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 second half of the 20th century. In this study we analysed multilocus microsatellite data to assess the genetic structure of the species. Our analysis revealed significant genetic structuring of Lesser Kestrel populations, not only at cross-continental scale, but also regionally within Central and Eastern (CE) 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 identify management units in the region, and inform the design of conservation actions aiming at 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

In many animal species, the patterns of genetic differentiation and gene flow are highly influenced by the geographical characteristics of their habitats as well as their migratory behavior (Willoughby *et al.*, 2017). Birds, and specifically raptors, can show long-distance migratory behavior and also exhibit natal and breeding site fidelity. As a general pattern, migratory populations of raptors have been found to have weaker genetic structure and higher genetic diversity than resident populations (Miller *et al.*, 2012; Willoughby *et al.*, 2017). For example some individuals might migrate through a different route upon their return on the breeding grounds, and consequently disperse and breed far from their natal site (Garcia *et al.*, 2011). On the other hand, philopatry reduces or even inhibits gene flow among populations leading to increased genetic differentiation. Patchily distributed and locally isolated populations are susceptible to a greater influence of genetic drift which may result in a decrease in genetic diversity and fitness, thus compromising a species' ability to adapt to a changing environment (Amos & Balmford, 2001). Genetic drift and inbreeding are expected to be stronger in peripheral populations relative to core ones, 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, identification of management units (MUs, Moritz, 1994; Taylor & Dizon, 1999; Palsbøll, Berube & Allendorf, 2007) would be central to delineate populations for monitoring and thus aid their short-term management.

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 (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, 2017a). Peripheral populations of smaller size, still exist in the Former Yugoslav Republic of Macedonia (Uzunova & Lisichanets, 2016), in Sicily (Sarà, 2004), the European part of Turkey (Kmetova *et al.*, 2012) and 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). Certain conservation actions, mainly implemented in Western Europe, have led to a stabilization and slightly positive population trend and subsequently the down-listing of the species from Vulnerable to Least Concern (BirdLife International, 2017b).

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,

99 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). At least at a continental
106 scale, Lesser Kestrels show strong migratory connectivity, i.e. there is a spatial segregation of
107 breeding populations at the wintering range: European populations winter in the Sahel,
108 while Asian populations winter in east and South Africa (Wink *et al*., 2004; Rodriguez *et al*.,
109 2009)

110 Herein, we examine the genetic structure of the Lesser Kestrel, 1) across the broader
111 species range and 2) within the CE Mediterranean. We aim to identify patterns of genetic
112 variation and gene flow among populations as well as their underlying processes. Such
113 information could be of crucial importance for conservation programs to identify the need of
114 local scale conservation actions and inform their design.

115 ## MATERIAL 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 sampled in Bulgaria originated from Spain and were used for the reinforcement
120 of the species, thus treated as if they were sampled in Extremadura (ES/BG; Fig. 1). A total of
121 295 individuals were sampled during four consecutive breeding seasons (2013 - 2016) with
122 the exception of Spanish samples (SES) that were collected in 2007. Birds were caught by

hand in the nest or using mist nets or spring traps close to the nest. To minimize biases associated with relatedness only a single fledgling per brood was sampled. Two drops of blood (≈50µl) were obtained from each individual by leg-pricking and immediately stored in blood storage cards (NucleoCards®) at room temperature until DNA extraction. DNA was extracted using the NucleoSpin Tissue kit (Macherey-Nagel) following the manufacturer's protocol.

MICROSATELLITE AMPLIFICATION AND GENOTYPING

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

148 Two loci (Fnd1.2 and Fnd2.1) were omitted from further analyses due to the presence of null
149 alleles and extensive stuttering respectively, and thus the full analysis was based on the
150 remaining 16 microsatellite loci.

151 GENETIC ANALYSES

152 To evaluate the genetic variability in each population, standard genetic diversity indices (*A*:
153 number of alleles, H_O: observed and H_E: expected heterozygosity) were calculated using the
154 program GENALEX v.6.5 (Peakall & Smouse, 2012). Rarefied Private Allelic richness (π)
155 estimates were produced using HP-RARE (Kalinowski, 2005). Allelic richness (A_R) corrected
156 for different sample sizes was calculated using FSTAT 2.9.3.2 (Goudet, 2002). The same
157 software was used to calculate the inbreeding coefficient (F_{IS}), test for deviations from
158 Hardy-Weinberg proportions at each locus and sampled population, as well as for linkage
159 disequilibrium (LD) using 1000 randomizations and adjusting significance for multiple
160 comparisons (adjusted P value < 0.0003). In addition we performed a Chi-Square test and
161 Fisher's method to confirm the Hardy Weinberg (HW) equilibrium results.

