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Do not disturb the family: roles of colony size and human disturbance in the genetic structure of lesser kestrel

R. Di Maggio[1], C. Mengoni[2], N. Mucci[2], D. Campobello[1], E. Randi[2,3] & M. Sarà[1]

1 Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy
2 Laboratorio di Genetica, Istituto per la Protezione e la Ricerca Ambientale (ISPRA), Bologna, Italy
3 Department 18/Section of Environmental Engineering, Aalborg University, Aalborg, Denmark

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Correspondence
Rosanna Di Maggio, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Via Archirafi 18, Palermo 90123, Italy. Tel: +39 3290842994
Email: rosannadimaggio@gmail.com; rosanna.dimaggio01@unipa.it

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Abstract

Dispersal and philopatry are fundamental processes influencing the genetic structure and persistence of populations, and might be affected by isolation and habitat perturbation. Habitat degradation induced by human activities could have detrimental consequences on the genetic structure of populations. Therefore, it is crucial to understand the role of human impact in promoting or disrupting the genetic structure. Here, we conducted a genetic analysis using 12 polymorphic microsatellite markers of 70 lesser kestrels *Falco naumanni* from 10 breeding colonies of two subpopulations in Sicily (southern Italy). Genetic differentiation between the two subpopulations was negligible, and linear distances played no role in the level of genetic relatedness recorded in the two sites. Linear distances between nests also resulted in no effects on the relatedness recorded within and between colonies in the largest subpopulation. Clusters of more-versus less-related individuals resulted when the two-dimensional positions of colonies (i.e., latitude and longitude) were tested as predictors of genetic proximity instead of linear distances. Specifically, analyses of colony features showed colony size and human disturbance as factors negatively affecting the relatedness among chicks from different nests. Regardless of colony size, less-related individuals were born in colonies located in the core of the agricultural plain, where we quantified a higher level of human disturbance. In contrast, more related individuals were in colonies located in the marginal, less disturbed, agricultural area. Given the high philopatry of this species, our results are consistent with disruption of colony fidelity related to intensification of agricultural practices. We discuss the possible implications of long-term effects of genetic variability in small and disturbed colonies on fitness and population viability.

Introduction

Understanding the mechanisms that influence animal distribution plays a fundamental role in ecology, especially when individuals occupy fragmented and disturbed environments (Hanski, 1998). From a genetic perspective, theoretical studies have suggested that deleterious alleles might accumulate more frequently in small and isolated populations than in larger and non-fragmented ones, increasing inbreeding and extinction probabilities (Saccheri *et al*., 1998; Madsen *et al*., 1999).

Dispersal and philopatry are fundamental processes influencing the genetic structure of populations (Banks, Skerratt & Taylor, 2002; Ando *et al*., 2011). Dispersal among populations maintains a higher level of gene flow and prevents inbreeding, whereas philopatry provides familiarity with foraging areas and conspecifics, avoiding travelling costs (Dieckmann, O'Hara & Weisser, 1999). Dispersal and philopatric predisposition might be affected by isolation and habitat fragmentation. These two occurrences, together with the number and size of populations may, in turn, result in severe genetic costs of populations, especially in social species (Frankham, Ballou & Briscoe, 2002; Temple, Hoffman & Amos, 2006).

Colonial species typically show a wide range of colony sizes, even within a single population, generally determined by food or nest-site availability (Moller, 2002). Living in groups could trigger fitness advantages such as reducing individual investment in vigilance (Campobello, Sarà & Hare, 2012), or enhancing survival probability (Di Maggio, Campobello & Sarà, 2013). Nevertheless, proximity with conspecific may impose fitness costs via the intensification of competition for resources (Bonal & Aparicio, 2008; Calabuig *et al*., 2010) or depression of genetic diversity by increasing opportunities to mate with kin (Serrano *et al*., 2004).

