DISTRUCT 1.1 (Rosenberg 2004).

246 To study how genetic differentiation is related to the locality characteristics and density of
247 heterospecific *Calopteryx* males, we used a Bayesian population based analysis of genetic
248 differentiation in the program GESTE2 (Foll & Gaggiotti 2006). GESTE2 incorporates non-
249 genetic information in the prior distribution of population specific F_{ST} -values via a
250 generalized linear model (Balding 2003; Foll & Gaggiotti 2006). GESTE2 analyses were run
251 for a total of 100 000 iterations with a 50 000 iteration burn-in period.

252 Since the sizes of the two datasets differed both in the amount of genetic information derived
253 from each individual (12 loci vs. 6 loci for *C. splendens* and *C. virgo*, respectively) and in
254 their geographic scope (18 sub-populations spanning c.a. 270km vs. 40 sub-populations
255 spanning c.a. 700km for *C. splendens* and *C. virgo*, respectively), subsets of the data were
256 analyzed to confirm the results obtained from the total dataset. To do this, we conducted
257 clustering in TESS for five subsets of the *C. splendens* dataset: three subsets each with six
258 randomly selected loci, one subset with the same loci that were used in the *C. virgo* dataset,
259 and one subset excluding the loci that showed some deviations from the assumptions (see
260 above; Supplementary material F, Fig. F1) In addition, a subset of the *C. virgo* dataset
261 including only the sympatric populations was analyzed. This was done for two reasons: (1) to
262 check if possible differences in the SGSs of the two *Calopteryx* species are associated with

263 the different geographic scales of sampling and different numbers of sampled sub-
264 populations, and (2) to account for the negative spatial autocorrelation in the density of *C.*
265 *splendens* males (see above).

266 RESULTS

267 The genetic diversities of the two species were related to different locality characteristics. The
268 uH_E of *C. splendens* sub-populations was related to potential connectivity, with increasing
269 potential connectivity being coupled with increasing uH_E (Table 1). Interestingly, *C.*
270 *splendens* uH_E was differentially related to the densities of conspecific versus heterospecific
271 males: increasing density of conspecific males was coupled with increasing uH_E , whereas the
272 increasing density of heterospecific males was coupled with decreasing uH_E . Surprisingly,
273 none of the locality characteristics were related to the AR of *C. splendens*. The genetic
274 diversity of *C. virgo* (both uH_E and AR) was related to latitude and longitude, with genetic
275 diversity increasing with decreasing latitude and increasing longitude (Table 1).

276 There was no statistically significant IBD pattern in pairwise individual kinship coefficients
277 (F_{ij}) of *C. splendens* (Fig 2a; jackknifed $\beta = -6.32 \times 10^{-5}$, S.E. = 1.34×10^{-5} ; 95% CI of permuted
278 null distribution = $[-6.67 \times 10^{-5}, -3.55 \times 10^{-5}]$; $P = 0.129$), although there was a distinct decrease
279 in the kinship coefficients after the first distance class. On the other hand, for *C. virgo*,
280 kinship coefficients decreased gradually from the first distance class onwards, and a
281 statistically significant IBD pattern was observed (Fig 2b; $\beta = -7.89 \times 10^{-6}$, S.E. = 3.16×10^{-6} ; 95%
282 CI of permuted null distribution = $[-2.31 \times 10^{-6}, -1.62 \times 10^{-7}]$; $P < 0.001$). However, although a
283 statistically significant IBD is observed, it must be noted that the F_{ij} values for *C. virgo* are
284 very low.

285 Furthermore, different levels of genetic differentiation were observed in the two species, both
286 in comparisons of pairwise genetic differentiation and in individual based clustering analyses.
287 For most of the pairwise comparisons between the *C. splendens* sub-populations, the lower
288 boundary of the 95% confidence interval was well above 0.01 for both F_{ST} and D_{est}
289 (Supplementary material D, Tables D1 and D2), whereas for majority of the pairwise
290 comparisons between *C. virgo* sub-populations, the 95% confidence intervals either included,
291 or were very close to zero at the lower boundaries (Supplementary material D, Tables D3 and
292 D4). Moreover, the individual based genetic clustering in TESS resulted in a different K_{max} for
293 the two species. For *C. splendens* six genetic clusters were detected, whereas for *C. virgo* only
294 two clusters were detected (Fig. 3). Five of the six *C. splendens* clusters each had a high
295 affinity with a single sub-population, and the remaining cluster had a high affinity with five
296 sub-populations in central Finland (Fig. 3a). In *C. virgo* the two genetic clusters seemed to
297 represent two ends of a cline, with one cluster having a high affinity with nine sub-
298 populations from southern and eastern Finland and the other with ten sub-populations in
299 northern Finland. Sub-populations geographically intermediate to those in the two clusters
300 showed different levels of admixture (Fig. 3b).

