Genetic structure of a patchily-distributed philopatric migrant: implications for management and conservation

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Abstract

The Lesser Kestrel (*Falco naumanni*) has suffered from both population decline and range fragmentation during the last century. In this study we used multilocus microsatellite genotypes to assess the genetic population structure of the species, with main focus on the patchily distributed populations of Central-Eastern (CE) Mediterranean. Our analysis revealed significant genetic structuring of Lesser Kestrel populations, not only at cross-continental scale, but also regionally within Central and Eastern Mediterranean where a significant correlation between genetic and geographic distance was observed. We detected signs of genetic bottlenecks in some of the peripheral populations, values of genetic differentiation among populations were rather small, albeit most of them significant, whereas gene flow levels were relatively low among the discontinuously distributed populations of CE Mediterranean region. We argue that the observed spatial genetic structure can be attributed to the combined influence of recent population declines, range fragmentation and species' philopatric behaviour. Finally, we discuss how management strategies should focus in the maintenance of current genetic diversity levels and the avoidance of inbreeding depression through restoration of gene flow rates among peripheral populations.

Keywords: Genetic Diversity, Genetic Structure, Falco naumanni, Microsatellites, Migration Rates, Population Fragmentation

Introduction

Measuring genetic variation and connectivity among populations allows the understanding of the influential impact of both historical events and contemporary processes. Besides, this constitutes an essential goal of conservation genetics (Frankham et al. 2010; Allendorf et al. 2013). Fragmentation and isolation of populations may lead to severe demographic alterations, which in turn can have major genetic consequences in wild populations. Depending on the degree of fragmentation, coupled with the species' dispersal capability, partially or totally impeded connectivity could eventually lead to increased genetic differentiation as well as to considerable reduction of population size (Fahrig 2003). Such highly fragmented and locally isolated populations are expected to be susceptible to the combined influence of genetic drift and inbreeding, which might result in loss of genetic diversity as well as in fitness reduction, further compromising their adaptive potential and viability in a changing environment (Amos & Balmford 2001; Frankham et al. 2010). Considering the spatial distribution of population fragments, the above impacts are expected to be more prominent in the peripheral ones, where population sizes can vary intensely with time, rather than in the core populations (Vucetich & Waite 2003; Hanski & Gaggiotti 2004). Therefore the assessment of genetic structure and the identification of its underlying processes become essential tasks providing valuable information towards the design and implementation of effective conservation strategies. For example, delineation of management units (Moritz 1994; Taylor & Dizon 1999; Palsbøll et al. 2007) would be valuable to identify populations or regions in need of localized conservation actions for the short-term management of the species in question.

The Lesser Kestrel (*Falco naumanni*) is a small migratory falcon breeding from the Mediterranean basin across Middle East and Central Asia to Mongolia and China, and wintering in sub-Saharan Africa (Cramp & Simmons 1980). The species underwent rapid

declines throughout its European range in the early 1960s mainly as a result of agricultural intensification and subsequent habitat degradation and land use changes (Iñigo & Barov 2010). In Central-Eastern Mediterranean region (hereafter CE Mediterranean) and especially in the Balkan Peninsula, the decline was dramatic, leading to local extinctions and consequently to significant range contraction and fragmentation (Iñigo & Barov 2010). Currently, the species shows a patchy distribution and is considered to have a depleted status in the region (BirdLife International 2017a), where the two largest populations located in the Apulia-Basilicata area of southern Italy and in Central Greece still hold several thousand pairs (BirdLife International 2004; Gustin et al. 2013). Smaller geographically peripheral populations, consisting of some hundreds of pairs, exist in the Former Yugoslav Republic of Macedonia (Uzunova & Lisichanets 2016), in Sicily (Sarà 2004) and in the European part of Turkey (Kmetova et al. 2012). In addition, populations with less than 100 breeding pairs (HOS/Birdlife Greece, unpublished data) can be found throughout continental Greece as well as on some Greek islands (Legakis & Maragou 2009). Finally, a small geographically isolated population is located in Croatia, at the northernmost edge of the species European distribution (Mikulic et al. 2013). Certain conservation actions, including population reinforcement, improvement of nesting conditions and maintenance of habitat quality, were mainly implemented in Western Europe and have led to a stabilization and slightly positive population trend thus recently leading to the down-listing of the species status from Vulnerable to Least Concern (BirdLife International 2017b). However, conservation actions have only recently been implemented in CE Mediterranean.

Several previous studies have been examining the genetic structure of the species and the underlying processes, at both continental (Wink et al. 2004; Alcaide et al. 2008a; Alcaide et al. 2008b) and finer spatial scales (Ortego et al. 2008a; Ortego et al. 2008b; Alcaide et al. 2009; Di Maggio et al. 2014). It has been proposed that Asian Lesser Kestrels are considerably differentiated from European conspecifics, whereas populations across the

97 Western Palearctic seem to follow an isolation by distance pattern while maintaining high
98 levels of genetic diversity (Wink et al. 2004; Alcaide et al. 2008b). On a regional scale though,
99 the size and the degree of spatial isolation of populations have been suspected as potential
100 factors causing varying levels of gene flow among populations that consequently could
101 create fine-scale genetic structuring (Ortego et al. 2008b; Alcaide et al. 2009). However, such
102 restricted gene flow patterns do not necessarily result from physical barriers to dispersal,
103 especially in efficient dispersers such as the Lesser Kestrel, but can be attributed also to their
104 philopatric behaviour. Indeed, the species shows high philopatry, returning to breed close to
105 its natal or previous breeding territory (Negro et al. 1997; Serrano & Tella 2003; Bounas et
106 al. 2016a). Such behavior could further enhance any effects of fragmentation (Greenwood
107 1980).

108 In this study, we examine the genetic structure of Lesser Kestrel populations firstly on a
109 larger scale across the species distribution as well as focusing on the patchily distributed
110 populations of CE Mediterranean. We aim to identify any patterns of genetic variation and
111 gene flow among populations shaped by their decline and subsequent fragmentation that
112 took place in the region. Such knowledge could be of crucial importance for conservation
113 programs to identify the need of local level conservation actions and thus help to determine
114 the appropriate conservation strategy in the region.