162 The software Arlequin 3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate F_{ST} values
163 between all pairs of sampled populations and test them for statistical significance using
164 10000 permutations. In addition to F_{ST}, we also calculated Jost's D (D_{est}) as an unbiased
165 estimator of differentiation that performs better than other relatives, in cases of markers
166 with different number of alleles (Gerlach *et al.*, 2010) as is the case in this study (see
167 Supporting information, Table S1). Pairwise D values (mean D_{est}) among populations were
168 calculated with the R-package "DEMEtics" (Gerlach *et al.*, 2010) and statistical significance
169 was tested using 1,000 bootstrap iterations. For both estimators, p-values were adjusted for
170 multiple comparisons after the B-H method (Benjamini & Hochberg, 1995). We used IBDWS
171 v.3.23 (Jensen, Bohonak & Kelley, 2005) in order to obtain any statistically significant
172 associations between pairwise genetic ($F_{ST}/[1-F_{ST}]$) and linear geographic (log km) distance

matrices using 30,000 randomizations. The analysis was performed both on the full and the reduced (i.e. CE Mediterranean) datasets.

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

Finally, the breeding populations of CE Mediterranean were grouped according to Structure results as well as F_{st} and D_{est} calculations, and the directional contemporary gene flow and its relative magnitude among them was estimated using the divMigrate function (Sundqvist et al. 2016) in the R-package “diveRsity” (Keenan et al. 2013). The method provides a relative (to within the analysis) migration network graph aiming to visualize the gene flow patterns among populations, with the resulting metric representing a proportion

198 of the gene flow among areas, scaled to the largest magnitude estimated. Populations are
199 represented as nodes and the properties of the lines connecting them are based on the
200 relative strength of gene flow. Populations that exhibit strong gene exchange between them
201 but weak gene flow with others tend to cluster closely together, reflecting patterns of
202 genetic structure. The method is described in detail in Sundqvist *et al.* (2016). We used N_M
203 (Alcala, Goudet & Vuilleumier, 2014) as a measure of genetic distance, and tested whether
204 gene flow among populations was asymmetrical using 10000 bootstrap iterations.

205 Signs of bottlenecks were evaluated in grouped populations using three approaches. First
206 we calculated *M*, a ratio based on the number of alleles to the allelic size range (Garza &
207 Williamson, 2001) in Arlequin. M will be smaller in populations that have suffered a decline
208 than in populations that are in mutation-drift equilibrium. A test for heterozygosity excess
209 was performed in BOTTLENECK (Piry, Luikart & Cornuet, 1999) using the Wilcoxon signed
210 rank test running 1000 iterations and using the two-phase model (TPM). As the
211 microsatellites we used are either of dinucleotide perfect repeats or of imperfect repeats,
212 both of which may tend toward the infinite allele model (IAM; Cornuet and Luikart, 1996),
213 we fixed the proportions of the TPM in favour of the IAM (Cristescu *et al.*, 2010) including
214 20% of the stepwise mutation model (SMM) and 80% of IAM. We finally tested for a mode-
215 shift distortion using a graphical approach, by plotting the number of alleles in 10 allele
216 frequency classes with 0.1 intervals, according to Luikart *et al.* (1998). In a population at
217 equilibrium, alleles with frequencies in the first class (<0.1) are expected to be more
218 numerous than those belonging to the second class, therefore allele frequencies present a
219 characteristic L-shaped distribution. Plots were examined for mode-shift distortions that
220 would be consistent with a bottlenecked population (Luikart *et al.*, 1998; Cristescu *et al.*,
221 2010). Plots were produced in R 3.2.2 and allele frequency calculations were performed
222 using the R package "Gstudio" (Dyer, 2014). Finally, the effective population size (N_E) along
223 with 95% confidence intervals (CIs) were estimated using the bias-corrected version of the

method based on linkage disequilibrium (Hill, 1981; Waples, 2006) as implemented in NeESTIMATOR v.2.01 (Do *et al.*, 2014). N_E estimates were obtained for all but the core populations (ES, APU, CNG) and Mongolia, as it is very difficult to obtain reliable estimates for large populations using this method (Waples & Do, 2010).