301 In addition to differences in the number of genetic clusters observed for the two *Calopteryx*
302 species, differentiation of the sub-populations was related to different locality characteristics
303 (Tables 1, 2 & 3; Fig. 4). In the GESTE2 analysis of *C. splendens* population specific F_{STs} ,
304 the models with potential connectivity were assigned higher posterior probabilities than most
305 other models (Table 2) resulting in a summed posterior probability of 0.605 for potential
306 connectivity. Of all the models, a model with constant and potential connectivity received the
307 highest posterior probability, indicating that population specific F_{ST} decreases with increasing

308 potential connectivity (Table 3; Fig. 4a). For *C. virgo*, however, latitude received the highest
309 summed posterior probability (0.994) and a model with constant and latitude had the highest
310 posterior probability, indicating that F_{ST} increases with increasing latitude (Table 3; Fig. 4b).
311 The population specific F_{ST} s of the *C. virgo* sub-populations were also lower than those of *C.*
312 *splendens* sub-populations (Fig. 4).

313 The difference between the two species in the number of observed genetic clusters was most
314 likely not an artifact of a difference in the number of microsatellite loci analyzed, since all of
315 the *C. splendens* subsets also detected more than two clusters (observed K_{max} was 3, 3, 5, 4
316 and 4 in the five respective subsets; Supplementary material F, Fig. F1). Also, the IBD pattern
317 observed in the SGS of *C. virgo* remained statistically significant even when the dataset was
318 reduced to a subset including only the sub-populations that are sympatric with *C. splendens*
319 ($\beta = -7.16 \times 10^{-6}$, S.E. = 3.49×10^{-6} ; 95% CI of permuted null distribution = $[-5.65 \times 10^{-6}, 3.98 \times 10^{-7}]$;
320 $P = 0.004$). However, other patterns observed from the *C. virgo* dataset disappeared when
321 the subset of sympatric sub-populations was analyzed: null models were selected for
322 population specific F_{ST} , uH_E and AR (Supplementary material F, Tables F1 and F2).

323 *DISCUSSION*

324 Despite the ecological similarities of the sympatric *C. splendens* and *C. virgo* in Finland, they
325 exhibited markedly different spatial genetic structures (SGS). Rather than supporting the
326 hypothesis that sympatric species with similar ecologies exhibit similar SGS (Whiteley *et al.*
327 2006; Dawson 2012; Hughes *et al.* 2013), our results highlight the possibility that ecological
328 similarity can, in fact, create differences in the SGS of interacting species.

329 *C. splendens*

330 The positive relationship between unbiased expected heterozygosity (uH_E) and density of
331 conspecific males (Table 1) together with multiple genetic clusters, most corresponding to
332 individual sub-populations (Fig. 3), indicate predominant effects of drift and low gene flow
333 on SGS in *C. splendens*. Nonetheless, some legacy of previous gene flow is apparent in the
334 positive relationship between potential connectivity (i.e. total length of streams within a 5 km
335 buffer zone) and uH_E and the negative relationship between potential connectivity and
336 population specific F_{ST} (Tables 1 & 3; Fig. 4a). *C. splendens* apparently rarely disperse more
337 than few kilometers and prefer dispersal along streams (Stettmer 1996; Schutte *et al.* 1997;
338 Ward & Mill 2007; Chaput-Bardy *et al.* 2008). Previous studies have also found dispersing *C.*
339 *splendens* to have lower mating success than residents, which can further inhibit gene flow
340 and strengthen differentiation between *C. splendens* sub-populations (Svensson *et al.* 2006;
341 Wellenreuther *et al.* 2010).

342 In contrast with previous population genetic studies on this species (Svensson *et al.* 2004;
343 Chaput-Bardy *et al.* 2008; Viitaniemi 2009) we did not observe IBD (Fig. 2). The absence of

344 IBD could be attributed to genetic disequilibrium due to recent colonization followed by rapid
345 range expansion (e.g. as seen in *Erythromma viridulum*, Watts *et al.* 2010; and *Coenagrion*
346 *sticulum*, Swaegers *et al.* 2013) or strong overall differentiation due to range-margin effects
347 (Eckert *et al.* 2008). Regarding recent colonization, no major shifts in the species range in
348 Finland have been recorded in recent decades. Although post-glacial colonization can still be
349 reflected in the SGS, it remains unclear if short-term effects (i.e. within the range of decades;
350 Watts *et al.* 2010; Swaegers *et al.* 2013) influence the SGS of *C. splendens*. Regarding range-
351 margin effects, the overall genetic differentiation of *C. splendens* observed here did not differ
352 greatly from that observed in different regions (overall $F_{ST}=0.11$ vs. 0.05 in southern Sweden
353 and 0.14 in western France; Svensson *et al.* 2004; Chaput-Bardy *et al.* 2008). Although our
354 results cannot be directly compared to those of previous studies because different types of
355 markers with different levels of polymorphism were used (microsatellites vs. AFLPs;
356 Jakobsson *et al.* 2013), similar overall differentiation between sub-populations in the Finnish
357 *C. splendens* population were also found using AFLPs ($\Phi_{PT}=0.08$; Viitaniemi 2009). Most
358 likely, the lack of IBD is due to strong genetic differentiation between some neighboring sub-
359 populations (e.g. pairwise $F_{ST}=0.20$ between sub-populations AK & CJ, Fig. 1;
360 Supplementary material D, Table D1), as a result of our sampling, which focused on sub-
361 populations at different rivers separated by land and/or lakes.