115 Materials and Methods

116 *Population sampling and DNA extraction*

117 Samples were obtained from 12 breeding sites in CE Mediterranean, where the species
118 shows a fragmented distribution as well as from Mongolia, Israel and two sites from Spain.
119 Individuals from Extremadura, Spain (ES/BG) were used for the reinforcement of the species
120 in Bulgaria (Fig.1). A total of 295 unrelated individuals were sampled during four consecutive

121 breeding seasons (2013 - 2016) with the exception of Spanish samples (SES) that were
122 collected in 2007. A single fledgling per brood, to avoid relatedness, and few adult birds
123 were caught by hand in the nesting crevice whereas in some cases adults were trapped by
124 mist nets close to the nest. Two drops of blood (≈50µl) were obtained from each individual
125 by leg-pricking and immediately stored in blood storage cards (NucleoCards®) at room
126 temperature until DNA extraction. DNA was extracted using the NucleoSpin Tissue kit
127 (Macherey-Nagel) following the manufacturer's protocol.

128 *Microsatellite amplification and genotyping*

129 Each individual was genotyped at a total of 18 microsatellite loci. Seven loci were originally
130 isolated from the Peregrine Falcon *Falco peregrinus* (Nesje et al. 2000; Alcaide et al. 2008a),
131 whereas 11 were developed specifically for the Lesser Kestrel (Ortego et al. 2007; Padilla et
132 al. 2009). Details on loci properties and primers used for their amplification are presented in
133 Table S1. All loci were amplified in five multiplex reactions using forward 5'-fluorescent-
134 labelled primers and the KAPA2G Fast Multiplex PCR Kit (Kapa Biosystems). Each 12.5 µl
135 reaction contained 2pM of each primer and 1x KAPA2G Mix and was carried out using the
136 following profile: an initial denaturation step of 3 min at 95 °C, 30 cycles of 15 s at 95°C, 30 s
137 at 60 °C,30 s at 72 °C, with a final extension step of 10 min at 72 °C. PCR products were
138 separated and visualized using an ABI 3730xl capillary sequencer (Applied Biosystems) and
139 genotypes were scored by eye with STRand v.2.4.59 (Toonen & Hughes 2001).
140 Randomization of samples was employed throughout lab processes to avoid any plate/gel
141 specific errors that might lead in population specific biases (Meirmans 2015). In addition, a
142 subset of 50 individuals was re-genotyped to quantify error rates due to allelic dropout or
143 false alleles but no inconsistencies were detected. We used the package "MsatAllele"
144 (Alberto 2009) in R 3.2.2 (R Core Team 2015) to allocate alleles to their respective size
145 classes. Genotyping errors, due to null alleles and stuttering, were examined for all loci and

146 sampled populations using Micro-Checker (Van Oosterhout et al. 2004). Two loci (Fnd1.2
147 and Fnd2.1) were omitted from further analyses due to the presence of null alleles and
148 extensive stuttering respectively, and thus the full analysis was based on the remaining 16
149 microsatellite loci.

150 *Genetic analyses*

151 To evaluate the genetic variability in each breeding population, standard genetic diversity
152 indices (A: number of alleles, P: number of private alleles, Ho: observed and He: expected
153 heterozygosity and Fis: inbreeding coefficient) were calculated using the program GenAlEx
154 version 6.5 (Peakall & Smouse 2012). Allelic richness (Ar) corrected for different sample
155 sizes, was calculated using FSTAT 2.9.3.2 (Goudet 2002). Deviations from Hardy-Weinberg
156 proportions at each locus and sampled population and linkage disequilibrium (LD) were
157 tested in Genepop 4.2 (Rousset 2008) following program's default settings. Signs of recent
158 bottlenecks were evaluated using the software Bottleneck (Piry et al. 1999). A test for
159 heterozygosity excess was performed using the Wilcoxon signed rank test running 1000
160 iterations and using the Two-Phase model (TPM). As the microsatellites we used are either
161 of dinucleotide perfect repeats or of imperfect repeats, both of which may tend toward the
162 IAM (Cornuet and Luikart 1996), we fixed the proportions of the TPM to include 5% of SMM
163 and 95% of IAM (Cristescu et al. 2010). We also tested for a mode-shift distortion using a
164 graphical approach, by plotting the number of alleles in 10 allele frequency classes with 0.1
165 intervals, according to Luikart et al. (1998). In a population at equilibrium, alleles with
166 frequencies in the first class (<0.1) are expected to be more numerous than those belonging
167 to the second class, therefore allele frequencies present a characteristic L-shaped
168 distribution. Plots were examined for mode-shift distortions that would be consistent with a
169 bottlenecked population (Luikart et al. 1998; Cristescu et al. 2010). Plots were produced in R

170 3.2.2 and allele frequency calculations were performed using the R package "Gstudio" (Dyer
171 2014).

172 To evaluate the genetic population structure, the Bayesian clustering software Structure
173 2.3.4 (Pritchard et al. 2000) was used to infer the number of genetically homogeneous
174 clusters present in the dataset. The analysis was conducted both on the full dataset as well
175 as only on the populations of CE Mediterranean region. For both analyses we assumed the
176 admixture ancestry model and correlated allele frequencies (Falush et al. 2003), using
177 sampling location as prior information (Hubisz et al. 2009) as it is deemed to be more
178 sensitive at inferring population structure at lower levels of divergence, which is expected in
179 good dispersers such as birds. Runs were set with a burn-in period of 2×10^5 iterations
180 followed by 10^6 MCMC steps with 20 replicates for each K value (1 to 15 for the full dataset
181 and 1 to 12 for CE Mediterranean). Structure runs were implemented on a beowulf cluster
182 using ParaStructure Perl script (Lagnel 2015) and Structure plots were constructed using
183 Distruct (Rosenberg 2004). The most likely value of genetic clusters, K, was evaluated
184 following the ΔK method (Evanno et al. 2005) implemented in Structure Harvester (Earl &
185 Vonholdt 2012), as well as by calculating the posterior probability for each K. We present all
186 cases with high probability that warrant a biological interpretation (Meirmans 2015).