Land-use change and fragmentation represent major drivers of the current decline in biodiversity because of the

destruction, reduction and transformation of natural habitats (Bellia, Campobello & Sarà, 2011). A multi-scale level of investigation has been recommended to understand the role of human impact in promoting or disrupting the natural dynamics of dispersal or philopatry of species (Simmons, 1996). Habitat fragmentation enhances genetic differentiation of populations and increases the negative effects of genetic drift (Martinez-Cruz, Godoy & Negro, 2004) by reducing dispersal or increasing philopatry. For colonial species living in steppe-like habitats, intensification of farming practices (Sokos *et al.*, 2013) can be another potential driver of the reduction of genetic diversity of populations as a result of land-use change. *Ascaphus montanus* frogs modify their dispersal pattern in response to human disturbance (Spear & Storfer, 2010), but nobody, to our knowledge, has analysed the influence of land-use intensification on genetic relatedness.

Lesser kestrel *Falco naumanni* is an excellent model species in which to investigate the effects of anthropogenic disturbance, colony size and spatial isolation on relatedness because populations are composed of several colonies (Serrano *et al.*, 2004; Sarà, 2010) that persist in areas with different degrees of disturbance (Sarà, Campobello & Zanca, 2012). Yet, colony size is known to affect the reproductive success of lesser kestrels differently (Serrano *et al.*, 2004; Calabuig *et al.*, 2008), and to enhance nestling survival via an interactive effect between breeder abundance and nest distance (Di Maggio *et al.*, 2013). Although genetic consequences of dispersal, size and spatial isolation of colonies of this species have been studied in the Iberian Peninsula (Ortego *et al.*, 2008a,b), they are still completely unexplored in the remaining part of the breeding range.

The aims of this study were to: (1) investigate the genetic structure of two lesser kestrel subpopulations using nestling relatedness; (2) evaluate whether a spatial pattern of genetic differentiation might be described; (3) estimate the relationship between nestling relatedness and spatial isolation. Finally, to explain the obtained results, we verified whether human disturbance, together with breeder abundance and proximity, might have affected patterns of inter- and intra-colony relatedness. Specifically, intensive agriculture in proximity of colonies could influence nest success at various levels (i.e., nest abandonment, eggs/chicks survival); therefore, altering the tendency of adults to return to the same site after a nest success (Serrano *et al.*, 2004). We hypothesized a negative effect of disturbance on nestling relatedness as a result of the disruption of philopatry previously reported in this species. To address our questions, we used microsatellite markers to describe individual genetic diversity and relatedness (Manel *et al.*, 2003).

Materials and methods

Study species

The lesser kestrel is a small raptor that lives in open and dry cereal steppes of the Western Palaearctic and breeds in colonies of two to 60 pairs (Catry *et al.*, 2009). It is a secondary-cavity nester, which finds its nests in cliff holes, wall crevices and under roof tiles of rural buildings (Sarà *et al.*, 2012). After a sharp decline in the 1950s (Birdlife International, 2004), the lesser kestrel recently has had its conservation status improved to 'least concern' because of conservation actions (Iñigo & Barov, 2011). Lesser kestrels in Sicily (southern Italy) are concentrated in two main subpopulations: one in the Sicani area and the other, the largest in Sicily and one of the most important in Italy, in the Gela Plain (Sarà, 2010; Sarà *et al.*, 2014).

Study area

The Sicani area (37° 44′ N, 13° 19′ E) is located on the north-western part of Sicily, with an altitude of 626.2 ± 34.93 m [mean ± standard error (SE)] because of the presence of the Sicani Mountains. Most of the area is composed of Mediterranean xeric grasslands and wheat croplands, but large parts of this habitat are now being replaced by intensive cultivations (EEA, 2000). The Gela Plain in the south-eastern portion of Sicily (474 km^2, 37° 07′ N, 14° 19′ E) is 160.3 ± 14.27 m above sea level, and is a mosaic of pseudo-steppes dominated by artichoke (*Cynara* spp.) fields, wheat and leguminous cultivation (Triolo, Campobello & Sarà, 2011). Across the both sample areas, several farmhouses and rural buildings host numerous lesser kestrel nests inside wall crevices and under roof tiles (Di Maggio *et al.*, 2013).