RESULTS

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

GENETIC DIVERSITY

We did not detect any 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 not significant in all cases (Table 1). Private alleles were present in all sampled populations with the exception of Croatia (CRO), Komotini (KAL) and one subpopulation in Central Greece (TRI). 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.

POPULATION STRUCTURE

Pairwise F_{ST} and D_{est} values were highly correlated (Pearson's correlation: $r = 0.95$, $P < 0.001$). Estimates of both F_{ST} and D_{est} (Table 2) varied between population pairs (F_{ST}: 0.003 – 0.06; D_{est}: 0.02 – 0.19), with all the core populations of Europe (Spain, Apulia, Central Greece) showing low differentiation among them. On the other hand, Mongolia and Israel seem

248 differentiated from all others, while the small peripheral CE Mediterranean populations
249 showed higher levels of differentiation from all other populations. This was particularly
250 evident for the populations of Croatia and Limnos where the highest values of D_{est} were
251 recorded when compared to all other populations (Table 2). Mantel tests showed that
252 genetic distance is not significantly correlated with geographical distance across all sampled
253 populations (r = 0.33, P = 0.1, Supporting information, Figure S1). Conversely, genetic
254 divergence of CE Mediterranean populations correlated significantly with the geographical
255 distance among them (r = 0.57, P = 0.01, Supporting information, Figure S1).

256 The Bayesian clustering method implemented in STRUCTURE suggested the presence of
257 population structuring. When all individuals were modeled, the ΔK-method suggested two
258 clusters (K = 2) as the most likely population structure (although the posterior probability
259 was higher for K = 3; Supporting information, Figure S2, Table S2). For K = 2 the admixture
260 model indicated two gene pools with all European populations showing high membership
261 coefficients in the first cluster, and the MON and LIM populations belonging to the second
262 (Fig. 2). This second gene pool seems to be present in all populations in Northern and
263 Central Greece (CNG) whereas absent from all other western populations. Lesser Kestrels
264 from Israel (ISR) were found to be highly admixed, exhibiting a mixed ancestry from both
265 clusters (Fig. 2). For K = 3, the model adds another gene pool that is mostly represented by
266 the Trans-Adriatic populations of Croatia (CRO), Apulia (APU) and Sicily (SIC) whereas Israel
267 (ISR) still seems to be of mixed ancestry. All subpopulations within Spain (SES, ES/BG) and
268 Central Greece (TRI, LAR, VOL) showed identical admixture proportions among them,
269 implying no further substructure (Fig. 2).

270 When individuals from the CE Mediterranean were modeled separately, the ΔK-method
271 suggested K = 2 as the optimal number of clusters, whereas posterior probability estimates
272 suggested the presence of four clusters (Supporting information, Figure S3, Table S3). The

populations of Croatia (CRO) and Limnos (LIM) constitute two different genetic clusters, the one present in Limnos (LIM) extending throughout Greek populations from Northern to Central Greece (CNG), while two other clusters are present in all other populations in different proportions (GIA and LES populations of western Greece form a single group). The maximum value of five clusters further partitions the Sicilian population (SIC) as a separate group. It is apparent that there is no substructure in the Central and Northern Greek population, CNG (Fig. 2).

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

DEMOGRAPHIC PARAMETERS

M ratios were found to be lower than the threshold value of 0.68 in all populations, which according to Garza & Williamson (2001) suggests that all populations have suffered a past

298 bottleneck event. The Wilcoxon signed rank test conducted in BOTTLENECK software,
299 detected signs of a recent population bottleneck for Sicily (SIC), Limnos island (LIM) and
300 Israel (ISR) populations (Table 3). On the other hand, inspection of plots of allele frequency
301 classes for recent bottlenecks did not reveal any mode-shift distortion in any of the
302 populations. However, they did show that the Croatian population (CRO) is moving towards
303 allele fixation, since this was the only population that exhibited an allele frequency in the
304 class 0.9-1.0 (see Supporting information, Figure S4). Most of the peripheral populations
305 were found to have small effective population sizes (Table 3), ranging from 24.2 (LIM) to
306 88.4 (LES). However, in some cases (SIC, LES and ISR populations) results should be treated
307 with caution as 95% CIs were broad.