187 The software Arlequin 3.5.1.3 (Excoffier & Lischer 2010) was used to calculate F_{st} values
188 between all pairs of sampled populations, as a measure of population differentiation, and
189 test them for statistical significance (10,000 permutations). In addition to F_{st}, we also
190 calculated Jost's D (D_{est}) as an unbiased estimator of differentiation that performs better
191 than other relatives, in cases of markers with different number of alleles (Gerlach et al.
192 2010) as is the case in this study (Table S1). Pairwise D values (mean D_{est}) among populations
193 were calculated with the R-package "DEMEtics" (Gerlach et al. 2010) and statistical
194 significance was tested using 1,000 bootstrap iterations. For both estimators, p-values were

195 adjusted for multiple comparisons after the B-H method (Benjamini & Hochberg 1995). We
196 used IBDWS v.3.23 (Jensen et al. 2005) in order to obtain any statistically significant
197 associations between pairwise genetic (F_{st}/[1-F_{st}]) and linear geographic (log km) distance
198 matrices using 30,000 randomizations. The analysis was performed both on the full and the
199 reduced (i.e. CE Mediterranean) datasets.

200 Finally, the breeding populations of CE Mediterranean were grouped according to
201 Structure results as well as F_{st} and D_{est} calculations, and the directional contemporary gene
202 flow and its relative magnitude among them was estimated using the divMigrate function
203 (Sundqvist et al. 2016) in the R-package "diveRsity" (Keenan et al. 2013). The method
204 provides a relative (to within the analysis) migration network graph aiming to visualize the
205 gene flow patterns among populations, with the resulting metric representing a proportion
206 of the gene flow among areas, scaled to the largest magnitude estimated. Populations are
207 represented as nodes and the properties of the lines connecting them are based on the
208 relative strength of gene flow. Populations that exhibit strong gene exchange between them
209 but weak gene flow with others tend to cluster closely together, reflecting patterns of
210 genetic structure. The method is described in detail in Sundqvist et al. (2016). We used Nm
211 (Alcala et al. 2014) as a measure of genetic distance, and tested whether gene flow among
212 populations was asymmetrical using 10,000 bootstrap iterations.

213 Results

214 *Genetic diversity*

215 All microsatellite markers were highly polymorphic across populations and the number of
216 alleles per locus ranged from four (loci Fnd1.4, Fp86-2, Fp89) to 38 (Locus Fnd1.6; Table S1).
217 We did not detect any notable differences in the mean allelic richness across populations,
218 however, the two island populations of Croatia (CRO) and Limnos (LIM) exhibited lower

219 values than the others. The average Ho was 0.65, Ho and He were similar and F was low in
220 all cases (Table 1). Private alleles were present at a low frequency in all sampled populations
221 but absent in those from Croatia (CRO), Komotini (KAL) and one subpopulation in Central
222 Greece (TRI). Generally, loci and populations conformed to Hardy-Weinberg proportions but
223 some deviations were detected: three populations (CRO, ISR, MON) showed deviations at
224 two, one and three out of the 16 loci respectively. Since these loci did not show consistent
225 deviations across all populations, we included them in subsequent analyses attributing this
226 disequilibrium in processes specific to those populations. No LD was detected between any
227 of the loci across all populations. The Wilcoxon signed rank test conducted in Bottleneck
228 software, detected signs of a population bottleneck for Sicily (SIC), Agrinio (LES), Limnos
229 island (LIM) and Israel (ISR) populations (Table S2). On the other hand, inspection of plots of
230 allele frequency classes for recent bottlenecks did not reveal any mode-shift distortion in
231 any of the populations. However, they did show that the Croatian population (CRO) is
232 moving towards allele fixation, since this was the only population that exhibited an allele
233 frequency in the class 0.9-1.0 (Figure S1).

234 *Population structure*

235 The Bayesian clustering method implemented in Structure suggested the presence of
236 considerable population structuring. The results were consistent for both the full and the
237 reduced dataset. When all individuals were modeled, the ΔK-method suggested two clusters
238 (K = 2) as the most likely population structure (although the posterior probability was higher
239 for K = 3; Figure S2, Table S3). For K = 2 the admixture model indicated two gene pools that
240 probably represent a European-Asian (or a western-eastern) transition, with all European
241 populations showing high membership coefficients in the 'western' cluster, and the MON
242 and LIM populations belonging to the 'eastern' one (Fig. 2). This eastern gene pool seems to
243 be present in all populations up to Central Greece (CGR) whereas absent from all other

244 western populations. Lesser Kestrels from Israel (ISR) were found to be highly admixed,
245 exhibiting a mixed ancestry from both clusters (Fig. 2). For K = 3, the model adds another
246 gene pool that is mostly represented by the Trans-Adriatic populations of Croatia (CRO),
247 Apulia (APU) and Sicily (SIC) whereas Israel (ISR) still seems to be of mixed ancestry (Fig. 2).
248 Finally, for K = 5, the western and eastern clusters are further partitioned: the two
249 populations of western Greece (GIA, LES) stand out as a single group, Mongolia (MON) is
250 represented as a single cluster and one cluster is present in Limnos Island (LIM), gradually
251 extending to all populations up to Central Greece (CGR). Israel (ISR) was found to be
252 extensively admixed, while subpopulations within Spain (SES, ES/BG) and Central Greece
253 (TRI, LAR, VOL) showed identical admixture proportions among them, implying no further
254 substructure (Fig. 2).

255 When individuals from the CE Mediterranean were modeled separately, the ΔK-method
256 suggested K = 2 as the optimal number of clusters, whereas posterior probability estimates
257 suggested the presence of four clusters (Figure S3, Table S4). Population structure proved to
258 be rather consistent with the previous runs when the full dataset was used: the populations
259 of Croatia (CRO) and Limnos (LIM) constitute two different genetic clusters, the one present
260 in Limnos (LIM) extending throughout Greek populations up to Central Greece (CGR), while
261 two other clusters are present in all other populations in different proportions (again the GIA
262 and LES populations of western Greece form a single group). The maximum value of five
263 clusters further partitions the Sicilian population (SIC) as a separate group. It is apparent that
264 there is no substructure in the Central Greek core population, CGR (Fig. 2).