362 An interesting finding in our analysis is that interspecific interactions could contribute to the
363 strength of drift experienced by *C. splendens* sub-populations (Table 1): when *C. virgo* males
364 are abundant, sympatric *C. splendens* sub-populations are less genetically diverse.
365 Alternatively, such a relationship could arise due to habitat partitioning and local adaptation
366 of the two species to different habitats. In Central Europe, the two *Calopteryx* species do

367 show different preferences to water temperature (Sternberg & Buchwald 1999; Schütte &
368 Schrimpf 2002). If locally adapted, the population sizes of the two species would be expected
369 to show opposing trends with water temperature. However, our data show that in Finland the
370 preferred water temperatures of the two species overlap. Although we have not quantified
371 habitat partitioning with respect to other environmental variables, densities of the two species
372 in sympatric sub-populations do not correlate negatively, suggesting that possible habitat
373 partitioning between the species is not strong (Supplementary material A).

374 Our results are concordant with a study by Viitaniemi (2009), who suggested that interspecific
375 interactions explained a decline in the heterozygosity of *C. splendens* with increasing
376 proportion of *C. virgo* individuals in sympatric sub-populations. We believe that the density
377 of *C. virgo*, rather than its relative abundance in sympatric populations, better describes how
378 frequently heterospecific individuals are encountered and is a more appropriate measure of
379 the frequency of interspecific interactions. Unfortunately, with our data it is not possible to
380 determine how interspecific interactions shape the effective population sizes of sub-
381 populations directly, or reduce gene flow, or both (e.g. as suggested by Ranta *et al.* 2009). It
382 is known that increasing aggression of *C. virgo* males towards *C. splendens* males decreases
383 the proportion of *C. splendens* males with a territorial mating strategy in Finnish sub-
384 populations (Tynkkynen *et al.* 2004, 2006). Since territorial males have better mating success
385 than non-territorial males (Plaistow & Siva-Jothy 1996), it is likely that *C. virgo* influences
386 the effective population size by accentuating the reproductive skew of *C. splendens*.
387 However, it is also possible that interspecific aggression limits gene flow by reducing either
388 the immigration of *C. splendens* (i.e. males immigrate to sites with fewer *C. virgo*), or the
389 probability that immigrants obtain matings (i.e. immigrant males are not able to hold

390 territories at sites with high *C. virgo* abundance). Future work should examine the role of
391 interspecific aggression as a likely explanation for the observed negative relationship between
392 *C. splendens* genetic diversity and *C. virgo* density.

393 Although uH_E of *C. splendens* was negatively related to *C. virgo* density, the effect of *C.*
394 *virgo* density was not identified in the analysis of population specific F_{ST} s of *C. splendens*,
395 which could be due to the low statistical power of GESTE2 (Balkenhol *et al.* 2009). It is more
396 puzzling, however, that we did not observe similar locality-specific patterns with AR as we
397 did with uH_E (Table 1, [Supplementary material E, Table E1](#)), since allelic richness and
398 heterozygosity often exhibit concordant patterns even though allelic richness is more sensitive
399 to changes in effective population size (Luikart *et al.* 1998; Eckert *et al.* 2008). A reasonable
400 explanation for this counterintuitive pattern could be that allelic richness of the Finnish *C.*
401 *splendens* population is already low, and most variation in AR between sub-populations is in
402 the relative frequencies of common alleles ([Supplementary material C, Table C3](#)). Indeed, the
403 average number of alleles per locus observed across the whole *C. splendens* dataset is 3.38
404 (S.D. = 1.59) and the average frequency of the most common allele is 0.72 (S.D. = 0.20),
405 leading to a situation in which the relative amount of variation is higher in uH_E than in AR
406 (Coefficient of variation = 0.130 and 0.086 respectively). This could be a consequence of the
407 markers, since variability of microsatellite loci in many dragonflies and damselflies is
408 generally low (Watts 2009), or alternatively reflect the fact that populations close to range-
409 margins generally have low genetic diversities (Eckert *et al.* 2008; Watts *et al.* 2010).

