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

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Running Title: Conservation Genetics of the Lesser Kestrel

Significant demographic fluctuations can have major genetic consequences in wild populations. The Lesser Kestrel (*Falco naumanni*) has suffered from both population declines 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. We detected signs of genetic bottlenecks in some of the peripheral populations coupled with small effective population sizes. Values of genetic differentiation among the largest populations were low, albeit significant, whereas the small peripheral CE Mediterranean populations showed higher levels of differentiation from all other populations. Gene flow levels were relatively low among the discontinuously distributed populations of the CE Mediterranean region. We argue that the observed spatial genetic structure can be attributed at some level to the past demographic decline experienced by the species. Finally, we discuss how management strategies should focus in the maintenance of current genetic diversity levels and the recovery of bottlenecked populations through the increase of population sizes and dispersal rates among peripheral populations.

Keywords: *Falco naumanni* -Genetic Diversity-Lesser Kestrel-Management Units-Mediterranean-Microsatellites-Migration Rates

INTRODUCTION

Significant demographic fluctuations and isolation, can have major genetic consequences in wild populations. Partially or totally impeded connectivity could eventually lead to increased genetic differentiation as well as to considerable reduction of population size (Fahrig, 2003). Patchily distributed and locally isolated populations are susceptible to the combined influences of genetic drift and inbreeding, which may result in a decrease in genetic diversity and fitness. Decreases in genetic variability compromise a species' ability to adapt to a changing environment (Amos & Balmford, 2001; Frankham, Ballou & Briscoe, 2010). Genetic drift and inbreeding are expected to be stronger in peripheral populations relative to core populations, due to their small population sizes and low immigration rates (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 (MUs, Moritz, 1994; Taylor & Dizon, 1999; Palsbøll, Berube & Allendorf, 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,* Fleischer, 1818) 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 (Fig.1; 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). The two largest (core) populations are located in the Apulia-Basilicata area of southern Italy and in Central Greece, still holding several thousand pairs (BirdLife International, 2004; Gustin *et al.*, 2013). Peripheral populations of smaller size, 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 of the 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). Conservation actions such as population reinforcement, construction of artificial nesting sites and maintenance of habitat quality have led to increases in population sizes in Western Europe and subsequently the down-listing of the species from Vulnerable to Least Concern (BirdLife International, 2017b). However, such conservation actions are only recently starting to be implemented in CE Mediterranean.

Several previous studies have examined the genetic structure of the species and the underlying processes, at both continental (Wink, Sauer-Gurth & Pepler, 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 Western Palearctic seem to follow an isolation by distance pattern while maintaining high levels of genetic diversity (Wink *et al.*, 2004; Alcaide *et al.*, 2008b). On a regional scale though, it seems that the species' philopatry does not lead to fine-scale genetic structuring (Alcaide *et al.*, 2009); nevertheless, population structure could emerge, depending on the size and the degree of spatial isolation and the levels of gene flow among

100 populations (Ortego *et al.*, 2008b). Such restricted gene flow patterns do not necessarily
101 result from physical barriers hindering dispersal, especially in efficient dispersers such as the
102 Lesser Kestrel, but can be attributed also to their philopatric behaviour. Indeed, the species
103 shows high philopatry, with adult birds returning to breed close to their previous breeding
104 territories (Negro, Hiraldo & Donázar, 1997), however, juveniles disperse more and in
105 greater distances (Serrano & Tella, 2003; Bounas *et al.*, 2016a).

106 Herein, we examine the genetic structure of the Lesser Kestrel, 1) across the broader
107 species range and 2) within the CE Mediterranean. We aim to identify any patterns of
108 genetic variation and gene flow among populations that could be of crucial importance for
109 conservation programs to determine the appropriate conservation strategy in the region and
110 design local scale conservation actions.

111 MATERIAL AND METHODS

112 POPULATION SAMPLING AND DNA EXTRACTION

113 Samples were obtained from 12 breeding sites in CE Mediterranean, where the species
114 shows a fragmented distribution as well as from Mongolia, Israel and two sites from Spain.
115 Individuals sampled in Bulgaria originated from Spain and were used for the reinforcement
116 of the species, thus treated as if they were sampled in Extremadura (ES/BG; Fig. 1). A total of
117 295 individuals were sampled during four consecutive breeding seasons (2013 - 2016) with
118 the exception of Spanish samples (SES) that were collected in 2007. Birds were caught by
119 hand in the nest or using mist nets or spring traps close to the nest. To minimize biases
120 associated with relatedness only a single fledgling per brood was sampled. Two drops of
121 blood (≈50µl) were obtained from each individual by leg-pricking and immediately stored in
122 blood storage cards (NucleoCards®) at room temperature until DNA extraction. DNA was

123 extracted using the NucleoSpin Tissue kit (Macherey-Nagel) following the manufacturer's
124 protocol.