265 Pairwise F_{st} and D_{est} values were highly correlated (Pearson's correlation: $r = 0.94$, $p<$
266 0.001) and consistent with Structure groupings. However, differentiation values among
267 populations were not very high albeit most of them significant (Table 2). Estimates of both
268 F_{st} and D_{est} varied between population pairs (F_{st}: 0.00 – 0.06; D_{est}: 0.00 – 0.18), with

269 geographically proximate populations showing no differentiation at all (e.g. subpopulations
270 of Spain and Central Greece). In addition, the population of Kilkis (KIL) showed no
271 differentiation from the Central Greek one (CGR) and all the core populations of Europe
272 (Spain, Apulia, Central Greece) showed very low or no differentiation among them. On the
273 other hand, Mongolia and Israel seem rather differentiated from all others, while the small
274 peripheral CE Mediterranean populations showed low to medium levels of differentiation
275 from all other populations. This was particularly evident for the populations of Croatia and
276 Limnos where the highest values of D_{est} were recorded when compared to all other
277 populations (Table 2). Mantel tests showed that genetic distance is not significantly
278 correlated with geographical distance across all sampled populations ($r = 0.33$, $p = 0.1$, Fig.
279 3a). Conversely, genetic divergence of CE Mediterranean populations correlated significantly
280 with the geographical distance among them ($r = 0.57$, $p = 0.01$, Fig. 3b).

281 Because of the lack of any structure among the subpopulations of Central Greece (TRI,
282 LAR, VOL) and Kilkis (KIL), we pooled the individuals from KIL into the central Greek group
283 (CGR) to estimate the directional relative migration networks using divMigrate. The
284 migration network (Fig. 4) reflects the genetic structuring revealed by previous analyses, as
285 the core populations of the region (APU, CGR) cluster closely in the network space showing
286 high gene flow and relatively low differentiation between them. The GIA and KAL
287 populations also exhibited a relatively high gene flow with the core populations, with the
288 former (GIA) showing connection with both APU and CGR while the latter (KAL) presents
289 high gene flow rates only with CGR. The rest of the groups showed relatively reduced gene
290 flow towards the core populations. It should be noted that as a general pattern, all
291 peripheral populations seem to exchange migrants exclusively with the core populations but
292 not between them, appearing rather isolated from each other (i.e. a star-shaped pattern of
293 the migration networks; Fig. 4), resembling a mainland-island metapopulation type (and not

294 a patchy population type). However, there was no evidence of significantly asymmetric gene
295 flow between any pair of populations.

296

297 Discussion

298 Across all sampled populations, Structure analysis suggested the presence of two major
299 clusters (Fig. 2) concurring with the longitudinal distribution of the species; a 'western'
300 (European) and an 'eastern' (Asian) cluster that are both represented in the population of
301 Israel. This result reflects the proposed phylogeographic pattern of the species; based on the
302 mitochondrial Cyt b region, European and Asian populations were found to be rather
303 divergent whereas birds from Israel seem to cluster with both of them indicating a degree of
304 genetic mixing (Wink et al. 2004). Interestingly, a comparison of plumage patterns of Lesser
305 Kestrels unveiled substantial differences between individuals of European and Chinese
306 descent whereas birds from Asian/Middle East populations (including Israel) exhibit an
307 intermediate plumage pattern (Corso et al. 2016). In addition, this eastern cluster is highly
308 represented in the eastern Greek population of Limnos Island (LIM) and extends gradually
309 up to central Greece suggesting a population consisting of birds of eastern origin (thus a
310 western-ward dispersal of individuals) possibly from populations of Asia Minor. Future
311 inclusion of samples from Western Anatolia (i.e. Turkey) and the Middle East could shed
312 light on such hypotheses.

313 Despite the presence of private alleles, suggesting that gene flow is restricted (at some
314 level), our analysis showed low F_{st} values among the core populations of Europe (Spain,
315 Apulia region in Italy and Central Greece) which is in concordance with previous larger-scale
316 studies (Alcaide et al. 2008b). This is an expected pattern since in populations that exhibit
317 high genetic diversity and similar allele frequency patterns albeit different frequencies of

318 rare alleles, the degree that F_{st} reflects population differentiation can be affected (Hedrick
319 1999; Meirmans & Hedrick 2011; Jakobsson et al. 2013). D_{est} on the other hand, generally
320 showed higher values of differentiation among populations including the core ones (ES and
321 APU). We found low to medium but statistically significant levels of differentiation among CE
322 Mediterranean populations (Table 2) and a correlation of pairwise genetic and geographic
323 distance. We should note that Structure may overestimate genetic structure in datasets
324 characterized by such correlation (Frantz et al. 2009) thus explaining the high number of
325 clusters supported in the region (Fig. 2). Correlation between genetic and geographic
326 distance has been previously reported for the species at both local (Ortego et al. 2008b) and
327 large spatial scales (Alcaide et al. 2008a; Alcaide et al. 2008b) and can be driven by the
328 distance-dependent dispersal exhibited by the species (Serrano & Tella 2003; Serrano et al.
329 2003; Ortego et al. 2008b). It has been proposed that sufficient gene flow and large effective
330 population sizes could prevent any genetic impoverishment that is due to random genetic
331 drift in the species (Alcaide et al. 2008b). This is partially true for our study. Some long-
332 distance dispersal events that could have facilitated gene flow among populations have been
333 reported across CE Mediterranean (Gustin et al. 2011; Bounas et al. 2016a). Nevertheless,
334 some of these movements took place in the '50s when the species showed a wider
335 distribution. Actually, restricted dispersal in a fragmented range, coupled with high
336 philopatry rates, along with a relatively short generation time of the species (average
337 lifespan of 4-6 years; Newton & Olsen 1990; Negro 1997), might have contributed to a more
338 frequent individual turnover in the breeding colonies thus allowing relatively quick changes
339 in allele frequencies that led to the patterns of genetic differentiation observed in the region
340 (Ortego et al. 2008a).