410 *C. virgo*

411 To our knowledge the SGS of *C. virgo* has not been described before, and to our surprise, we
412 observed an SGS that was very different from that observed in *C. splendens*. Latitude was
413 consistently related to all aspects of the SGS of *C. virgo* (uH_E , AR , IBD, clustering and
414 population specific F_{ST} , Tables 1, 2 & 3; Figures 2b, 3b, and 4b). In addition to latitude, the
415 genetic diversity of *C. virgo* was also related to longitude, with increasing longitude being
416 coupled with increasing genetic diversity (Table 1). The relationship of *C. virgo*'s SGS with
417 latitude and longitude probably reflects a persistent signature of colonization after the last
418 glacial maximum, and latitudinal gradients in genetic diversity at geographic scales similar to
419 ours have been reported before (Hewitt 2000; Schmitt & Seitz 2001). Alternatively, the
420 latitudinal trends in diversity and differentiation can result from range margin effects, which
421 are difficult to differentiate from the effects of post glacial colonization (Eckert *et al.* 2008).
422 On the other hand, low genetic differentiation between sub-populations and a latitudinal cline
423 in genetic diversity could be due genetic disequilibrium caused by recent colonization or
424 range expansion (Watts *et al.* 2010; Swaegers *et al.* 2013); but, similarly to the case of *C.*
425 *splendens*, we cannot determine this since we do not have adequate temporal data on changes
426 in SGS or know the timing of colonization of Finland by *C. virgo*. However, although two
427 genetic clusters are discovered and some pairwise genetic differences between southern and
428 northern *C. virgo* sub-populations are significant (Supplementary material D, Tables D3 and
429 D4) the genetic differentiation is low, which likely reflects gene flow between populations.
430 Indeed, this is supported by the cline of admixture between the genetic clusters and low
431 population specific F_{ST} s (Figures 3b & 4b).

432 *Why is there a difference between the SGSs of the two Calopteryx species?*

433 Although it is not unprecedented that closely related species show different SGSs (Hodges *et*
434 *al.* 2007; Lehrian *et al.* 2009; Fortuna *et al.* 2009; Johansson *et al.* 2013), our results are
435 surprising considering the similarities in the life-history characteristics of the two species and
436 extent of habitat overlap in Fennoscandia. The different SGSs of the two *Calopteryx* species
437 are unlikely to be explained by statistical artifacts, such as differences in the information
438 content of the loci (Supplementary material C, Tables C2, C3 and C4; Jakobsson *et al.* 2013),
439 different numbers of sampled loci, or different geographic ranges of sampling (Supplementary
440 material F). However, since latitudinal and longitudinal variation were not significantly
441 related to the SGS of *C. virgo* in a subset consisting only of the sympatric sub-populations, we
442 cannot rule out the possibility that the lack of relationship between latitude and longitude with
443 *C. splendens* SGS is partly due to a difference in scale.

444 Our study shows that the SGS of *C. splendens* is influenced more by genetic drift than is the
445 SGS of *C. virgo*. Furthermore, our results suggest that interspecific interactions between *C.*
446 *splendens* and *C. virgo* contribute to this difference by strengthening the magnitude of genetic
447 drift experienced by *C. splendens* sub-populations while the SGS of *C. virgo* is not affected.
448 A likely explanation for this discrepancy is that, in Finland, *C. virgo* is the stronger
449 competitor for the best breeding habitats (Tynkkynen *et al.* 2004, 2005, 2006). The
450 competitive advantage of *C. virgo* could be due to its ability to occupy a wider thermal niche
451 in Fennoscandia (Wellenreuther *et al.* 2012; Supplementary material A). Indeed, *C. virgo* is
452 more common in Finland than *C. splendens* and this seems to be also reflected in its SGS: the
453 abundance of sub-populations offer more stepping stones for dispersal and gene flow, leading
454 to less genetic differentiation between *C. virgo* sub-populations.

455 *Conservation implications*

456 Since characterizing SGS of multiple species for landscape level conservation planning is
457 often unfeasible, surrogate approaches are in high demand from practicing conservationists to
458 aid prediction of the effects of different management and land-use actions (Margules &
459 Pressey 2000; Rodrigues & Brooks 2007). Although neither *C. splendens* nor *C. virgo* is
460 endangered, the two species system can function as a test of the surrogate approach. Our
461 results highlight the fact that use of a surrogate requires careful consideration. Interspecific
462 interactions are an important part of the community that can have a role in shaping the SGS of
463 natural populations, and should not be overlooked in conservation planning.

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654 *DATA ACCESSIBILITY*

655 Genotypes for both *Calopteryx splendens* and *Calopteryx virgo*, accompanied with sub-

656 population specific environmental data uploaded to Dryad (doi:10.5061/dryad.v1ss7).

657 *AUTHOR CONTRIBUTIONS*

658 All the authors (AK, IK, KEK, KK and JSK) contributed to the design of the research;
659 AK, IK and KK conducted the field work; AK, IK and KEK designed the laboratory
660 protocol and conducted the laboratory work; AK and KEK conducted the analyses; AK
661 led the writing process of the manuscript, to which all authors contributed.