125 MICROSATELLITE AMPLIFICATION AND GENOTYPING

126 Each individual was genotyped at a total of 18 microsatellite loci. Seven loci were originally
127 isolated from the Peregrine Falcon *Falco peregrinus* (Nesje *et al.*, 2000; Alcaide *et al.*, 2008a),
128 whereas 11 were developed specifically for the Lesser Kestrel (Ortego *et al.*, 2007; Padilla *et*
129 *al.*, 2009). Details on loci properties and primers used for their amplification are presented in
130 Supporting information, Table S1. All loci were amplified in five multiplex reactions using
131 forward 5'-fluorescent-labelled primers and the KAPA2G Fast Multiplex PCR Kit (Kapa
132 Biosystems). Each 12.5 µl reaction contained 2pM of each primer and 1x KAPA2G Mix and
133 was carried out using the following profile: an initial denaturation step of 3 min at 95 °C, 30
134 cycles of 15 s at 95°C, 30 s at 60 °C, 30 s at 72 °C, with a final extension step of 10 min at 72
135 °C. PCR products were separated and visualized using an ABI 3730xl capillary sequencer
136 (Applied Biosystems) and genotypes were scored by eye with STRand v.2.4.59 (Toonen &
137 Hughes, 2001). Randomization of samples was employed throughout lab processes to avoid
138 any plate/gel specific errors that might lead in population specific biases (Meirmans, 2015).
139 In addition, a subset of 50 individuals was re-genotyped to quantify error rates due to allelic
140 dropout or genotyping errors but no inconsistencies were detected. We used the package
141 "MsatAllele" (Alberto, 2009) in R 3.2.2 (R Core Team, 2015) to allocate alleles to their
142 respective size classes. Genotyping errors, due to null alleles and stuttering, were examined
143 for all loci and sampled populations using MICROCHECKER (Van Oosterhout *et al.*, 2004).
144 Two loci (Fnd1.2 and Fnd2.1) were omitted from further analyses due to the presence of null
145 alleles and extensive stuttering respectively, and thus the full analysis was based on the
146 remaining 16 microsatellite loci.

147 GENETIC ANALYSES

148 To evaluate the genetic population structure, the Bayesian clustering software
149 STRUCTURE 2.3.4 (Pritchard, Stephens & Donnely, 2000) was used to infer the number of
150 genetically homogeneous clusters present in the dataset. The analysis was conducted both
151 on the full dataset as well as only on the populations of CE Mediterranean region. For both
152 analyses we assumed the admixture ancestry model and correlated allele frequencies
153 (Falush, Stephens & Pritchard, 2003), using sampling location as prior information
154 (LOCPRIOR model; Hubisz *et al.*, 2009) as it is deemed to be more sensitive at inferring
155 population structure at lower levels of divergence, which is expected in good dispersers such
156 as birds. Runs were set with a burn-in period of 2 x 10^5 iterations followed by 10^6 MCMC
157 steps with 20 replicates for each K value (1 to 15 for the full dataset and 1 to 12 for CE
158 Mediterranean). STRUCTURE runs were implemented on a beowulf cluster using
159 PARASTRUCTURE Perl script (Lagnel, 2015) and STRUCTURE plots were constructed using
160 DISTRUCT (Rosenberg, 2004). The most likely value of genetic clusters, K, was evaluated
161 following the ΔK method (Evanno, Regnaut & Goudet, 2005) implemented in STRUCTURE
162 HARVESTER (Earl & Vonholdt, 2012), as well as by calculating the posterior probability for
163 each K. We present all cases with high probability that warrant a biological interpretation
164 (Meirmans, 2015) and are supported by both STRUCTURE runs.

165 To estimate the directional contemporary gene flow and its relative magnitude among
166 the breeding populations of CE Mediterranean we used the divMigrate function (Sundqvist
167 *et al.*, 2016) in the R-package "diveRsity" (Keenan *et al.*, 2013). The method provides a
168 relative (to within the analysis) migration network graph aiming to visualize the gene flow
169 patterns among populations, with the resulting metric representing a proportion of the gene
170 flow among areas, scaled to the largest magnitude estimated. Populations are represented
171 as nodes and the properties of the lines connecting them are based on the relative strength
172 of gene flow. Populations that exhibit strong gene exchange between them but weak gene
173 flow with others tend to cluster closely together, reflecting patterns of genetic structure.

174 The method is described in detail in Sundqvist *et al.* (2016). We used N_M (Alcala, Goudet &
175 Vuilleumier, 2014) as a measure of genetic distance, and tested whether gene flow among
176 populations was asymmetrical using 10,000 bootstrap iterations.