341 Our results suggest that the past population declines have left genetic bottleneck signs in
342 four populations across the species' range. The Israeli population (ISR) has gone through a
343 steep decline and is estimated to be less than 10% of the population prior to 1950 (Liven-

344 Schulman et al. 2004). However the high levels of diversity we found could suggest its
345 recovery. Bottleneck signs were also detected in three of the peripheral populations in the
346 CE Mediterranean region (SIC, LES, LIM), that were also differentiated and showed a
347 relatively limited gene flow with other populations (Fig. 4) but nevertheless similar diversity
348 patterns. Different areas seem to be differentially impacted by population declines as we
349 found no evidence of bottleneck in the rest of the peripheral populations. Ioannina (GIA) and
350 Komotini (KAL) populations for example although they experienced a population decline in
351 the past, they exhibit high levels of gene flow with the core populations, a process that can
352 annihilate the bottleneck effects. In addition, genetic drift could also play a role on the
353 observed genetic patterns of populations in the region, since they exhibit small size and
354 limited gene flow with other populations. This is particularly evident in the small Croatian
355 population. This population consists of only 25 pairs and was recently discovered (Mikulic et
356 al. 2013). Therefore we are ignorant regarding its history, that is if the population was
357 always present there, but failed to detect it, or this is a recently colonized area. The
358 population did not show any signs of bottleneck but there seems to be a loss of rare alleles,
359 including private alleles, as well as signs of allele fixation (Table 1, Fig. S1). Despite that,
360 there is no observed decrease in heterozygosity, which could be consistent with a scenario
361 of a recently founded population that experienced the effects of genetic drift, since
362 heterozygosity can be relatively insensitive to the loss of rare alleles due to drift (Allendorf
363 1986; Allendorf et al. 2013). While the case of the Croatian population might be attributed
364 to its relative large distance from any core population, this does not seem to apply to the
365 other insular population of Limnos which is located at a relatively short distance from both
366 the Anatolian and the continental Greek population. Therefore we could consider the
367 hypothesis that insularity per se, plays some role in population isolation (Frankham 1997),
368 either due to effective competition for nesting sites by the first colonizers, or due to some
369 behavioral barriers.

370 Finally, we should consider that the genetic structure of long-distance migrants cannot be
371 unambiguously defined based solely on geography and life stage (Esler 2000). Despite its
372 pronounced philopatry, the Lesser Kestrel is a potentially good disperser thus not limited by
373 geographical barriers. However, barriers to dispersal may be caused by other factors such as
374 non-breeding distributions (Szczys et al. 2017), these being either wintering grounds
375 (Rodríguez et al. 2011), migration routes or even premigratory sites that exist in the study
376 region (Minias et al. 2009; Bounas et al. 2016b), as well as breeding phenology that could for
377 example lead to assortative mating and consequently affect the genetic structure of
378 populations (Casagrande et al. 2006; Friesen 2015). Given the recent advances in genomics,
379 future work examining such factors could provide a better understanding of the underlying
380 processes shaping the genetic structure of the species in the region.

381 *Conservation implications*

382 According to our results, the genetic structure of the CE Mediterranean populations of the
383 Lesser Kestrel appears to reflect to some extent the demographic decline that led to its
384 range fragmentation. In addition, in spite of bottleneck signs in some of the peripheral
385 populations within the CE Mediterranean, we did not observe any extensive loss of alleles
386 that could render them under immediate risk of genetic erosion. Therefore, we suggest the
387 management of the whole CE Mediterranean population as a single unit. However,
388 peripheral populations should be of increased concern and especially the Croatian one, since
389 limited immigration along with its small size could lead to inbreeding. Thus a mixed
390 conservation strategy that aims to preserve the existent high diversity of the core
391 populations of Italy and Greece, while focusing on the recovery of bottlenecked peripheral
392 populations, should be appropriate for the conservation of the species in the region.
393 Prevention of the negative effects of drift in the peripheral populations could imply actions
394 towards increasing population sizes and dispersal rates among them, by promoting the

395 expansion of natural populations and the foundation of new colonies. Although such actions
396 could be challenging in the absence of conspecific cues (Serrano & Tella 2003; but see
397 Calabuig et al. 2010), they might be of crucial importance to restore the connectivity among
398 populations thus resembling the former continuous distribution of the species in the region.

399 Additionally, increase of population sizes could be achieved by translocations of
400 individuals from the core populations to the peripheral or newly founded colonies
401 (Rodríguez et al. 2013). In fact, reintroductions could be a more effective way to establish
402 populations than the natural expansion of existing ones (Morandini et al. 2017). As
403 mentioned before, the individuals from Bulgaria used in this study were actually of Spanish
404 origin (ES/BG), that have been translocated in the country for the reinforcement of the
405 species within the framework of LIFE11 NAT/BG/360 Project (Gradev et al. 2015). Based
406 solely on our results from the microsatellite analysis, it seems to be an acceptable action
407 since this population was found to exhibit high diversity and low differentiation from all the
408 others. However, the fact that our results were based solely on the analysis of selectively
409 neutral loci prevents us from conclusions regarding the adaptive and evolutionary
410 consequences of the specific action (Holderegger et al. 2006). Although translocations could
411 restore the genetic variation in the short-term (Bouzat et al. 2009), the Iberian and CE
412 Mediterranean Lesser Kestrels follow clearly different migration routes and could possibly
413 have different genetic adaptations (Pulido 2007; Rodríguez et al. 2011). Thus any future
414 translocation programmes should consider maximizing both the genetic and the adaptive
415 similarity between populations (Edmands 2007). Undoubtedly, genetic monitoring (Schwartz
416 et al. 2007) would be a useful practice for this reintroduced population especially for future
417 captive breeding schemes in the region. In the meanwhile, since the proposed conservation
418 strategies could be fruitful in the long-term, monitoring of both demographic and genetic
419 parameters of the small peripheral populations would be highly desirable.