662 TABLES

663 Table 1. The relationships between locality characteristics and genetic diversity of *Calopteryx*
 664 species. The results considering uH_E and AR represent beta regression and multiple linear
 665 regression models, respectively. For *C. splendens* only a beta regression model for the
 666 relationship between uH_E and locality characteristics is displayed, as AR showed no
 667 statistically significant relationship with any of the locality characteristics. Descriptions of
 668 model selection can be found in Supplementary material E.

Species	Dependent	Independent	Estimate	S.E.	z (uH_E); t (AR)	P
<i>C. splendens</i>	uH_E	intercept	-0.884	0.139	-6.345	< 0.001
		$\log_{10}(\text{CsD})$	0.156	0.073	2.131	0.033
		PC	0.011	0.005	2.126	0.033
		$\log_{10}(\text{CvD})$	-0.183	0.052	-3.479	< 0.001
<i>C. virgo</i>	uH_E	intercept	1.565	0.560	2.794	0.005
		N	-3.432×10^{-7}	8.436×10^{-8}	-4.068	< 0.001
		E	3.474×10^{-7}	1.593×10^{-7}	2.181	0.029
	AR	intercept	6.365	0.698	9.120	< 0.001
		N	-5.720×10^{-7}	1.051×10^{-7}	-5.436	< 0.001
		E	4.451×10^{-7}	2.012×10^{-7}	2.213	0.033

669 Abbreviations of independent variables of the models: $\log_{10}(\text{CsD})$ = logarithm transformed density estimates of *C. splendens*
 670 males; PC = potential connectivity (in kilometers; see text for description); $\log_{10}(\text{CvD})$ = logarithm transformed density
 671 estimates of *C. virgo* males; N = latitude in UTM coordinates; E = longitude in UTM coordinates.

672 Table 2. The highest posterior probability models for the relationship between population
 673 specific F_{ST} and locality characteristics obtained using GESTE2.

Model #	Probability	Locality characteristics included
<i>C. splendens</i>		
3	0.455	PC
1	0.282	Null
4	0.062	$\log_{10}(\text{CsD})$, PC
<i>C. virgo</i>		
9	0.522	N
25	0.204	N, E
13	0.100	N, $\log_{10}(\text{CsD})$

674 Abbreviations of independent variables of the models: PC = potential connectivity
 675 (see text for description); N = latitude in UTM coordinates; $\log_{10}(\text{CsD})$ =
 676 logarithm transformed estimates of density of *C. splendens* males; E = longitude
 677 in UTM coordinates. Null stands for a null-model including only the intercept but
 678 none of the explanatory variables.

679 Table 3. Coefficients of the models with the highest posterior probabilities for population
 680 specific F_{ST} (bold in Table 1).

Species	Estimates	Regression coefficient		HDPI 95%	
		Mean	Mode	LL	UL
<i>C. splendens</i>					
	Constant (α_0)	-2.14	-2.19	-2.47	-1.81
	PC (α_2)	-0.393	-0.400	-0.699	-0.085
	Variance (σ^2)	0.336	0.257	0.121	0.616
<i>C. virgo</i>					
	Constant (α_0)	-4.26	-4.21	-4.62	-3.92
	N (α_4)	0.628	0.622	0.338	0.929
	Variance (σ^2)	0.347	0.262	0.112	0.658

681 Abbreviations of independent variables of the models: PC = potential connectivity (see text
 682 for description); N = latitude in UTM coordinates.

683 FIGURE CAPTIONS

684 Figure 1. Map of *Calopteryx* sampling localities within Finland. Open symbols represent sites
685 with both *C. virgo* and *C. splendens* in sympatry and solid symbols represent sites with *C.*
686 *virgo* in allopatry. Letters indicate the locality codes (see Supplementary material B, Table B1
687 for more information on the localities). The dashed and dotted lines represent the range limit
688 of *C. splendens* and *C. virgo*, respectively.

