Zoology

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--Manuscript Draft--

Manuscript Number:	ZOOL-D-20-00067R1
Article Type:	Research Paper
Section/Category:	Other
Keywords:	Columba livia; feral pigeons; domestic pigeons; genetic structure; microsatellite loci
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Feral pigeon populations: their gene pool and links with local domestic breeds

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Abstract

Columba livia is a wild bird whose domestication has led to a large number of pigeon breeds. The occasional loss or straying of domestic birds determined the origin of feral pigeons, which are now widespread all around the world. In this study, we assumed that the main contribution to feral populations is provided by domestic breeds reared in the same areas. We tested this hypothesis by analysing the variability of 12 microsatellite loci in nine Italian feral populations sampled in areas with different intensities of breeding and selecting domestic breeds. We included in the analysis samples belonging to ten domestic lineages commonly bred in Italy. The pattern of geographic differentiation of feral populations turned out to be rather complex and only partially explained by the geographic distance between populations. This pattern can be understood only when the domestic breeds were included in the analysis. In particular, feral populations located in regions with a long-lasting tradition of pigeon breeding showed a high level of admixture with domestic breeds, in particular with Racing Homer and Piacentino. Ferals from Bolzano, Venice and Sassari were characterized by unique genetic components, mostly not shared by other feral populations and by the considered domestic breeds. Our results further emphasize the complex genetic structure of feral populations whose origin can be properly investigated by taking into account the pool of domestic pigeons bred in the considered area.

Keywords: *Columba livia*, feral pigeons, domestic pigeons, genetic structure, microsatellite loci

1. Introduction

Feral pigeons *Columba livia* are one of the most common inhabitants of cities all around the world, being a virtually cosmopolitan taxon (Lever, 1987). The Neolithic domestication of the wild rock dove, dating back to about 6,000 years BC (Sossinka, 1982) and the subsequent selection of the various pigeon domestic breeds represent the initial steps of their origin. Indeed, feral pigeons originated from domestic pigeons abandoned or escaped from farms and then settled in urban habitat (Johnston and Janiga, 1995). This process began in the Old World and it is still ongoing in almost every place where domestic pigeons were introduced or bred (Lever, 1987; Johnston, 1994). Synanthropic wild rock doves seem to have contributed only marginally to the constitution of feral populations and only within their original range (Ballarini et al., 1989; Johnston and Janiga, 1995).

The different ways pigeons established themselves in European and North-American urban habitats have been reviewed from a historical point of view by Johnston and Janiga (1995), Haag-Wackernagel (1998) and Baldaccini and Giunchi (2006). Given that nearly any domestic breed had (and still has) the potential to contribute to the feral gene pool, at least two main contributions to feral populations have been identified. Dovecotes had been rather widespread in several European countries until the 19th century [e.g. The British Isles (Ritchie, 1920; Gompertz, 1957); France (Van der Linden, 1950); Italy (Giachetti, 1894)]. Pigeons breeding in dovecotes could revert to a free life in towns and sometimes they were even forced to leave their dovecotes e.g. during the French Revolution, when large dovecotes owned by aristocrats were destroyed (Van der Linden, 1950). Urban dovecotes are now rare in Europe so they do not represent an important source of individuals for current feral populations. The main contribution to feral populations both in Europe and North America probably was and is still represented by homing pigeons that failed to return to their home loft (Goodwin, 1960; Simms, 1979; Stringham et al., 2012). In particular, Simms (1979) suggested that juveniles homing pigeons lost at the beginning of their training period constituted an important component of feral populations at least in the UK.

In Italy, pigeon breeding has been an embedded activity since ancient times especially in Northern and Central regions, where several domestic breeds have been selected (McNeillie, 1976; Bigi et al., 2016). It can be assumed that these local breeds have mostly contributed to the Italian feral populations, possibly with birds (both dovecote and wild individuals) imported mainly from Egypt (from the range of *C. l. gaddi* and *C. l. schimperi* subspecies) and Spain, used in pigeon shooting ranges till the first half of the last century (Ghigi in Toschi, 1939).