177 Sampled populations were pooled according to the STRUCTURE and DivMigrate analyses,
178 and F_{ST} values between all pairs of populations were calculated with the software Arlequin
179 3.5.1.3 (Excoffier & Lischer, 2010). F_{ST} values were tested for statistical significance using
180 10000 permutations. In addition to F_{ST}, we also calculated Jost's D (D_{est}) as an unbiased
181 estimator of differentiation that performs better than other relatives, in cases of markers
182 with different number of alleles (Gerlach *et al.*, 2010) as is the case in this study (see
183 Supporting information, Table S1). Pairwise D values (mean D_{est}) among populations were
184 calculated with the R-package "DEMEtics" (Gerlach *et al.*, 2010) and statistical significance
185 was tested using 1,000 bootstrap iterations. For both estimators, p-values were adjusted for
186 multiple comparisons after the B-H method (Benjamini & Hochberg, 1995). We used IBDWS
187 v.3.23 (Jensen, Bohonak & Kelley, 2005) in order to obtain any statistically significant
188 associations between pairwise genetic ($F_{ST}/[1-F_{ST}]$) and linear geographic (log km) distance
189 matrices using 30,000 randomizations. The analysis was performed both on the full and the
190 reduced (i.e. CE Mediterranean) datasets.

191 To evaluate the genetic variability in each population, standard genetic diversity indices
192 (A: number of alleles, P: number of private alleles, H_O: observed and H_E: expected
193 heterozygosity) were calculated using the program GENALEX v.6.5 (Peakall & Smouse, 2012).
194 Allelic richness (A_R) corrected for different sample sizes and inbreeding coefficient (F_{IS}), were
195 calculated using FSTAT 2.9.3.2 (Goudet, 2002). The same software was used to test for
196 deviations from Hardy-Weinberg proportions at each locus and sampled population, as well
197 as for linkage disequilibrium (LD) using 1000 randomizations and adjusting significance for
198 multiple comparisons. Signs of recent bottlenecks were evaluated using the software

199 BOTTLENECK (Piry, Luikart & Cornuet, 1999). A test for heterozygosity excess was performed
200 using the Wilcoxon signed rank test running 1000 iterations and using the two-phase model
201 (TPM). As the microsatellites we used are either of dinucleotide perfect repeats or of
202 imperfect repeats, both of which may tend toward the infinite allele model (IAM; Cornuet
203 and Luikart, 1996), we fixed the proportions of the TPM in favour of the IAM (Cristescu *et al.*,
204 2010) including 20% of the stepwise mutation model (SMM) and 80% of IAM. We also tested
205 for a mode-shift distortion using a graphical approach, by plotting the number of alleles in
206 10 allele frequency classes with 0.1 intervals, according to Luikart *et al.* (1998). In a
207 population at equilibrium, alleles with frequencies in the first class (<0.1) are expected to be
208 more numerous than those belonging to the second class, therefore allele frequencies
209 present a characteristic L-shaped distribution. Plots were examined for mode-shift
210 distortions that would be consistent with a bottlenecked population (Luikart *et al.*, 1998;
211 Cristescu *et al.*, 2010). Plots were produced in R 3.2.2 and allele frequency calculations were
212 performed using the R package "Gstudio" (Dyer, 2014). Finally, the effective population size
213 (N_e) along with 95% confidence intervals (CIs) were estimated using the bias-corrected
214 version of the method based on linkage disequilibrium (Hill, 1981; Waples, 2006) as
215 implemented in NeESTIMATOR v.2.01 (Do *et al.*, 2014). N_E estimates were obtained for all
216 but the core populations (ES, APU, CNG) and Mongolia, as it is very difficult to obtain reliable
217 estimates for large populations using this method (Waples & Do, 2010).

218 RESULTS

219 All microsatellite markers were highly polymorphic across populations and the number of
220 alleles per locus ranged from four (loci Fnd1.4, Fp86-2, Fp89) to 38 (locus Fnd1.6; Supporting
221 information, Table S1).

222 POPULATION STRUCTURE

223 The Bayesian clustering method implemented in STRUCTURE suggested the presence of
224 considerable population structuring. When all individuals were modeled, the ΔK-method
225 suggested two clusters (K = 2) as the most likely population structure (although the posterior
226 probability was higher for K = 3; Supporting information, Figure S1, Table S2). For K = 2 the
227 admixture model indicated two gene pools that probably represent a European-Asian (or a
228 western-eastern) transition, with all European populations showing high membership
229 coefficients in the 'western' cluster, and the MON and LIM populations belonging to the
230 'eastern' one (Fig. 2). This eastern gene pool seems to be present in all populations in
231 Northern and Central Greece (CNG) whereas absent from all other western populations.
232 Lesser Kestrels from Israel (ISR) were found to be highly admixed, exhibiting a mixed
233 ancestry from both clusters (Fig. 2). For K = 3, the model adds another gene pool that is
234 mostly represented by the Trans-Adriatic populations of Croatia (CRO), Apulia (APU) and
235 Sicily (SIC) whereas Israel (ISR) still seems to be of mixed ancestry. All subpopulations within
236 Spain (SES, ES/BG) and Central Greece (TRI, LAR, VOL) showed identical admixture
237 proportions among them, implying no further substructure (Fig. 2).