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Fig. 1 Map of the Lesser Kestrel populations used in the present study. SES: Andalucia, ES/BG: Extremadura, APU: Apulia, SIC: Sicily, CRO: Croatia, GIA: Ioannina, LES: Agrinio, TRI: Trikala, LAR: Larisa, VOL: Volos, KIL: Kilkis, KAL: Komotini, LIM: Limnos, ISR: Israel, MON: Mongolia. Shaded areas represent the breeding distribution of the Lesser Kestrel (modified from BirdLife International). Sampling sites where the species is continuously distributed are circled (ES: Spain; CGR: Central Greece)

Fig. 2 Admixture proportions (proportions of membership to each of K inferred clusters) of individual Lesser Kestrels. Upper plots correspond to the number of clusters when the full dataset was modeled (K = 2, K = 4 and K = 5), whereas the lower 3 plots show admixture proportions of the CE Mediterranean populations only, for K=2, K=4 and K=5 inferred clusters respectively

Fig. 3 Correlation of genetic divergence with linear geographic distance for (a) all Lesser Kestrel populations sampled in this study and (b) Lesser Kestrel populations across CE Mediterranean

Fig. 4 Directional relative migration networks of grouped Lesser Kestrel populations from CE Mediterranean. Networks were visualized with divMigrate using Alcala's Nm. (a) all relative migration values (b) only values above 0.2 threshold (c) only gene flow values estimated >0.4. Line shading and thickness increases with the relative strength of gene flow. APU: Apulia, SIC: Sicily, CRO: Croatia, GIA: Ioannina, LES: Agrinio, CGR: Central Greece, KAL: Komotini, LIM: Limnos.

665 **Table 1** Measures of genetic variation of the 15 Lesser Kestrel populations used in the
666 present study. Number of genotyped individuals (n), number of alleles per locus (A), allelic
667 richness (Ar), observed (Ho) and expected (He) heterozygosity, number of private alleles (P)
668 and inbreeding coefficient (Fis). Values are presented as means ± standard error (in
669 brackets)

Code	Location	n	A	Ar	Ho	He	P	Fis
SES	Andalucia	19	7.1 (0.9)	5.4 (0.6)	0.635 (0.037)	0.696 (0.037)	1	0.082 (0.041)
ES/BG	Extremadura	25	8.3 (1.3)	5.6 (0.7)	0.674 (0.040)	0.699 (0.037)	6	0.029 (0.037)
APU	Apulia	44	9.4 (1.5)	5.5 (0.6)	0.669 (0.038)	0.707 (0.036)	3	0.053 (0.025)
SIC	Sicily	12	5.9 (0.8)	5.1 (0.6)	0.682 (0.038)	0.667 (0.041)	2	-0.045 (0.045)
CRO	Croatia	14	5.6 (0.6)	4.7 (0.5)	0.654 (0.048)	0.650 (0.048)	0	-0.023 (0.043)
GIA	Ioannina	24	7.6 (1.1)	5.2 (0.6)	0.634 (0.051)	0.654 (0.048)	2	0.036 (0.031)
LES	Agrinio	16	6.7 (0.8)	5.4 (0.6)	0.603 (0.058)	0.684 (0.042)	2	0.136 (0.047)
TRI	Trikala	20	7.6 (1.3)	5.5 (0.7)	0.626 (0.056)	0.680 (0.046)	0	0.087 (0.046)
LAR	Larisa	20	7.4 (1.1)	5.4 (0.6)	0.669 (0.053)	0.680 (0.044)	1	0.024 (0.045)
VOL	Volos	20	7.6 (1.2)	5.5 (0.7)	0.678 (0.051)	0.662 (0.048)	1	-0.027 (0.035)
KIL	Kilkis	13	6.4 (0.9)	5.3 (0.7)	0.621 (0.062)	0.661 (0.048)	1	0.067 (0.064)
KAL	Komotini	20	7.1 (1.077)	5.3 (0.6)	0.689 (0.053)	0.677 (0.046)	0	-0.019 (0.040)
LIM	Limnos	11	5.1 (0.6)	4.8 (0.5)	0.661 (0.060)	0.667 (0.041)	1	0.024 (0.060)
ISR	Israel	20	7.3 (1.1)	5.5 (0.7)	0.619 (0.062)	0.682 (0.042)	5	0.124 (0.055)
MON	Mongolia	17	7.2 (1.0)	5.4 (0.6)	0.585 (0.057)	0.655 (0.049)	10	0.155 (0.046)