Quantification of colony parameters

Investigations were conducted between April and July 2011. For this study, we chose two colonies in the Sicani area and eight colonies in the Gela Plain that represent, respectively, 10 and 10.4% of total known colonies (20 and 77, respectively) of these two areas. The number of studied colonies mirrored the different colony abundance and species occupancy within each study area (Sarà, 2010), and therefore, the subsamples were representative of the lesser kestrel subpopulations. We visited each colony at least three times during the breeding season. We used a standardized protocol (Di Maggio *et al.*, 2013) to collect data on reproductive biology and ecology and colony structure by minimizing disturbance at the reproductive sites. We classified each colony in terms of size and human disturbance, whereas each nest was characterized by a measure of spatial isolation. Colony size, defined as the number of resident pairs nesting within colonies, proved to be stable across the long-term study period (i.e., 14 years, Supporting Information, Table S1). We quantified human disturbance in each colony as the percentage of roads, urban networks and intensive cultivated areas within a 1-km radius. We selected the following Corine Land Cover (EEA, 2000) related to agricultural intensification and here indicated as numbers in brackets according to a 3-level hierarchical classification system: urban fabric (1.1), road networks (1.2.2), permanent crops (2.2) and heterogeneous agricultural areas (2.4) using ArcGIS 9.0 (ESRI, 2004). Spatial isolation of each nest was quantified by two linear distance measurements determined at different scales: intra- and inter-colony distances and by the two-dimensional (2-D) geographic position of colonies.

The first linear measure was the distance between one nest and all the others within the colony, while the inter-colony distance was the linear measure between one colony and all the others in both the Gela Plain and Sicani. Furthermore, pair abundance and intra-colony distance were combined together in a neighbour index (NI; Campobello & Hare, 2007) to provide an interactive measure between number and proximity of conspecifics (i. e. the higher the index, the more and closer the pairs in proximity of the focal nest). To compute linear and geographic distances among colonies, we recorded coordinates of colonies with a Garmin (eTrex, Olathe, KS, USA) global positioning system device and then reported their positions on Google™ Earth maps (version 7.1, Google Inc., Mountain View, CA, USA).

Blood sample collection and genetic analyses

To collect sufficient blood without causing stress to the nestlings, we used FTA™ Classic Cards (Whatman® BioScience, FTA™ Blood Collection Kit, Buckinghamshire, UK). They are made of a chemically treated paper with a circle designed to hold approximately 100 μL of blood, providing an effective matrix to preserve blood for DNA analysis. The FTA paper allowed us to store blood samples at room temperature without any special precaution or contamination. Blood samples (≈0.1 mL), obtained by puncture of the brachial vein of nestlings, were placed on the FTA cards and then stored in plastic bags until analysis.

We genotyped 70 lesser kestrel nestlings: 12 nestlings belonging to the Sicani subpopulation and 58 to the Gela Plain subpopulation. We used a ZR Genomic DNA II Kit (Zymo Research, Irvine, CA, USA) to extract and purify genomic DNA from the blood samples. The DNA samples were genotyped across 12 polymorphic microsatellite markers designed for *F. naumanni* species: FND1_2, FND1_4, FND1_5, FND1_6, FND1_7, FND1_8, FND2_1, FND2_2, FND2_3, FND2_4, FND2_5, and FND2_6 (Padilla *et al*., 2009; Ortego *et al*., 2008a). We used GIMLET 1.33 (Valiere, 2002) to perform two independent polymerase chain reaction (PCR) replicates to check the absence of allelic dropout or false alleles. We used the following PCR protocol: an initial denaturing step at 94°C for 3 min; 35 cycles at 94°C for 40 s, 55°C for 40 s, and 72°C for 40 s; and a final step at 60°C for 30 min. The PCR products were identified using an ABI 3130XL sequencer with GeneScan™-350 ROX (Life Technologies, Carlsbad, CA, USA) as a marker ladder. Allele sizes were scored with Genemapper 4.0 (Life Technologies).

GENALEX 6.1 (Peakall & Smouse, 2006) was used to estimate allele frequency by locus and colony, observed and expected unbiased heterozygosity, a chi-square test for deviations from Hardy–Weinberg equilibrium (HWE), mean number of alleles per locus, and number of private alleles per colony. Partitions of genetic diversity within and among colonies were computed by analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 1992) using PhiPT analogues of Wright's (1965) *F*-statistics.