In recent years, domestic pigeons have been the subject of several genetic investigations, mainly aimed at understanding their relationships and their geographic origins (Stringham et al., 2012;

Shapiro et al., 2013; Biala et al., 2015; Bigi et al., 2016; Domyan and Shapiro, 2017), whereas feral pigeons, on the contrary, received rather less attention. While a number of the above-mentioned studies on domestic breeds actually included one group of feral pigeons in their analyses (e.g. Stringham et al., 2012; Biala et al., 2015), only a few studies were specifically focused on feral pigeons, with the aim of clarifying the pattern of genetic differentiation within and between urban areas (Jacob et al., 2015; Tang et al., 2018; Carlen and Munshi-South, 2020). To our knowledge, no study has systematically investigated the degree of influence of different domestic breeds on the genetic composition of feral populations.

In this paper, we hypothesized that the gene pool of feral pigeons living in a specific urban context should at least partially reflect the prevalent breeding activities of domestic breeds in the area, given the low dispersal propensity of ferals (Hetmanski, 2007; Jacob et al., 2015). In particular, we would like to test the hypothesis put forward by Stringham et al. (2012), that almost all feral populations should show strong affinities with racing breeds.

We compared the genetic pattern of nine Italian feral populations distributed in areas with different domestic pigeon breeding traditions with the aim of: 1) characterizing their genetic composition and structure; 2) testing their affinities with a number of domestic pigeons commonly bred in Italy that could have contributed to their actual genetic pool (Bigi et al., 2016). For this reason, we included in the sampling a number of feral populations belonging to the Pianura Padana (Pavia, Reggio Emilia, Modena), where the breeding of racing pigeons is traditionally widespread and where a significant number of Italian breeds originated (Ghigi, 1950; Bigi et al., 2016), to Central (Pisa, Livorno) and Northeastern Italy (Venezia, Treviso, Bolzano), where racing pigeon breeding is rather less common, and to Sardinia (Sassari) where racing pigeon breeding is not present (Fig. 1). Sardinia possibly still hosts colonies of wild rock doves (Ragionieri et al., 1991; Baldaccini et al., 2000) which might have contributed to the gene pool of feral populations in the area.

2. Materials and methods

A total of 194 samples were obtained from nine urban areas (Fig. 1, Table 1) in North and Central Italy. Birds were captured using walk-in traps (Bolzano, Venice, Treviso, Pisa, Reggio Emilia, Pavia) or were hosted in wildlife rehabilitation centres (Modena, Livorno, Sassari). At least five contour feathers (belonging to the breast and the back) were collected from each individual and stored as soon as possible (usually during the same day of sampling) in ethanol 95% at -20 °C. All the procedures were performed with the permission of the local authorities and complied with the

Italian law on animal welfare.

2.1. DNA extraction and microsatellite genotyping

DNA was extracted using the ZR Genomic DNA II kit (Zymo Research) and amplified at 12 loci microsatellite (*Cliμd01*, *Cliμt13*, *Cliμd17*, *CliμT17*, *Cliμd16*, *Cliμd19* - (Traxler et al., 2000); *CliuD11*, *CliuT47*, *CliuT24*, *UUCli10*, *UUCli13*, *UUCli14n*, *UUCli12*, *UUCli08* - (Stringham et al., 2012). Two independent replicates were performed in 8 μl of volume containing 0.5 U of Hot Start Taq polymerase (Qiagen), 0.18 μM of each primer and 0.04 mg of Bovine Serum Albumin Fraction V (Roche), with the following thermal protocol: (94 °C x 5'), 10 cycles at (94 °C x 40'') (55 °C-0.5 °C x 40'') (72 °C x 60''), 35 cycles at (94 °C x 40'') (50 °C x 40'') (72 °C x 60''), and a final extension at 72 °C for 10'.