308 DISCUSSION

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

Despite the high private allelic richness, suggesting that gene flow could be restricted (at some level), our analysis showed low F_{ST} values among the core populations of Europe (Spain, Apulia region in Italy and Central Greece) which is in concordance with previous larger-scale studies (Alcaide *et al.*, 2009). D_{est} on the other hand, generally showed higher values of differentiation among populations including the core ones (ES and APU). We found significant levels of differentiation among CE Mediterranean populations and a correlation of pairwise genetic and geographic distance. We should note that STRUCTURE may overestimate genetic structure in datasets characterized by such correlation (Frantz *et al.*, 2009). Correlation between genetic and geographic distance has been previously reported for the species at both local (Ortego *et al.*, 2008b) and large spatial scales (Alcaide *et al.*, 2008a; Alcaide *et al.*, 2008b) and can be driven by the distance-dependent dispersal exhibited by the species (Serrano & Tella, 2003; Serrano *et al.*, 2003; Ortego *et al.*, 2008b). Some long-distance dispersal events that could have facilitated gene flow among populations have been reported across the CE Mediterranean (Gustin, Mendi & Pedrelli, 2011; Bounas *et al.* 2016a). Nevertheless, some of these movements took place in the '50s when the species showed a wider distribution. Actually, restricted dispersal in a fragmented range, coupled with high philopatry rates, along with a relatively short generation time of the species (average lifespan of 4-6 years; Newton & Olsen, 1990; Negro, 1997), might have contributed to a more frequent individual turnover in the breeding colonies thus allowing relatively quick changes in allele frequencies that led to the patterns of genetic differentiation observed in the region (Ortego *et al.*, 2008a).

Our results for genetic bottlenecks seem to be contrasting, at first glance. *M* ratios suggest that the reported past population declines have indeed left genetic bottleneck signs in all populations throughout the species' range while tests for heterozygosity excess suggested bottlenecks only in three populations. Detection of a bottleneck using *M* ratios but not heterozygosity excess is expected when a bottleneck is older, more severe, and/or

349 the population has recovered (Williamson-Natesan, 2005). Thus Lesser Kestrel populations
350 have at some point in the past undergone a severe, prolonged bottleneck, whereas in some
351 areas (ISR, SIC, LIM) they seem to have experienced more recent, population declines. The
352 Israeli population (ISR) has gone through a steep decline and is estimated to be less than
353 10% of the population prior to 1950 (Liven-Schulman *et al.*, 2004). The geographic position
354 of Israel population could explain the increased levels of diversity despite its small N_E as a
355 result of the genetic admixture of immigrants from other European or Asian populations in
356 the area. Recent bottleneck signs were also detected in two of the peripheral populations in
357 the CE Mediterranean region (SIC, LIM), that were also differentiated and showed a
358 relatively limited gene flow with other populations (Fig. 3) and small N_E but nevertheless
359 similar diversity patterns. Despite its small N_E, Ioannina (GIA) population exhibits high levels
360 of diversity and gene flow with both core populations of CE Mediterranean (APU, CNG), a
361 process that can alleviate the bottleneck effects. Since Ioannina is a known premigratory site
362 for the species (Bounas *et al.*, 2016b), these high levels of diversity could be explained by
363 immigrants from other populations, that is individuals that visit the site during premigration
364 and return to breed there. Besides, it has been suggested that non-breeding distributions
365 can shape the genetic structure of populations (Szczys, Oswald & Arnold, 2017).

366 Finally, genetic drift could also play a role on the observed genetic patterns of
367 populations in the region, since they exhibit small size and limited gene flow with other
368 populations. This is particularly evident in the small Croatian population. This population
369 consists of only 25 pairs and was recently discovered (Mikulic *et al.*, 2013). Therefore we are
370 ignorant regarding its history, that is if the population was recently founded or present
371 historically but not detected. The population did not show any signs of bottleneck but there
372 seems to be a loss of rare alleles as well as signs of allele fixation (Table 1, Supporting
373 information, Figure S4). Despite that, there is no observed decrease in heterozygosity, which
374 could be consistent with a scenario of a recently founded population that experienced the

375 effects of genetic drift, since heterozygosity can be relatively insensitive to the loss of rare
376 alleles due to drift (Allendorf, 1986; Allendorf, Aitken & Luikart, 2013).