238 When individuals from the CE Mediterranean were modeled separately, the ΔK-method
239 suggested K = 2 as the optimal number of clusters, whereas posterior probability estimates
240 suggested the presence of four clusters (Supporting information, Figure S2, Table S3). The
241 populations of Croatia (CRO) and Limnos (LIM) constitute two different genetic clusters, the
242 one present in Limnos (LIM) extending throughout Greek populations from Northern to
243 Central Greece (CNG), while two other clusters are present in all other populations in
244 different proportions (GIA and LES populations of western Greece form a single group). The
245 maximum value of five clusters further partitions the Sicilian population (SIC) as a separate
246 group. It is apparent that there is no substructure in the Central and Northern Greek
247 population, CNG (Fig. 2).

248 Because of the lack of any structure among the subpopulations of Central Greece (TRI,
249 LAR, VOL) and Kilkis (KIL), we pooled the individuals from KIL into the central Greek group
250 (CGR) to estimate the directional relative migration networks using divMigrate. The
251 migration network (Fig. 3) reflects at some level the genetic structuring revealed by
252 STRUCTURE analysis, as the core populations of the region (APU, CGR) cluster closely in the
253 network space showing high gene flow and relatively low differentiation between them. The
254 GIA and KAL populations also exhibited a relatively high gene flow with the core populations,
255 with the former (GIA) showing connection with both APU and CGR while the latter (KAL)
256 presents high gene flow rates only with CGR. Thus KAL was considered to be a part of the
257 Central-North Greek population in all subsequent analysis. However, in contrast to
258 STRUCTURE results the sampled populations of western Greece (GIA, LES) showed reduced
259 gene flow between them and appear to be differentiated. The rest of the groups showed
260 relatively reduced gene flow towards the core populations. It should be noted that as a
261 general pattern, all peripheral populations seem to exchange migrants exclusively with the
262 core populations but not between them, appearing isolated from each other (i.e. a star-
263 shaped pattern of the migration network; Fig. 3), resembling a mainland-island
264 metapopulation type (and not a patchy population type). However, there was no evidence
265 of significantly asymmetric gene flow between any pair of populations.

266 Pairwise F_{ST} and D_{est} values were highly correlated (Pearson's correlation: $r = 0.95$, $P <$
267 0.001). All but one of the differentiation values among populations were significant (Table
268 1). Estimates of both F_{ST} and D_{est} varied between population pairs (F_{ST}: 0.003 – 0.06; D_{est}:
269 0.02 – 0.19), with all the core populations of Europe (Spain, Apulia, Central Greece) showing
270 low differentiation among them. On the other hand, Mongolia and Israel seem
271 differentiated from all others, while the small peripheral CE Mediterranean populations
272 showed higher levels of differentiation from all other populations. This was particularly
273 evident for the populations of Croatia and Limnos where the highest values of D_{est} were

recorded when compared to all other populations (Table 1). Mantel tests showed that genetic distance is not significantly correlated with geographical distance across all sampled populations (r = 0.33, P = 0.1, Fig. 4a). Conversely, genetic divergence of CE Mediterranean populations correlated significantly with the geographical distance among them (r = 0.57, P = 0.01, Fig. 4b).

GENETIC DIVERSITY

We did not detect any notable differences in the mean allelic richness across populations. The average H_O was 0.64, H_O and H_E were similar and F_{IS} was low in all cases (Table 2). Private alleles were present in all sampled populations with the only exception of Croatia (CRO). Generally, populations did not deviate from Hardy-Weinberg proportions but some deviations of individual loci were detected: four populations (CRO, LIM, ISR, MON) showed deviations at two, one, one and two out of the 16 loci respectively (see Supporting information, Table S4). Since these loci did not show consistent deviations across all populations, we included them in subsequent analyses attributing this disequilibrium in processes specific to those populations. No LD was detected between any of the loci across all populations. The Wilcoxon signed rank test conducted in BOTTLENECK software, detected signs of a population bottleneck for Sicily (SIC), Limnos island (LIM) and Israel (ISR) populations (Supporting information, Table S5). On the other hand, inspection of plots of allele frequency classes for recent bottlenecks did not reveal any mode-shift distortion in any of the populations. However, they did show that the Croatian population (CRO) is moving towards allele fixation, since this was the only population that exhibited an allele frequency in the class 0.9-1.0 (see Supporting information, Figure S3). Most of the peripheral populations were found to have small effective population sizes (Supporting information, Table S5), ranging from 24.2 (LIM) to 88.4 (LES). However, in some cases (SIC, LES and ISR populations) results should be treated with caution as 95% CIs were broad.