2.2. Data analysis

The number of alleles (*Na*), the number of effective (*Ne*) and private alleles, and the expected (*He*) and observed (*Ho*) heterozygosity were obtained using GenAlEx 6.503 (Peakall and Smouse, 2006, 2012; Smouse et al., 2015). Allelic richness (*Ar*) was computed in Fstat to minimize the effect of a different sample size (Goudet, 2001). Departure from Hardy-Weinberg equilibrium was estimated with the exact test in Genepop on the web (Raymond and Rousset, 1995) (http://genepop.curtin.edu.au/) using 100 batches and 1000 iterations per batch.

Pairwise *Fst* computation and AMOVA test (Excoffier et al., 1992) were performed in Genetix 4.05.02 (Belkhir et al., 2002) and GenAlEx 6.503, respectively, to evaluate the significance of genetic differentiation between groups. The matrix of P-values corresponding to each pairwise *Fst* computation was adjusted using the Bonferroni correction (Sokal and Rohlf, 1995) for multiple comparisons (nominal level for multiple tests = 0.05).

Genetic divergence among geographical groups was estimated using distance based on the Stepwise Mutation Model (SSM) and the Infinite Allele Model (IAM). Cavalli-Sforza chord distance (*Dc*) (Cavalli-Sforza and Edwards, 1967) and the proportion of shared alleles (*Dps*) (Bowcock et al., 1994) were computed in MSA (Dieringer and Schlotterer, 2003) and the resulting networks were visualized in SplitTree 4.13.1 (Huson and Bryant, 2006). *Da* distance (Nei, 1973) and *Fst* distance (Latter, 1972) with sample size bias correction, Goldstein distance $(\delta\mu)^2$ (Goldstein et al., 1995) and

Shriver distance (*Dsw*) (Shriver et al., 1995) were computed and visualized in a Neighbour-Joining tree using Poptree on the web (http://poptree.med.kagawa-u.ac.jp/). A total of 10,000 bootstraps were used to reconstruct the tree topology.

Clustering was computed using a Bayesian model in STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003; Hubisz et al., 2009) with no admixture and independent allele frequencies models. The USEPOPINFO selection flag column was considered = 0. A total of five independent runs for a number of subpopulations (K) from 1 to 10, was run with a burn-in period of 30,000 followed by 300,000 MCMC repetitions. STRUCTURE HARVESTER on the web (Dent and von Holdt, 2012) was used to process the data and identify the best number of clusters according to both ΔK and Mean Likelihood (Janes et al., 2017). CLUMPP (Jakobsson and Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) were used respectively to merge the five independent results for each K and to display the final data. A Discriminant Analysis of Principal Components (DAPC) that is not affected by Hardy-Weinberg disequilibrium was also performed to verify the substructure of feral populations using the Adegenet package (Jombart, 2008; Jombart and Ahmed, 2011) for R software (R Core Team, 2019). A total of 80 PCs and four discriminant functions were retained to draw the plot.

All individuals belonging to the same urban area were characterized by identical coordinates. The hypothesis of Isolation by Distance among populations was tested in GenAlEx by means of the Mantel test calculated on the geographic distance and the *Fst* obtained by GenAlEx computation.

Genetic migration levels between populations was estimated using the function divMigrate of the DiveRsity R pakage (Sundqvist et al., 2016). Nei's *Gst* and filter_threshold = 0.5 were set to plot the network and the relationships among populations.

2.3. Relationship between feral pigeons and domestic breeds

The considered domestic samples were the same already analysed in Bigi et al. (2016) and were used to verify the affinity of feral populations with Italian domestic breeds. In details, a total of 250 samples belonging to 10 Italian native breeds (Florentine, Italian Beauty Homer, Italian Owl, Italian Owl Rondone, Piacentino, Romagnol, Runt, Sottobanca, Triganino Schietto and Triganino Gazzo) and one international breed (Racing Homer) commonly bred in Italy, were included in the analysis (Table 1, see Bigi et al., 2016 for further details). *Dc*, *Dps*, *Da*, *Fst*, $(\delta\mu)^2$ and *Dsw* were computed as for the previous analysis on feral populations to identify the phylogenetic affinities. Cluster

155 analysis was conducted in STRUCTURE using the models applied in the previous section with K
156 ranging from 1 to 20. DAPC was calculated using the same number of PCs and discriminant
157 functions used in the previous section. Cluster analysis and DAPC were run as reported in the
158 previous paragraph.