377 CONSERVATION IMPLICATIONS

378 According to our results, the genetic structure of the CE Mediterranean populations of the
379 Lesser Kestrel appears to reflect to some extent the demographic decline that led to its
380 range fragmentation. Therefore, we suggest the management of the whole CE
381 Mediterranean population as a single conservation unit (ESU). However, the population
382 could be divided into four different management units (MUs) since three peripheral
383 populations (CRO, LIM, SIC) showed limited migration rates and different allele frequencies
384 from all the other populations. Thus a mixed strategy that aims to preserve the high diversity
385 of the core populations of Italy and Greece, while focusing on the recovery of bottlenecked
386 peripheral populations (SIC, LIM), should be appropriate for the conservation of the species
387 in the region.

388 Translocations of individuals from the core populations to the peripheral or newly
389 founded colonies could be used as an effective conservation action (Morandini *et al.*, 2017).
390 Based solely on our results from the microsatellite analysis, the use of birds from the core
391 populations seems to be an acceptable action since they were found to exhibit high
392 diversity and low differentiation from all the others. However, the fact that our results were
393 based solely on the analysis of selectively neutral loci prevents us from conclusions
394 regarding the adaptive and evolutionary consequences of such action (Holderegger, Kamm
395 & Gugerli, 2006). Thus any future translocation programmes should consider maximizing
396 both the genetic and the adaptive similarity between populations.

397

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618 **Figure 1.** Map of the Lesser Kestrel populations used in the present study. SES: Andalucia,
619 ES/BG: Extremadura, APU: Apulia, SIC: Sicily, CRO: Croatia, GIA: Ioannina, LES: Agrinio, TRI:
620 Trikala, LAR: Larisa, VOL: Volos, KIL: Kilkis, KAL: Komotini, LIM: Limnos, ISR: Israel, MON:
621 Mongolia. Shaded areas represent the breeding distribution of the Lesser Kestrel (modified
622 from BirdLife International). Sampling sites pooled for the analyses are circled (ES: Spain;
623 CNG: Central-North Greece)

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

628 **Figure 3.** Directional relative migration networks of grouped Lesser Kestrel populations from
629 CE Mediterranean. Networks were visualized with divMigrate using Alcala's Nm. (a) all relative
630 migration rate values (b) only values above 0.2 threshold (c) only gene flow values estimated
631 >0.4. Line shading and thickness increases with the relative strength of gene flow.

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636 **Table 1** Measures of genetic variation of all sampled Lesser Kestrel populations. Number of
637 genotyped individuals (*N*), number of alleles per locus (*A*), allelic richness (A_R), observed (H_O)
638 and expected (H_E) heterozygosity, private allelic richness (π) and inbreeding coefficient (F_{IS}).
639 Values are presented as means ± standard error (in brackets)

Code	Location	*N*	*A*	A_R	H_O	H_E	π	F_{IS}
SES	Andalucia	19	7.1 (0.9)	5.4 (0.6)	0.635 (0.037)	0.696 (0.037)	0.17	0.082 (0.041)
ES/BG	Extremadura	25	8.3 (1.3)	5.6 (0.7)	0.674 (0.040)	0.699 (0.037)	0.26	0.029 (0.037)
APU	Apulia	44	9.4 (1.5)	5.5 (0.6)	0.669 (0.038)	0.707 (0.036)	0.13	0.053 (0.025)
SIC	Sicily	12	5.9 (0.8)	5.1 (0.6)	0.682 (0.038)	0.667 (0.041)	0.18	-0.045 (0.045)
CRO	Croatia	14	5.6 (0.6)	4.7 (0.5)	0.654 (0.048)	0.650 (0.048)	0.03	-0.023 (0.043)
GIA	Ioannina	24	7.6 (1.1)	5.2 (0.6)	0.634 (0.051)	0.654 (0.048)	0.16	0.036 (0.031)
LES	Agrinio	16	6.7 (0.8)	5.4 (0.6)	0.603 (0.058)	0.684 (0.042)	0.18	0.136 (0.047)
TRI	Trikala	20	7.6 (1.3)	5.5 (0.7)	0.626 (0.056)	0.680 (0.046)	0.08	0.087 (0.046)
LAR	Larisa	20	7.4 (1.1)	5.4 (0.6)	0.669 (0.053)	0.680 (0.044)	0.09	0.024 (0.045)
VOL	Volos	20	7.6 (1.2)	5.5 (0.7)	0.678 (0.051)	0.662 (0.048)	0.13	-0.027 (0.035)
KIL	Kilkis	13	6.4 (0.9)	5.3 (0.7)	0.621 (0.062)	0.661 (0.048)	0.09	0.067 (0.064)
KAL	Komotini	20	7.1 (1.1)	5.3 (0.6)	0.689 (0.053)	0.677 (0.046)	0.04	-0.019 (0.040)
LIM	Limnos	11	5.1 (0.6)	4.8 (0.5)	0.661 (0.060)	0.667 (0.041)	0.09	0.024 (0.060)
ISR	Israel	20	7.3 (1.1)	5.5 (0.7)	0.619 (0.062)	0.682 (0.042)	0.28	0.124 (0.055)
MON	Mongolia	17	7.2 (1.0)	5.4 (0.6)	0.585 (0.057)	0.655 (0.049)	0.52	0.155 (0.046)