299

300 DISCUSSION

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

315 Despite the high number of private alleles, suggesting that gene flow could be restricted
316 (at some level), our analysis showed low F_{ST} values among the core populations of Europe
317 (Spain, Apulia region in Italy and Central Greece) which is in concordance with previous
318 larger-scale studies (Alcaide *et al.*, 2008b). However, we should note that the degree that F_{ST}
319 reflects population differentiation can be affected in populations that exhibit high genetic
320 diversity and similar allele frequency patterns, albeit different frequencies of rare alleles
321 (Hedrick, 1999; Meirmans & Hedrick, 2011; Jakobsson, Edge & Rosenberg, 2013). D_{est} on the
322 other hand, generally showed higher values of differentiation among populations including
323 the core ones (ES and APU). We found significant levels of differentiation among CE

324 Mediterranean populations (Table 1) and a correlation of pairwise genetic and geographic
325 distance. We should note that STRUCTURE may overestimate genetic structure in datasets
326 characterized by such correlation (Frantz *et al.*, 2009) thus explaining the high number of
327 clusters supported in the region (Fig. 2). Correlation between genetic and geographic
328 distance has been previously reported for the species at both local (Ortego *et al.*, 2008b) and
329 large spatial scales (Alcaide *et al.*, 2008a; Alcaide *et al.*, 2008b) and can be driven by the
330 distance-dependent dispersal exhibited by the species (Serrano & Tella, 2003; Serrano *et al.*,
331 2003; Ortego *et al.*, 2008b). It has been proposed that sufficient gene flow and large
332 effective population sizes could prevent any genetic impoverishment that is due to random
333 genetic drift in the species (Alcaide *et al.*, 2008b). This is partially true for our study. Some
334 long-distance dispersal events that could have facilitated gene flow among populations have
335 been reported across the CE Mediterranean (Gustin, Mendi & Pedrelli, 2011; Bounas *et al.*
336 2016a). Actually, restricted dispersal in a fragmented range, coupled with high philopatry
337 rates, along with a relatively short generation time of the species (average lifespan of 4-6
338 years; Newton & Olsen, 1990; Negro, 1997), might have contributed to a more frequent
339 individual turnover in the breeding colonies thus allowing relatively quick changes in allele
340 frequencies that led to the patterns of genetic differentiation observed in the region (Ortego
341 *et al.*, 2008a).

342 Our results suggest that the past population declines have left genetic bottleneck signs in
343 four populations across the species' range. The Israeli population (ISR) has gone through a
344 steep decline and is estimated to be less than 10% of the population prior to 1950 (Liven-
345 Schulman *et al.*, 2004). However, the high levels of diversity we found despite the small N_E
346 could be a result of its genetic admixture. Bottleneck signs were also detected in two of the
347 peripheral populations in the CE Mediterranean region (SIC, LIM), that were also
348 differentiated and showed a relatively limited gene flow with other populations (Fig. 3) and
349 small N_E but nevertheless similar diversity patterns. Despite its small N_E, Ioannina (GIA)

350 population exhibits high levels of diversity and gene flow with both core populations of CE
351 Mediterranean (APU, CNG), a process that can alleviate the bottleneck effects. In addition,
352 genetic drift could also play a role on the observed genetic patterns of populations in the
353 region, since they exhibit small size and limited gene flow with other populations. This is
354 particularly evident in the small Croatian population. This population consists of only 25
355 pairs and was recently discovered (Mikulic *et al.*, 2013). Therefore we are ignorant regarding
356 its history, that is if the population may have been recently colonized or present historically
357 and simply not detected. The population did not show any signs of bottleneck but there
358 seems to be a loss of rare alleles, including private alleles, as well as signs of allele fixation
359 (Table 2, Supporting information, Figure S3). Despite that, there is no observed decrease in
360 heterozygosity, which could be consistent with a scenario of a recently founded population
361 that experienced the effects of genetic drift, since heterozygosity can be relatively
362 insensitive to the loss of rare alleles due to drift (Allendorf, 1986; Allendorf, Aitken & Luikart,
363 2013).

364 Finally, we should consider that the genetic structure of long-distance migrants cannot be
365 unambiguously defined based solely on the spatial distribution of their breeding sites (Esler,
366 2000). Despite its pronounced philopatry, the Lesser Kestrel is a potentially good disperser
367 thus not limited by geographical barriers. However, barriers to dispersal may be caused by
368 other factors such as non-breeding distributions (Szczys, Oswald & Arnold, 2017), these
369 being either wintering grounds (Rodríguez *et al.*, 2011), migration routes or even
370 premigratory sites that exist in the study region (Minias *et al.*, 2009; Bounas *et al.*, 2016b), as
371 well as breeding phenology that could for example lead to assortative mating and
372 consequently affect the genetic structure of populations (Casagrande *et al.*, 2006). Given the
373 recent advances in genomics, future work examining such factors could provide a better
374 understanding of the underlying processes shaping the genetic structure of the species in
375 the region.