The genetic structure of the sampled colonies was inferred using the Bayesian clustering procedures implemented in STRUCTURE 2.0 (Pritchard, Stephens & Donnelly, 2000), and graphs were obtained with STRUCTURE HARVESTER (Earl & von Holdt, 2011). Structure was designed to identify the K number of distinct genetic populations (clusters) included in the sample, assuming HWE and linkage equilibrium within each subpopulation, and to assign the individuals to the inferred clusters. Burn-in periods of 30 000 steps followed by 100 000 Monte Carlo iterations were used to obtain convergence of the parameter values. Explorative analyses were performed first with K from 1 to 5 using all the samples ignoring sample locations. All simulations were independently replicated five times for each K, using the 'admixture' and the 'independent' allele frequency models (Falush, Stephens & Pritchard, 2003). The number of K populations was set at the value that maximized the increase in the posterior probability of the LnP(D) data according to the formula $[LnP\ (D)\ k - LnP\ (D)\ k - 1]$ (Garnier *et al*., 2004). We examined genetic relatedness using the mean correlation of genetic distances based on the estimator from Lynch & Ritland (1999).

Modelling relatedness

Relatedness coefficients estimated by GENALEX represented the mean relatedness of pair-wise combinations of nestlings from different colonies, from different nests within the same colony, and within the same nest ($n = 2{,}415$). We therefore distinguished these three relatedness values to which, for brevity, we refer as inter-colony ($n = 2{,}170$), intra-colony ($n = 214$) and intra-nest relatedness ($n = 31$), respectively. We tested the correlations between the logarithm of the linear distances in metres and PhiPT values (Excoffier *et al*., 1992) by performing Mantel's (1967) tests with the software RStudio (2012) 0.98 (package ade4, Chessel, Dufour & Thioulouse, 2004). An initial Mantel test evaluated the correlation between relatedness and the corresponding linear distances among the 10 colonies of Sicani and Gela Plain subpopulations. Then, because of the small sample size of the Sicani subpopulation, we focussed on the Gela Plain subpopulation. In a second Mantel test, we fitted PhiPT values measured among Gela Plain colonies with the corresponding inter-colony linear distances. We also explored the correlation of genetic similarity between pairs of individuals in relation to their geographical positions in the Gela subpopulation (Double *et al*., 2005). GENALEX computed the autocorrelation coefficient r based on the 2-D positions and squared genetic distance matrices (local spatial autocorrelation). The geographical position matrix was calculated from X- and Y-coordinates of each of the colonies sampled in the Gela Plain. All individual residents in the same colony were given identical coordinates.

Eventually, we tested whether intra-colony relatedness was predicted by colony size, NI, and human disturbance by a generalized linear mixed model (GLMM) (McCullagh & Searle, 2000). In order to control for potential non-independence of data represented by nestlings from the same

colony and avoid pseudoreplication, colony identity was included as a random effect (Millar & Anderson, 2004). Statistical analyses were performed with Statistica 8.0 (Statsoft Inc, 2001).

Results

We genotyped 70 lesser kestrel nestlings and 12 overall microsatellite markers. Each locus analysed was polymorphic with 63 alleles for the Sicani subpopulation and 110 for the Gela Plain subpopulation. The mean number of alleles per locus was 7.5 and ranged from 5.2 (Sicani subpopulation) to 9.1 (Gela Plain subpopulation). The mean value of heterozygosity was 0.597 ± 0.05 (mean ± SE) and ranged from 0.56 (Sicani) to 0.63 (Gela Plain), with no difference between the two subpopulations (analysis of variance, $F_{1,22} = 0.53$, $P = 0.47$). We found 53 private alleles for the Gela and five for the Sicani subpopulations. This difference was probably due to the different sample size between subpopulations. Hardy–Weinberg tests showed a significant deviation from equilibrium for one locus for the Sicani subpopulation and five loci for the Gela Plain subpopulation.

Mean F_{st} values were very low (AMOVA, $F_{st} = 0.025$, $P = 0.03$), suggesting only a negligible, although statistically significant population differentiation. As expected, relatedness between nestlings belonging to the same nest (mean ± SE, 0.438 ± 0.047; $n = 31$) was higher [unequal n Tukey's honest significant difference (HSD) test, $P < 0.001$] than both inter-colony (mean ± SE, –0.033 ± 0.005; $n = 2{,}170$) and intra-colony relatedness (mean ± SE, –0.001 ± 0.005; $n = 214$), whereas inter- and intra-colony relatedness showed no significant difference (unequal n Tukey's HSD test, $P = 0.383$).