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643 **Table 2** Pairwise F_{st}-values (below diagonal) and D_{est}-values (above diagonal) among Lesser Kestrel populations. Statistically significant values after B-H

644 correction for multiple comparisons are given in bold ($p < 0.039$)

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	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**	-

655 **Table 3.** Contemporary effective population sizes (N_E) and 95% CI, results of tests for genetic
656 bottlenecks: Garza-Williamson M values, Wilcoxon sign-rank tests for heterozygosity excess
657 and mode-shift tests for all Lesser Kestrel populations. The Wilcoxon tests were carried using
658 the TPM model (5% SMM, 95% IAM). Values in bold are indicative of a bottleneck (M<0.68
659 for the Garza-Williamson ratio and $P < 0.05$ for the Wilcoxon test)

Code	Location	N_E	M	P (TPM)	Allele frequency distribution
ES	Spain	Not estimated	**0.43**	0.083	L-shaped
APU	Apulia	Not estimated	**0.45**	0.148	L-shaped
SIC	Sicily	57.3 (29.8-327.7)	**0.39**	**0.007**	L-shaped
CRO	Croatia	47.7 (21.6-82.8)	**0.41**	0.216	L-shaped
GIA	Ioannina	26.6 (19.5-39.1)	**0.45**	0.390	L-shaped
LES	Agrinio	88.4 (40.5-167.8)	**0.45**	0.056	L-shaped
CNG	Central-North Greece	Not estimated	**0.45**	0.078	L-shaped
LIM	Limnos	24.2 (14.5-55.8)	**0.42**	**0.004**	L-shaped
ISR	Israel	54.9 (35.8-106.1)	**0.43**	**0.009**	L-shaped
MON	Mongolia	Not estimated	**0.42**	0.201	L-shaped