376 CONSERVATION IMPLICATIONS

377 According to our results, the genetic structure of the CE Mediterranean populations of the
378 Lesser Kestrel appears to reflect to some extent the demographic decline that led to its
379 range fragmentation. In addition, in spite of bottleneck signs in some of the peripheral
380 populations within the CE Mediterranean, allelic diversity was not lower than in core
381 populations. Therefore, we suggest the management of the whole CE Mediterranean
382 population as a single conservation unit (ESU). However, the population could be divided
383 into four different management units (MUs) since three peripheral populations (CRO, LIM,
384 SIC) showed limited migration rates and different allele frequencies from all the other
385 populations. These processes along with their small effective and census population sizes
386 could eventually render them vulnerable to extinction. Thus a mixed conservation strategy
387 that aims to preserve the existent high diversity of the core populations of Italy and Greece,
388 while focusing on the recovery of bottlenecked peripheral populations (SIC, LIM), should be
389 appropriate for the conservation of the species in the region. Prevention of the negative
390 effects of drift in the peripheral populations could imply actions towards increasing
391 population sizes and dispersal rates among them, by promoting the expansion of natural
392 populations and the foundation of new colonies.

393 Increase of population sizes could be achieved by translocations of individuals from the
394 core populations to the peripheral or newly founded colonies. In fact, reintroductions could
395 be a more effective way to establish populations than the natural expansion of existing ones
396 (Morandini *et al.*, 2017). As mentioned before, the individuals from Bulgaria used in this
397 study were actually of Spanish origin (ES/BG), that have been translocated in the country for
398 the reinforcement of the species within the framework of LIFE11 NAT/BG/360 Project
399 (Gradev *et al.*, 2015). Based solely on our results from the microsatellite analysis, it seems to
400 be an acceptable action since this population was found to exhibit high diversity and low

differentiation from all the others. However, the fact that our results were based solely on the analysis of selectively neutral loci prevents us from conclusions regarding the adaptive and evolutionary consequences of the specific action (Holderegger, Kamm & Gugerli, 2006). Although translocations could restore the genetic variation in the short-term (Bouzat *et al.*, 2009), the Iberian and CE Mediterranean Lesser Kestrels follow clearly different migration routes and could possibly have different genetic adaptations (Pulido, 2007; Rodríguez *et al.*, 2011). Thus any future translocation programmes should consider maximizing both the genetic and the adaptive similarity between populations (Edmands, 2007). Undoubtedly, genetic monitoring (Schwartz, Luikart & Waples, 2007) would be a useful practice for this reintroduced population especially for future captive breeding schemes in the region. In the meanwhile, since the proposed conservation strategies could be fruitful in the long-term, monitoring of demographic parameters such as population size, breeding success and survival, as well as the genetic diversity of the small peripheral populations after few generations, would be highly desirable.

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Figure 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 pooled for the analyses are circled (ES: Spain; CNG: Central-North Greece)

Figure 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 = 3), 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

Figure 3. 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 rate 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 and CGR: Central Greece are the largest (core) populations. SIC: Sicily, CRO: Croatia, GIA: Ioannina, LES: Agrinio, KAL: Komotini, LIM: Limnos are the smaller peripheral populations

Figure 4. 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

677 **Table 1.** Pairwise F_{ST} values (below diagonal) and D_{est} values (above diagonal) among Lesser Kestrel populations. Statistically significant values after B-H

678 correction for multiple comparisons are given in bold

	ES	APU	SIC	CRO	GIA	LES	CNG	LIM	ISR	MON
ES	-	**0.024**	**0.097**	**0.134**	**0.050**	**0.049**	**0.018**	**0.152**	**0.061**	**0.109**
APU	0.003	-	**0.100**	**0.103**	**0.076**	**0.072**	**0.025**	**0.143**	**0.054**	**0.121**
SIC	**0.031**	**0.029**	-	**0.167**	**0.105**	**0.128**	**0.092**	**0.175**	**0.115**	**0.183**
CRO	**0.032**	**0.026**	**0.048**	-	**0.152**	**0.117**	**0.116**	**0.191**	**0.117**	**0.168**
GIA	**0.015**	**0.019**	**0.035**	**0.044**	-	**0.077**	**0.044**	**0.143**	**0.088**	**0.151**
LES	**0.014**	**0.019**	**0.038**	**0.033**	**0.029**	-	**0.062**	**0.183**	**0.074**	**0.158**
CNG	**0.004**	**0.008**	**0.028**	**0.032**	**0.012**	**0.020**	-	**0.115**	**0.052**	**0.097**
LIM	**0.035**	**0.036**	**0.051**	**0.064**	**0.041**	**0.046**	**0.026**	-	**0.152**	**0.143**
ISR	**0.018**	**0.015**	**0.029**	**0.035**	**0.029**	**0.022**	**0.020**	**0.045**	-	**0.094**
MON	**0.034**	**0.042**	**0.055**	**0.059**	**0.052**	**0.053**	**0.033**	**0.042**	**0.033**	-