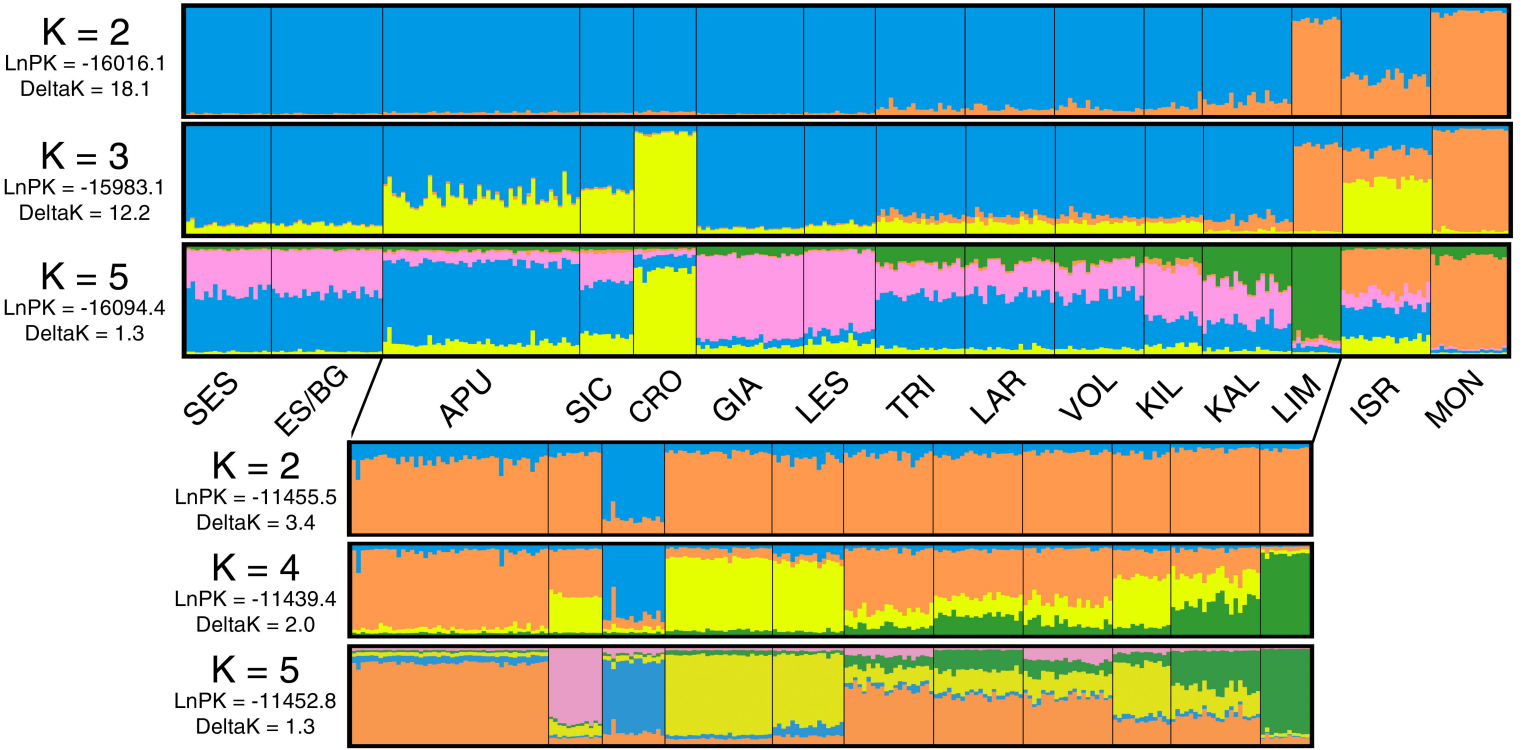
670

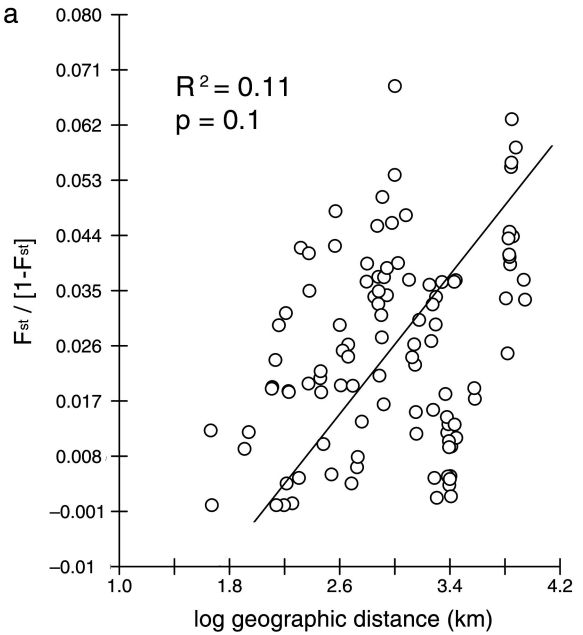
671

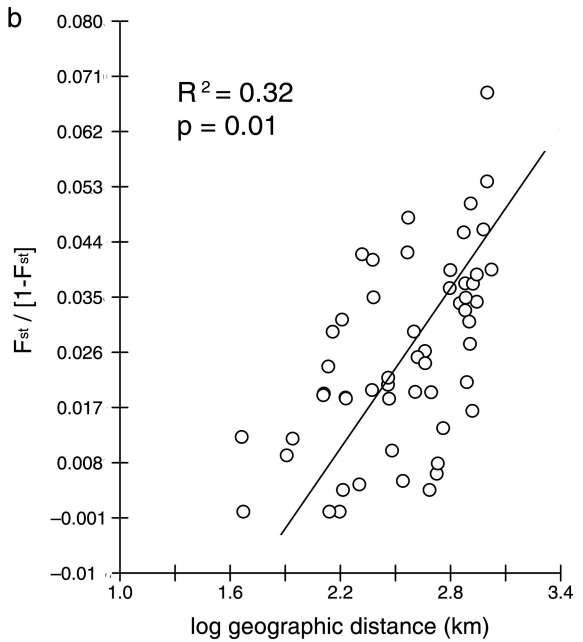
Table 2 Pairwise F_{st}-values (below diagonal) and D_{est}-values (above diagonal) among Lesser Kestrel populations. Statistically significant values after B-H correction for multiple comparisons are given in bold ($p < 0.039$)