159 After the preliminary results, five breeds (Florentine, Italian Owl, Italian Owl Rondone, Triganino
160 Schietto and Triganino Gazzo) that did not show any genetic relationship with feral pigeon
161 populations (ESM 1) were excluded from the analysis.

162

163 **3. Results**

164 *3.1. Genetic variability of feral populations*

165 The descriptive statistics for the genotypes are listed in Table 2. The allelic number ranged from 6.2
166 ± 0.5 SE (Venice) to 8.5 ± 0.9 (Reggio Emilia) with an average value of 7.6 ± 0.8. The average
167 number of effective alleles decreased to 4.4 ± 0.5. Mean values of expected (*He*) and observed (*Ho*)
168 heterozygosity were 0.718 ± 0.062 and 0.726 ± 0.050, respectively. Allelic richness was computed
169 on a minimum value of 14 samples and ranged from 5.6 (Venice) to 7.6 (Leghorn) with an average
170 value of 6.9. A total of eight different private alleles were detected in Sardinian ferals even if with a
171 low frequency (< 10%).

172 No departure from Hardy Weinberg equilibrium was detected. *Fst* was not significant among feral
173 populations in Central Italy (ESM 2). The highest values were retrieved between Venice and
174 Bolzano (0.093) and between Sassari and Venice (0.081). The lowest values were recorded between
175 Pisa and Treviso (0.016) and between Pavia and Treviso (0.019). AMOVA did not result in a
176 structured sampling with 90% of differences found within individuals and only 4% among
177 populations.

178 Phylogenetic trees built using *Da*, *Fst*, and $(\delta\mu)^2$ distances did not show any significant structure
179 (ESM 3). Ferals from Leghorn, Pisa, Pavia, Modena and Reggio Emilia clustered together in all the
180 trees, but the associated bootstrap values were so low as to make the topology not significant.
181 Acceptable bootstrap values were obtained only for the group constituted by Venice and Treviso.
182 Additional results from other distance computations [Cavalli-Sforza chord distance (*Dc*), proportion
183 of shared alleles (*Dps*) and Shriver distance (*Dsw*)] were not shown as they did not produce a
184 different structure.

185 Mantel test evidenced a positive correlation between the genetic and geographic distances (r = 0.50;
186 P = 0.03, ESM 4a); this correlation remained almost unchanged (r = 0.46; P = 0.01) when the
187 Sardinian sample was removed from the analysis (ESM 4b).

188 No defined genetic structure was identified by both multivariate and Bayesian analyses (Fig. 2a-c).
189 DAPC analysis showed an admixed pattern in Central Italy, whereas samples from Sassari,
190 Bolzano, and Venice clustered separately from the others. The population from Treviso plotted in
191 an intermediate position between the remaining populations and F_Venice.

192 Both Mean Likelihood and ΔK obtained from STRUCTURE HARVESTER identified the best
193 grouping at K = 4. At K = 2 Bolzano and Sassari populations split from the remaining populations.
194 K = 3 permitted an additional distinction of ferals from Treviso and Venice. At the most well
195 supported K value (K = 4), ferals from Bolzano, Venice, and Sassari were associated to different
196 clusters with high *q* individual membership values, while samples from Pavia, Reggio Emilia,
197 Modena, Pisa, and Leghorn remained not differentiated from each other and showed retraces of
198 admixture with F_Bolzano, F_Venice, F_Sassari. The genetic composition of F_Treviso was
199 mainly associated with F_Venice but showed traces of admixtures with the former five populations
200 (Fig. 2c).