The result of the Mantel test showed no significant difference in relatedness with respect to linear distance among the 10 colonies of the Gela Plain and Sicani subpopulations ($R = 0.006$, $P = 0.401$; $n = 45$, based on 9 999 permutations).

The detailed analysis of the Gela Plain subpopulation confirmed that intra- and inter-colony relatedness did not differ within and among colonies (Fig. 1). Furthermore, the Mantel test again showed a lack of correlation between linear distances of colonies and inter-colony relatedness ($R = –0.023$, $P = 0.522$; $n = 28$, based on 9,999 permutations). Contrary to linear distances, the 2-D spatial analysis, employing latitude and longitude (Fig. 2), showed the presence of a cluster of colonies corresponding to a hotspot (*sensu* Peakall & Smouse, 2006) of genetically related individuals.

Results of the GLMM evidenced that colony size and the human disturbance estimate both had a significant and negative effect (Table 1, Fig. 3) in determining intra-colony relatedness of nestlings, whereas intra-colony distance and NI did not affect nestling relatedness (Table 1).

The four colonies clustered by the 2-D spatial analysis proved to have a statistically lower human disturbance index with respect to the other four colonies (2011, $t = 744.95$; $P < 0.01$; $n = 8$). This result was not dependent on the effect of

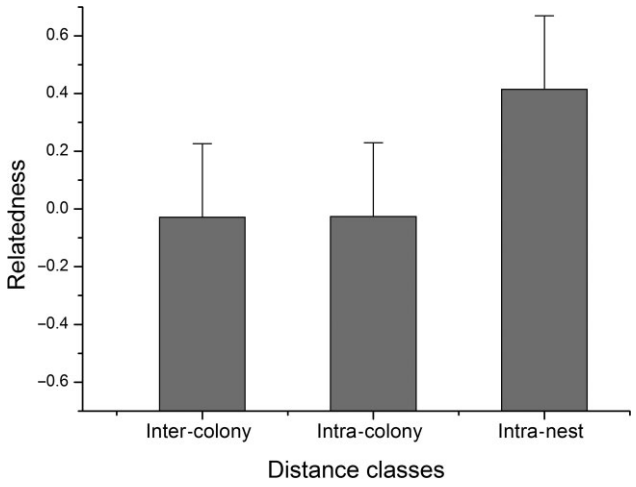

Figure 1 Different spatial scales of nestling relatedness in the Gela Plain subpopulation. In more detail: inter-colony relatedness (–0.029 ± 0.005, mean ± standard error), intra-colony relatedness (–0.026 ± 0.015) and intra-nest relatedness (0.414 ± 0.041).

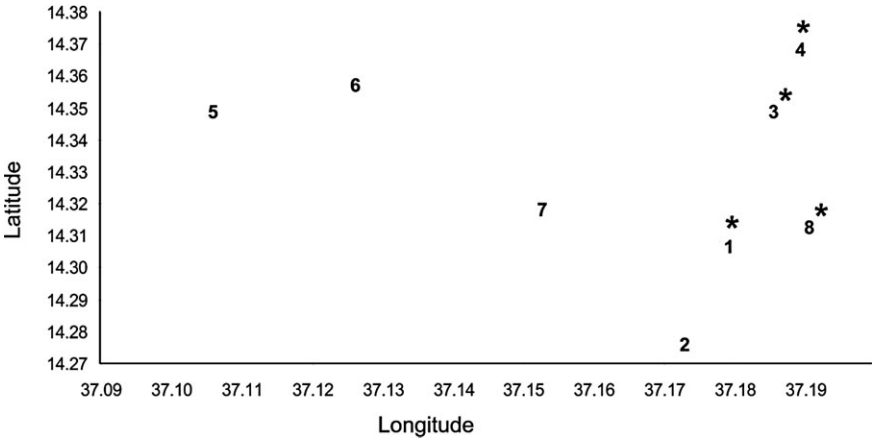

Figure 2 Results of the 2-D spatial genetic analysis showing heterogeneity in genetic autocorrelation across the Gela Plain subpopulation (2-D local spatial autocorrelation, $P < 0.05$). Asterisks indicate a cluster of high positive autocorrelation among colonies. Such a cluster of colonies is located at the edge of the agricultural Gela Plain whereas the other four colonies (i.e. colonies 2, 5, 6 and 7, respectively) insist in the core agricultural area.