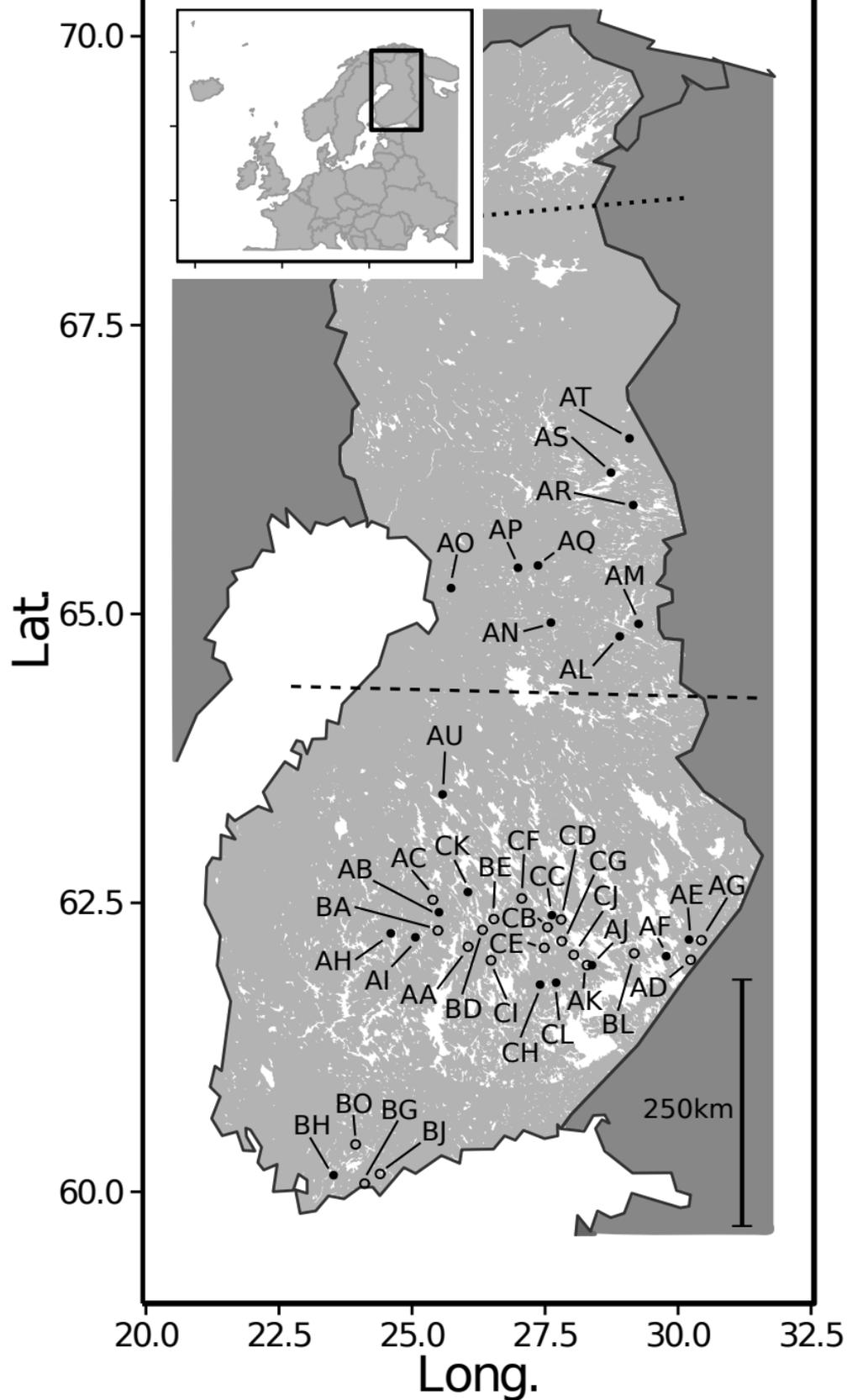
689 Figure 2. A correlogram of average pairwise kinship coefficients ($F_{(d)}$) between individuals of
690 (a) *C. splendens* and (b) *C. virgo* in different distance classes. The error bars represent \pm S.E.
691 for each $F_{(d)}$. Note the different scales on the x-axis of the two panels.