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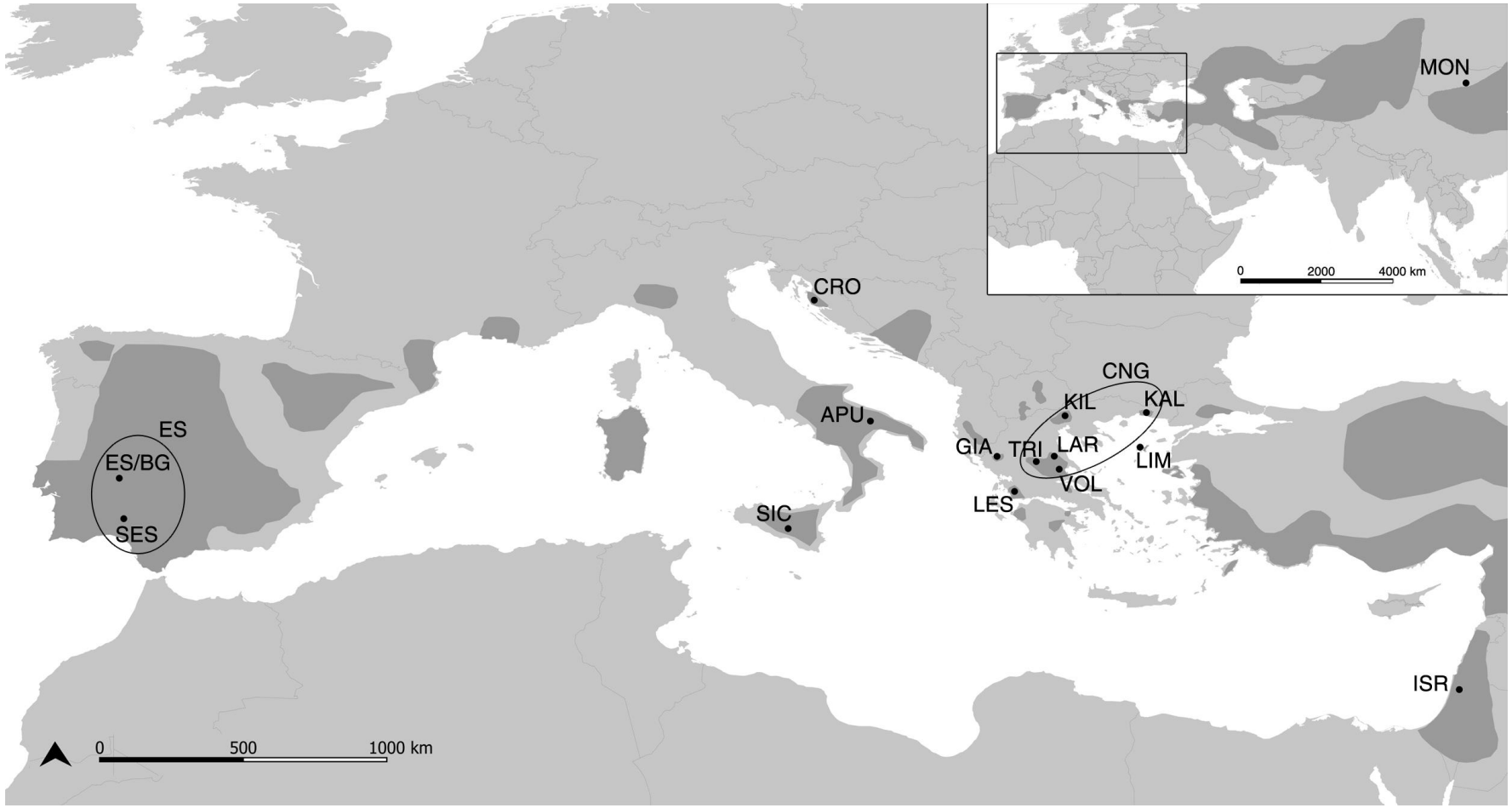

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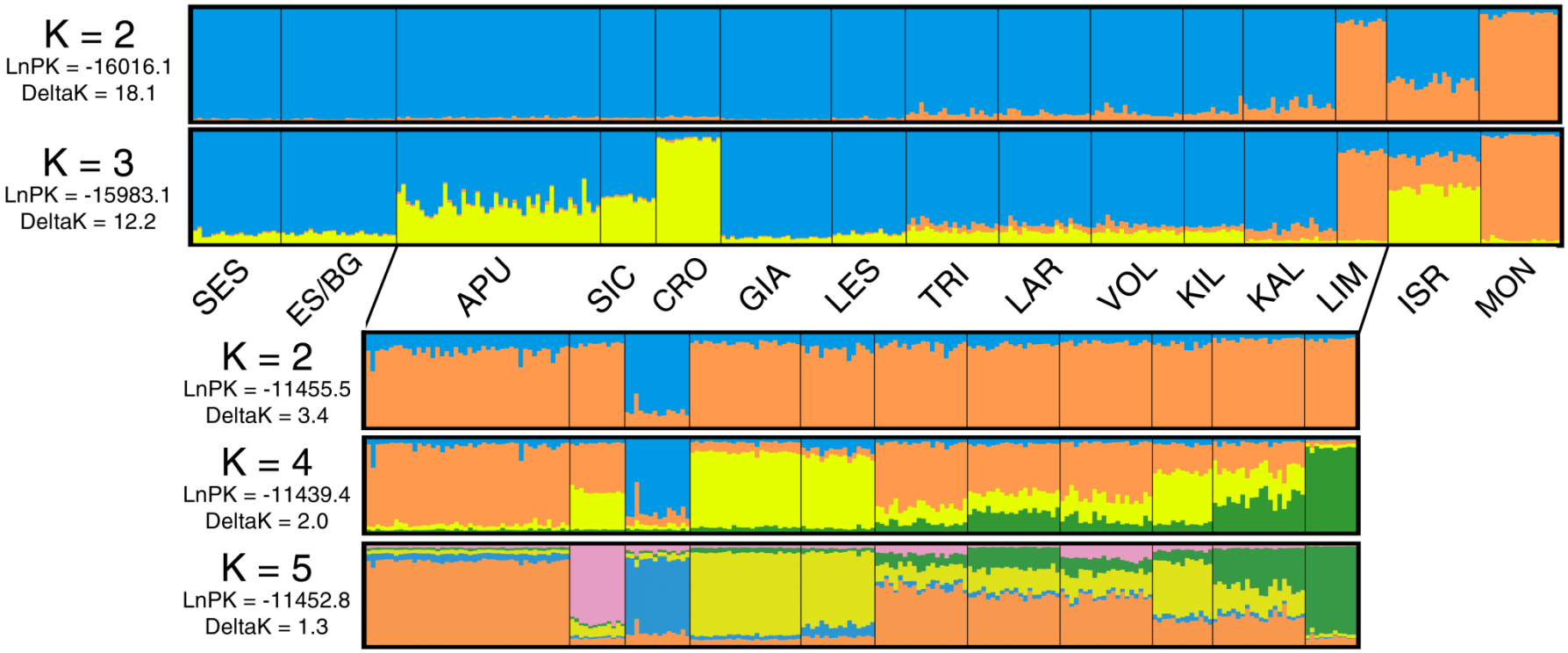
674 **Figure 1**