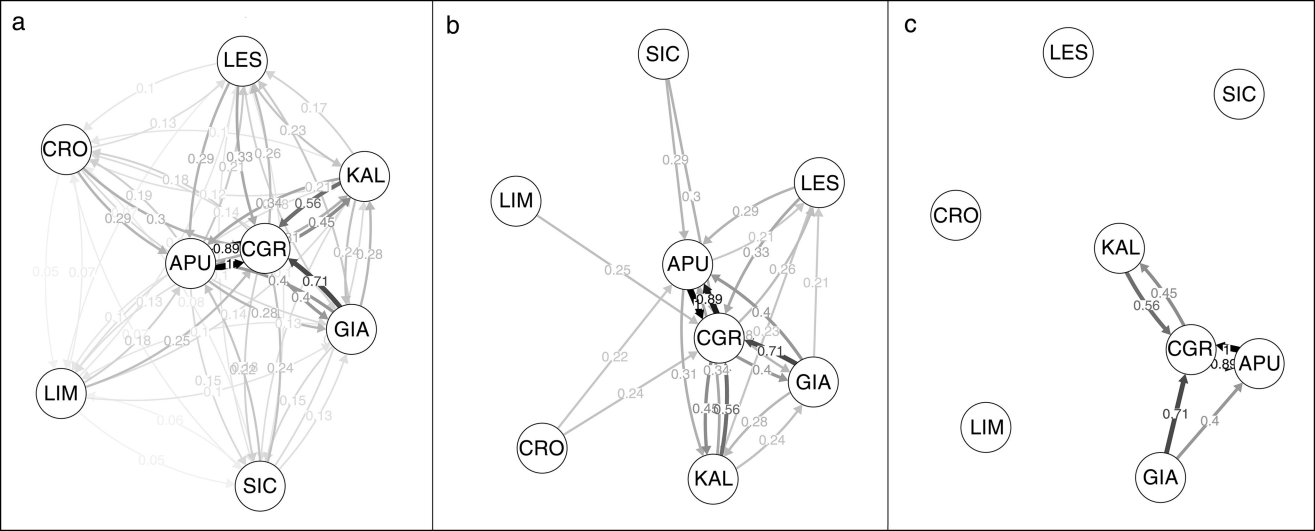
	SES	ES/BG	APU	SIC	CRO	GIA	LES	TRI	LAR	VOL	KIL	KAL	LIM	ISR	MON
SES	-	0.018	**0.038**	**0.099**	**0.132**	**0.048**	**0.053**	0.014	0.030	**0.053**	0.016	**0.027**	**0.147**	**0.059**	**0.093**
ES/BG	0.000	-	**0.035**	**0.096**	**0.138**	**0.056**	**0.049**	0.015	**0.046**	0.003	0.029	**0.041**	**0.160**	**0.069**	**0.126**
APU	0.001	0.004	-	**0.100**	**0.103**	**0.076**	**0.072**	0.014	**0.021**	**0.041**	**0.030**	**0.052**	**0.143**	**0.054**	**0.121**
SIC	**0.026**	**0.035**	**0.029**	-	**0.167**	**0.105**	**0.128**	**0.111**	**0.099**	**0.108**	**0.069**	**0.096**	**0.175**	**0.115**	**0.183**
CRO	**0.033**	**0.032**	**0.026**	**0.048**	-	**0.152**	**0.117**	**0.101**	**0.112**	**0.146**	**0.117**	**0.130**	**0.191**	**0.117**	**0.168**
GIA	**0.012**	**0.018**	**0.019**	**0.035**	**0.044**	-	**0.077**	**0.027**	**0.078**	**0.065**	0.026	**0.054**	**0.143**	**0.088**	**0.151**
LES	**0.013**	**0.014**	**0.019**	**0.038**	**0.033**	**0.029**	-	**0.065**	**0.084**	**0.072**	**0.050**	**0.067**	**0.183**	**0.074**	**0.158**
TRI	0.003	0.005	0.004	**0.033**	**0.027**	0.009	**0.023**	-	0.000	**0.035**	0.000	0.000	**0.100**	**0.030**	**0.110**
LAR	0.005	**0.010**	0.006	**0.032**	**0.036**	**0.019**	**0.030**	0.000	-	0.027	0.005	**0.028**	**0.153**	**0.064**	**0.112**
VOL	0.010	0.004	**0.013**	**0.030**	**0.037**	**0.018**	**0.018**	0.012	0.012	-	0.015	**0.047**	**0.132**	**0.080**	**0.121**
KIL	0.001	0.009	0.008	0.016	**0.034**	0.004	0.018	0.000	0.000	0.004	-	**0.037**	**0.136**	**0.020**	**0.095**
KAL	**0.011**	**0.013**	**0.021**	**0.038**	**0.044**	**0.025**	**0.024**	0.005	**0.010**	**0.021**	**0.019**	-	**0.092**	**0.088**	**0.085**
LIM	**0.035**	**0.035**	**0.036**	**0.051**	**0.064**	**0.041**	**0.046**	**0.020**	**0.034**	**0.040**	**0.039**	**0.019**	-	**0.152**	**0.143**
ISR	**0.017**	**0.019**	**0.015**	**0.029**	**0.035**	**0.029**	**0.022**	**0.015**	**0.026**	**0.024**	**0.012**	**0.035**	**0.045**	-	**0.094**
MON	**0.032**	**0.035**	**0.042**	**0.055**	**0.060**	**0.052**	**0.053**	**0.038**	**0.039**	**0.043**	**0.039**	**0.024**	**0.042**	**0.033**	-

MON
0
2000
4000 km
CRO
ES
ES/BG
SES
APU
SIC
KIL
KAL
GIA
TRI
LAR
LIM
VOL
LES
CGR
ISR
0
500
1000 km


K = 2
LnPK = -16016.1
DeltaK = 18.1
K = 3
LnPK = -15983.1
DeltaK = 12.2
K = 5
LnPK = -16094.4
DeltaK = 1.3
SES
ES/BG
APU
SIC
CRO
GIA
LES
TRI
LAR
VOL
KIL
KAL
LIM
ISR
MON
K = 2
LnPK = -11455.5
DeltaK = 3.4
K = 4
LnPK = -11439.4
DeltaK = 2.0
K = 5
LnPK = -11452.8
DeltaK = 1.3


a
R 2 = 0.11
p = 0.1
0.080
0.071
0.062
0.053
0.044
0.035
0.026
0.017
0.008
−0.001
−0.01
Fst / [1-Fst]
1.0
1.8
2.6
3.4
4.2
log geographic distance (km)


b
R 2 = 0.32
p = 0.01
0.080
0.071
0.062
0.053
0.044
0.035
0.026
0.017
0.008
−0.001
−0.01
Fst / [1-Fst]
1.0
1.6
2.2
2.8
3.4
log geographic distance (km)


a
LES
CRO
KAL
APU
CGR
GIA
LIM
SIC
b
SIC
LES
LIM
APU
CGR
GIA
CRO
KAL
c
LES
SIC
CRO
KAL
CGR
APU
LIM
GIA