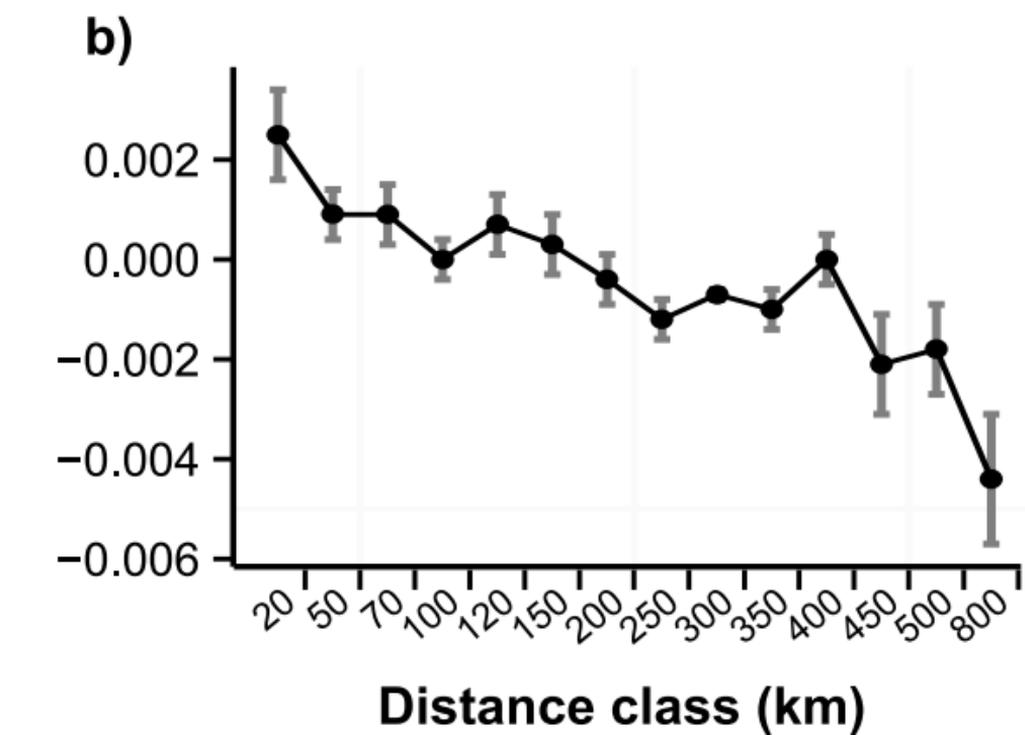
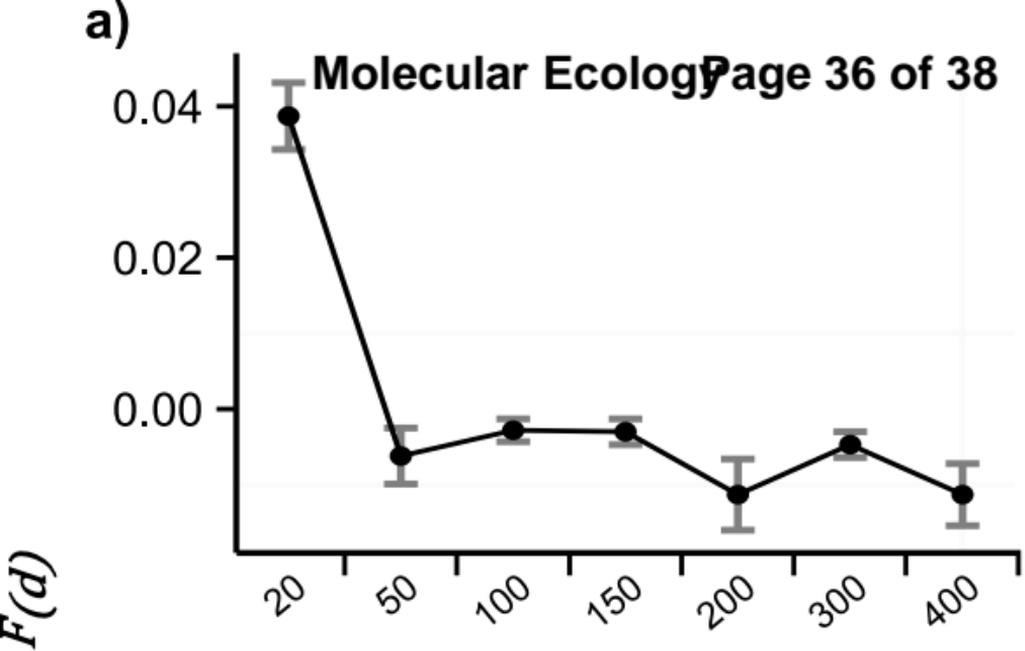
692 Figure 3. The admixture proportions of (a) *C. splendens* and (b) *C. virgo* individuals.
693 Different colors represent different genetic clusters with $K=6$ and $K=2$ for *C. splendens* and *C.*
694 *virgo*, respectively. The samples in the two panels are arranged according to the geographic
695 coordinate axis along which the range of variation is the largest. Thus, the samples of (a) *C.*
696 *splendens* are arranged according to longitude with the westernmost sampling localities on the
697 left hand side and the samples of (b) *C. virgo* are arranged according to latitude, with
698 sampling localities at low latitudes on the left hand side. Letters codes indicate the sub-
699 population (see Supplementary material B, Table B1 for more information on the localities).

700 Figure 4. A graphical representation of the GESTE2 models in Table 3. (a) The relationship
701 between *C. splendens* population specific F_{ST} and potential connectivity (i.e. length of rivers
702 within a five kilometer buffer zone), and (b) the relationship between *C. virgo* population
703 specific F_{ST} and latitude (according to UTM grid ETRS-TM35FIN).