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684 **Table 2** Measures of genetic variation of the Lesser Kestrel populations. Number of

685 genotyped individuals (*N*), number of alleles per locus (*A*), allelic richness (A_R), observed (H_O)

686 and expected (H_E) heterozygosity, number of private alleles (*P*) and inbreeding coefficient

687 (F_{IS}). Values are presented as means ± standard error (in brackets)

Code	Location	*N*	*A*	A_R	H_O	H_E	*P*	F_{IS}
ES	Spain	44	9.4 (1.6)	5.6 (0.6)	0.657 (0.039)	0.695 (0.038)	8	0.062 (0.036)
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)
CNG	Central-North Greece	93	10.8 (2.0)	5.5 (0.6)	0.660 (0.050)	0.694 (0.044)	3	0.053 (0.033)
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)

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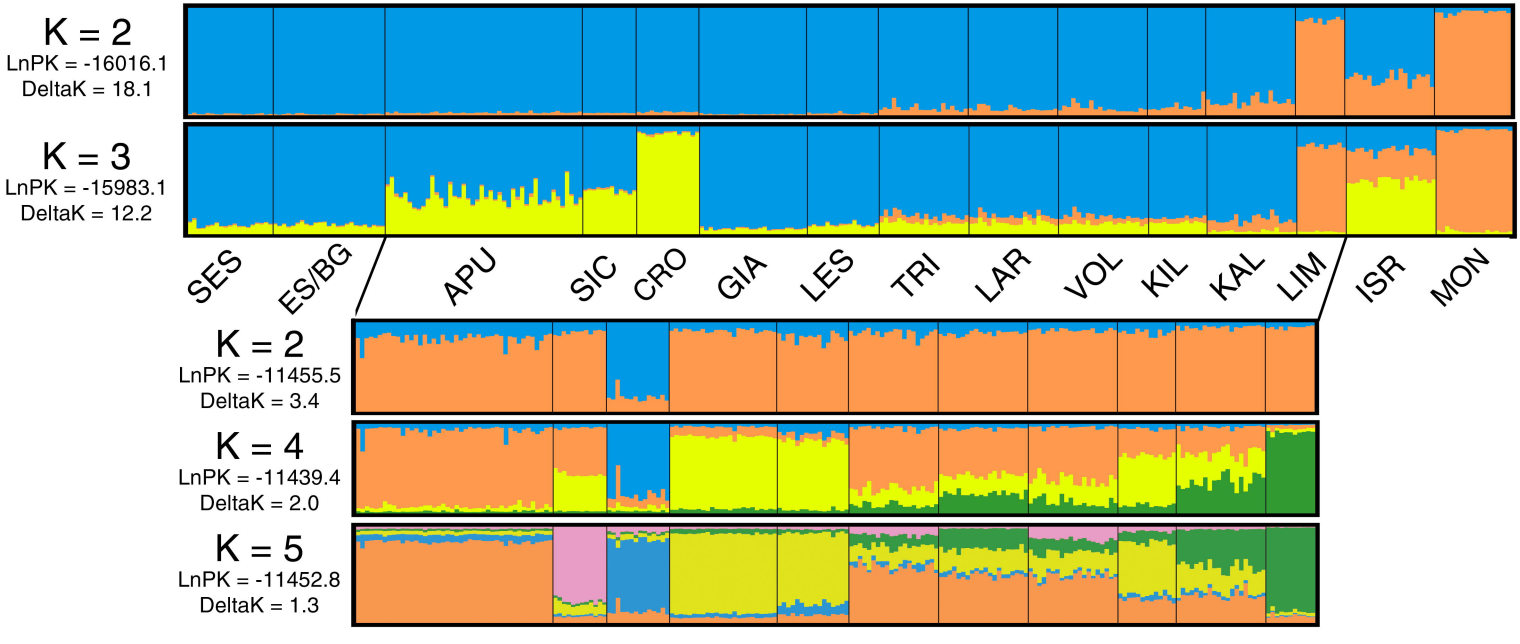
MON
0
2000
4000 km
CRO
CNG
KIL
KAL
APU
ES
ES/BG
GIA
TRI
LAR
LIM
VOL
LES
SIC
SES
ISR
0
500
1000 km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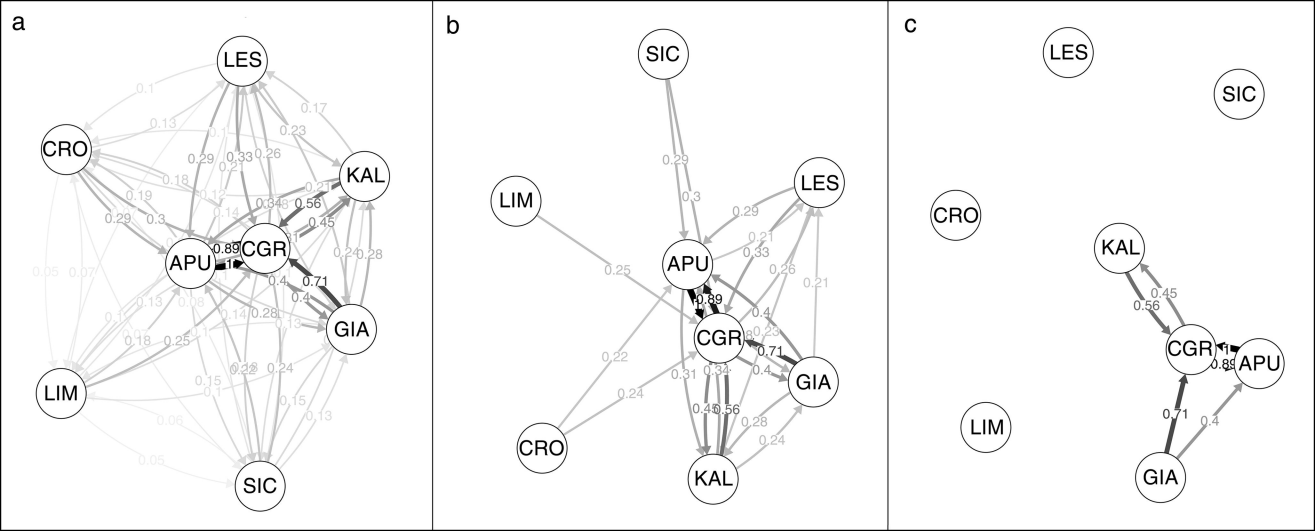
LnPK = -16016.1
DeltaK = 18.1
K = 3
LnPK = -15983.1
DeltaK = 12.2
SES
ES/BG
APU
SIC
CRO
GIA
LES
TRI
LAR
VOL
KIL
KAL
LIM
ISR
MON
K = 2
LnPK = -11455.5
DeltaK = 3.4
K = 4
LnPK = -11439.4
DeltaK = 2.0
K = 5
LnPK = -11452.8
DeltaK = 1.3