201 No genetic components shared between F_Bolzano, F_Sassari and the other feral populations was
202 estimated by the divMigrate function (Fig. 3). Gene flow towards F_Treviso from F_Venice and
203 F_Pavia resulted well supported (asymmetric values = 0.8 and = 0.52, respectively), as well as that
204 from F_Pavia to F_ReggioE. Populations from Modena, Reggio Emilia, Leghorn and Pisa resulted
205 to be quite interconnected. The highest values of gene flow (0.94 and 1.0) were reported between
206 F_Modena and F_ReggioE.

207

208 *3.2. Relationship between feral pigeons and domestic breeds*

209 Phylogenetic trees built using *Fst*, D*a* and $(\delta\mu)^2$ distances did not show any significant structure
210 (ESM 5) and feral groups and the domestic lineages considered in the analysis were differently
211 associated depending on the distance computation considered. As in the previous section, Cavalli-
212 Sforza chord distance (*Dc*), the proportion of shared alleles (*Dps*) and Shriver distance (*Dsw*) were
213 not informative and are not shown.

214 The DAPC of the nine feral populations and the six Italian breeds did not reveal a sharp separation
215 between the two groups (Fig. 4a). With the exception of domestic Sottobanca and Romagnol, all the

other breeds overlap with feral populations in the plot. F_Venice and F_Sassari did not show any tracks of an origin from the considered domestic lineages because in the plots they did not overlap with any of them. The Bayesian analysis partially confirmed the results of DAPC and the *q* individual membership values identified a relationship between domestic and local feral pigeons (Fig. 4c and 5). The ΔK computation in STRUCTURE HARVESTER identified eight main informative clusters (Fig. 4b). The main splits at K = 2, 4 and 6 were also plotted in Figure 4c to describe the main similarities among groups. Interestingly, the first split (K = 2) does not occur between feral and domestic pigeons but between Racing and Italian Beauty Homers and the other groups. At K = 4, Runt and Sottobanca separated from other domestic lineages while ferals from Sassari separated from peninsular populations. At K = 6, ferals from Sassari, Bolzano, Venice and Treviso separated from the other populations while Romagnol splits from Homers. The evaluation of the estimated membership coefficient for each population (*q*) at K = 8 identified the predominance of the domestic component characterizing the Homers (light blue bar in Fig. 5) in feral populations from Lombardy, Emilia-Romagna and Tuscany, particularly in F_ReggioE, F_Modena, and F_Leghorn. Rather high percentages of domestic Piacentino and lower values of domestic Sottobanca and Runt were found in all populations, including F_Bolzano and F_Sassari, although the latter populations were characterized by over 60% of a unique private component. Ferals from Venice showed a high percentage of a unique genetic component, that was also detected in the other feral populations but that was almost irrelevant in domestic lineages. As evidenced in Figure 5, the population of Venice did not show relevant traces of admixture with the considered domestic populations.

4. Discussion

This paper represents one of the few studies dealing with the genetic structure of feral pigeon populations. Up to now, only two studies were specifically focused on clarifying the pattern of genetic differentiation of these birds between urban areas (Jacob et al., 2015; Carlen and Munshi-South, 2020). Furthermore, while it is largely accepted that feral pigeons originated from domestic breeds (see for instance, Johnston and Janiga, 1995), this study is the first one that systematically investigates the affinities between feral pigeon populations and the domestic breeds commonly reared in the same area. To our knowledge only Stringham et al. (2012) and Biala et al. (2015) tried to quantify the gene flow between several domestic breeds and feral pigeons. However, both studies considered only a small number of feral populations (two and one respectively) and in Biala et al. (2015) the feral group was composed of birds sampled in different towns. In the present study we sampled a significant number of feral populations in

249 order to investigate the contribution of domestic breeds to their gene pool and how this contribution varies
250 among populations located in different areas.