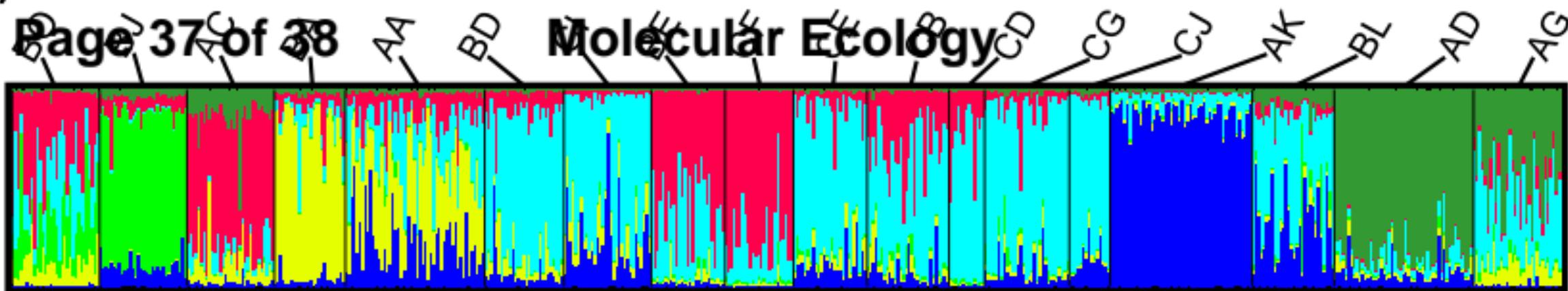
704 *SUPPORTING INFORMATION*

- 705 - Supplementary material A: Overlap in water temperature preferences of *Calopteryx*
706 species in Finland.
- 707 - Supplementary material B: Characteristics of *Calopteryx* sub-populations and locality
708 information.
- 709 - Supplementary material C: Genotyping protocols, characteristics of the microsatellite
710 loci and genetic characteristics of the sub-populations.
- 711 - Supplementary material D: Pairwise genetic differentiation of the *Calopteryx* sub-
712 populations.
- 713 - Supplementary material E: Model selection for beta regression and linear regression
714 models on the relationships between locality characteristics and genetic diversity.
- 715 - Supplementary material F: Analyses with subsets of the *Calopteryx* datasets.

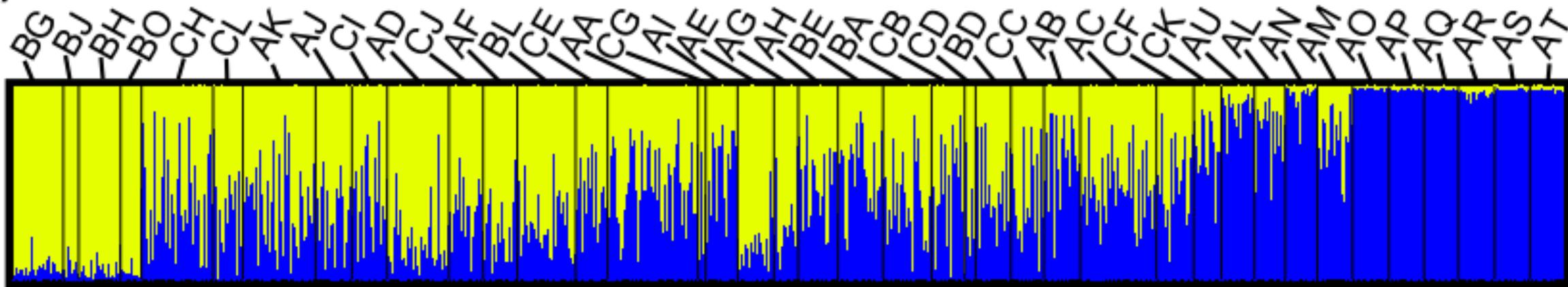




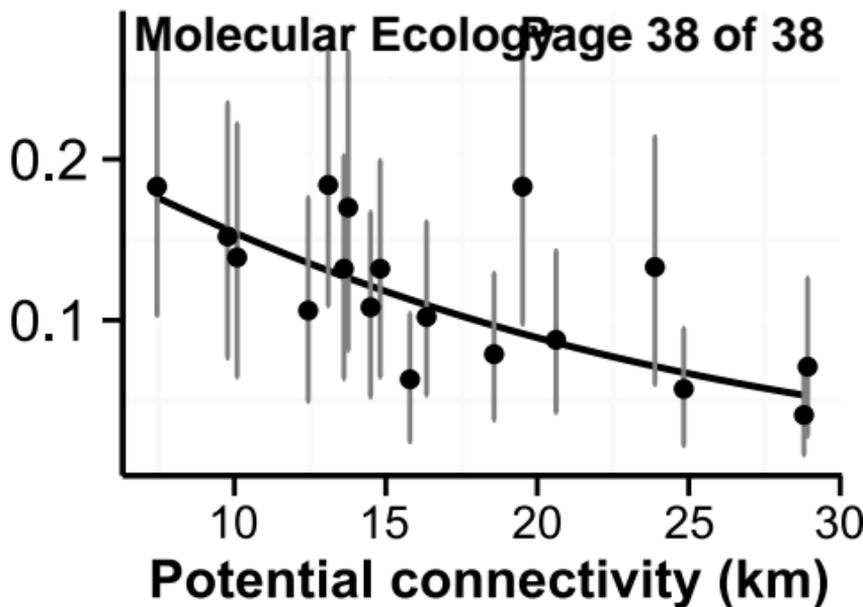
a)



b)



a)



b)