a
LES
CRO
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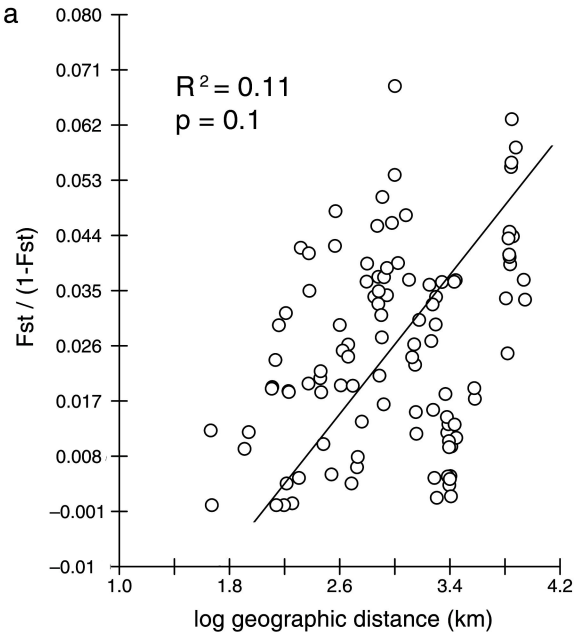
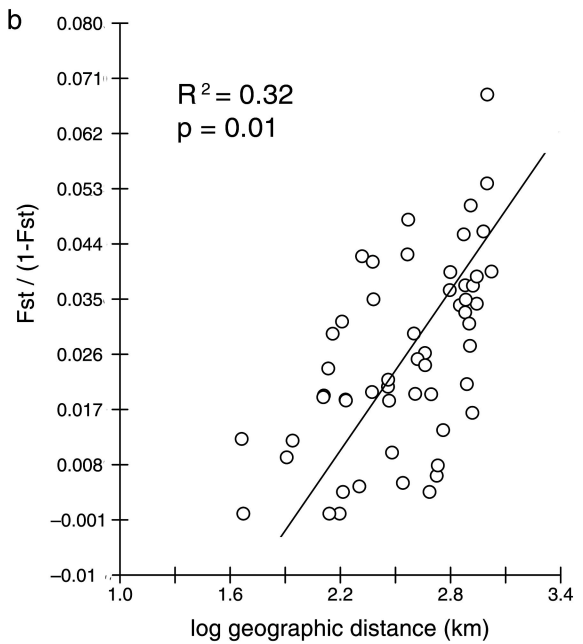

a
R 2 = 0.11
p = 0.1
Fst / (1-Fst)
0.080
0.071
0.062
0.053
0.044
0.035
0.026
0.017
0.008
−0.001
−0.01
1.0
1.8
2.6
3.4
4.2
log geographic distance (km)


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