Table 1 Generalized linear mixed model results showing the relationship between nestling relatedness in the Gela Plain subpopulation and colony size, intra-colony distance, neighbour index and human disturbance (n = 180)

Intra-colony relatedness	*F*	*P*
Intercept	1.314	0.253
Colony size	7.138	**0.008**
Linear distance	0.168	0.682
Neighbour index	0.908	0.342
Human disturbance	14.584	**<0.001**

Significant predictor *P*-values are indicated in bold. Colony identity was set as a random term to avoid pseudoreplication.

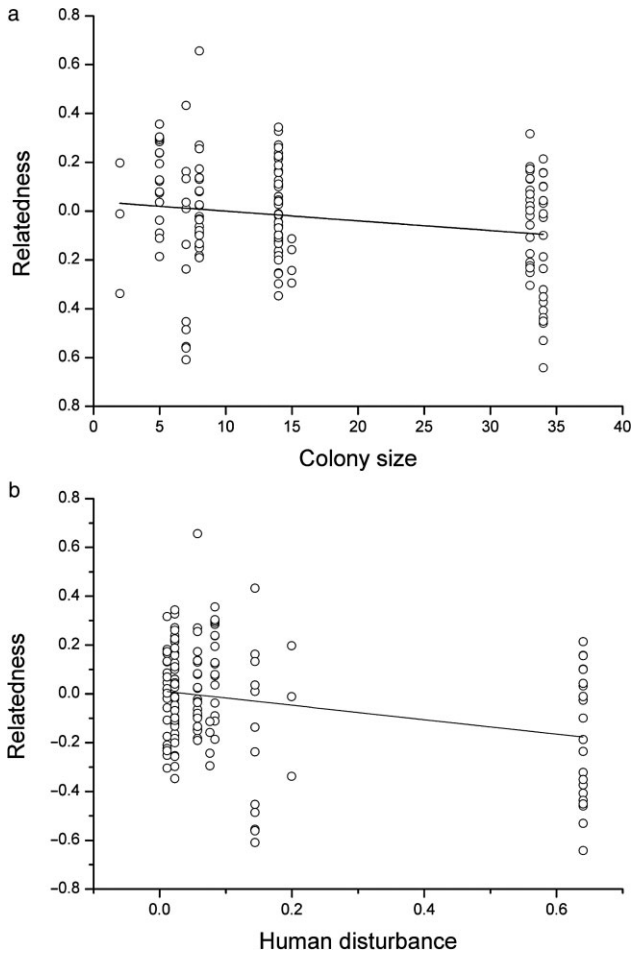

Figure 3 Relationships between colony size (a); r^2 = 0.038, P = 0.008), and human disturbance (b); r^2 = 0.132, P < 0.001) with relatedness among nestlings in the Gela Plain subpopulation.

colony size, as colonies within and outside the cluster were composed of a similar number of breeding pairs both in the year of the study (2011, t = 0.986, P 0.362; n = 8) and the previous one (2010, t = 0.556, P = 0.598; n = 8).

Discussion

Microsatellite analysis revealed, as showed in Spanish populations, a higher relatedness between chicks belonging to the same nest rather than between chicks of different nests. This finding would confirm also the low incidence of extra-pair paternity in lesser kestrels in southern Italy (Alcaide *et al.*, 2005). Genetic analyses showed only a slight genetic differentiation between our sampled subpopulations that fell within the range of values reported by Alcaide *et al.* (2009b). Nestling relatedness was, furthermore, not dependent on linear distances among colonies within subpopulations. Our results are consistent with Alcaide *et al.* (2009b), who showed low genetic differentiation among four geographically distinct populations and argued that a few long-distance dispersal events are sufficient to connect genetically distant patches and homogenize allele frequencies. Similarly, our low genetic differentiation might be explained by the fact that the Gela Plain and Sicani areas are approximately 110 km apart in distance, a value well within the maximum juvenile dispersal found so far (136 km; Serrano *et al.*, 2003).