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676 **Figure 2**

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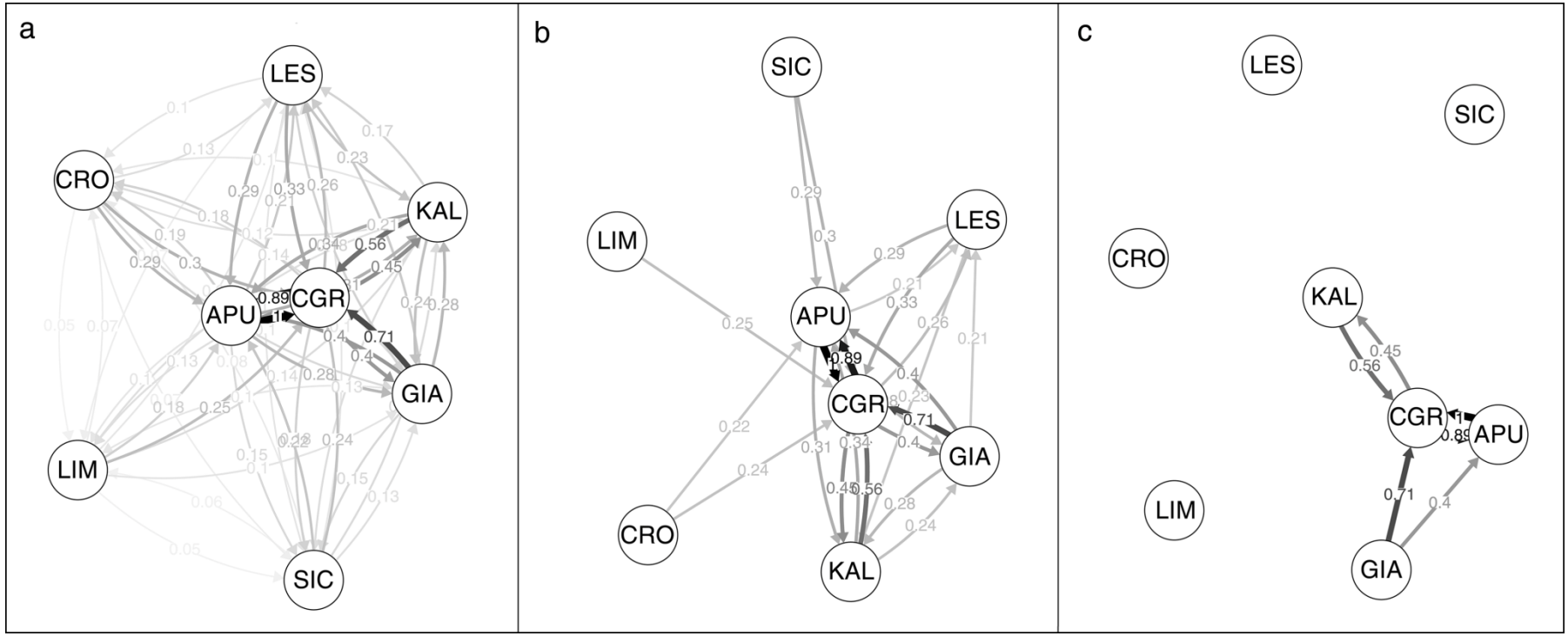

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681 **Figure 3**