In Gela colonies, intra- and inter-colony relatedness did not differ statistically. Adult lesser kestrels are philopatric to their previous breeding colony, and first-time breeders tend to disperse fairly close to their natal colony (Serrano *et al.*, 2003), with dispersal ranges equivalent to the average distance (7 km) among the 77 colonies of the Gela Plain. Such close network could facilitate exchange of individuals among colonies, homogenize the degree of relatedness, and, therefore, explain our results of comparable relatedness within and between colonies. In addition, the lack of any correlation among linear distance of colonies and nestling relatedness further suggested a widespread gene flow. Either way, this lack of correlation is in contrast to results found by Ortego *et al.* (2008b), where lesser kestrels born in isolated colonies were genetically less diverse. These colonies received a lower number of immigrants, supporting the idea that reduced gene flow was responsible for the observed genetic pattern.

Spatial isolation analysis in the Gela Plain subpopulation revealed a 2-D scale pattern of genetic differentiation, with four colonies showing higher genetic similarity that clustered together on the basis of their geographical position. Dixon *et al.* (2007) suggested an island model for bear populations *Ursus americanus floridanus* segregating in discrete genetic clusters. Accordingly, our results indicated that lesser kestrel subpopulations might follow a similar pattern rather than the isolation-by-distance documented in Ortego *et al.* (2008a,b) and Alcaide *et al.* (2009a).

In the Gela Plain, intra-colony relatedness of lesser kestrels was negatively influenced by colony size; thus, individuals born in larger colonies were less related than those born in smaller ones. A lower number of immigrants could arrive at small colonies, which, consequently, may have a lower chance of being explored by prospectors (Calabuig *et al.*, 2010). The reduced number of potential immigrants, together with the philopatric behaviour of kestrels, could explain why relatedness is higher in small colonies than in large ones. Our results are thus consistent with the observed positive relationship

between individual genetic diversity and colony size (Ortego *et al*., 2008b). In addition, neither the linear distance between nests in the same colony side nor the NI seemed to influence relatedness, suggesting that the main factors driving the genetic structure of colonial breeders act especially at a colony scale.

We found that human disturbance caused by agricultural intensification was able to explain the relatedness patterns predicted by 2-D geographic position. Our results hence provide a mechanism that might explain what was indicated by Dharmarajan *et al*. (2014) and Banks *et al*. (2013), thus that high levels of disturbance would promote lower relatedness. The four colonies clustered by 2-D analysis had a lower human disturbance estimate in the year of study. In the agricultural plain, these colonies occur at the edge with respect to the other four ones, categorized previously as insisting in the agricultural core (Sarà *et al*., 2012). It is therefore consistent with our results that increased disturbance, because of intensification of agricultural practices, would disrupt the philopatric behaviour reported in lesser kestrels (Ortego *et al*., 2008a) by driving relatives away from their natal sites with the consequent result of lower relatedness coefficients. If human disturbance prevents philopatry, our result would be consistent to imagine a paradoxical outcome where intense agricultural activities improve gene flow in kestrel populations and, on the contrary, low human presence would enhance inbreeding depression. However, gene flow among our subpopulations could be determined by other factors (i.e. immigration) acting alone or in interaction with human disturbance.

In conclusion, our results showed that genetic differentiation is low at a large geographical scale among populations of lesser kestrels nesting in a southern Mediterranean area. At a smaller spatial scale, we found evidence of more related individuals in small colonies and in clusters of colonies in the least-disturbed area of the agricultural plain. Accordingly, a topic to further address should examine what are the long-term effects and the incidence of such atypical source of genetic variability on population viability.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of Wilcoxon matched pairs test ($t = 11.0$, $P = 0.173$, $n = 10$) that show the consistent size between each colony size recorded in the study year (2011, Cs 2011) and colony size mean recorded across the long-term study period (i.e. from 2000 to 2014 except 2011, Cs study period) in the Gela Plain and Sicani subpopulations.