251 On the whole, the populations analyzed in our study showed levels of genetic variability lower than those
252 observed by Jacob et al. (2015). The low levels of variability indices which were found in the populations
253 lacking genetic admixture allowed us to argue that these values were influenced by the reduced incidence
254 of gene flow between these populations and other feral populations.

255 The genetic distance between sampled feral populations was correlated with their geographic distance,
256 both considering all samples or only the peninsular ones. This result is in agreement with the data provided
257 by Jacob et al. (2015) and further confirms that the exchange rate of individuals among cities is relatively
258 rare (Johnston and Janiga, 1995; Hetmanski, 2007), given the inability of feral pigeons to undergo long
259 flights, as experimentally demonstrated by Chelazzi and Pineschi (1974) and by Edrich and Keeton
260 (1977). Carlen and Munshi-South (2020) observed on the contrary relatively little genetic differentiation in
261 feral pigeons across a 750-km strip of large metropolitan areas in United States (the Northeastern
262 megacity). The high level of urbanization of this area creates a nearly continuous habitat for pigeons,
263 possibly facilitating their movements and thus gene flow (Carlen and Munshi-South, 2020). In our study
264 area the pattern of geographic differentiation is actually rather complex and only partially explained by the
265 geographic distance between populations. Indeed, while our data do not support a well defined genetic
266 structure, it is interesting to observe that Sardinia (F_Sassari) and North-eastern populations (F_Bolzano,
267 F_Venice and F_Treviso to a lesser extent) tended to cluster separately and showed a null or rather low
268 gene flow with the remaining populations. On the contrary, populations belonging to Tuscany, Emilia-
269 Romagna and Lombardy (F_Pisa, F_Leghorn, F_Modena, F_ReggioE and F_Pavia) showed a high level
270 of admixture, almost independent of their geographic distance. The genetic distinction of populations like
271 F_Bolzano, F_Sassari and F_Venice, can be attributed to their relative geographic isolation. Actually,
272 Bolzano is located in an Alpine valley surrounded by habitats mostly unsuitable for pigeons, while
273 Sardinia is an island and it is known that pigeons do not like to fly over large water bodies (Wagner et al.,
274 1972). The effect of the surrounding water should probably be taken into account also for Venice: for
275 instance, as reported by Soldatini et al. (2006), the number of Venetian pigeons involved in foraging
276 flights outside the city is very low considering the size of the population, which possibly confirm the low
277 propensity of these pigeons to fly over the lagoon and thus also to disperse inland. The relatively high gene
278 flow from F_Venice to F_Treviso estimated by divMigrate can be interpreted both considering the
279 relatively short distance between the two cities, but also the likely common origin of the two populations.

280 The level of admixture of the remaining populations (F_Pisa, F_Leghorn, F_Modena, F_ReggioE and
281 F_Pavia) and the high level of estimated gene flow among them as well as between F_Pavia and

F_Treviso are quite difficult to explain either considering the above mentioned low rate of dispersal among cities (Johnston and Janiga, 1995; Hetmanski, 2007; Jacob et al., 2015) or the effect of extreme urbanization (Carlen and Munshi-South, 2020). It can not be excluded, indeed, that dispersal events could regularly occur between very close cities (e.g. Modena and Reggio Emilia) connected by mainly urban landscape. This explanation seems rather unlikely, however, when considering the high similarity of populations (e.g. F_Modena, F_ReggioE and F_Pavia *vs* F_Pisa and F_Leghorn) separated by true geographical barriers such as the Apennine Mountains. The migration events described by divMigrate, which are estimated from the allele frequencies retrieved inside the populations, could be interpreted, however, not as real gene flow mediated by animal movements between populations. Instead, they could indicate that the considered populations have common genetic components, partially due to their origin from a shared domestic gene pool. The inclusion in the analysis of domestic samples confirms this hypothesis. Indeed, in these feral populations it is possible to identify a significant component belonging to the domestic breeds considered in this study. In particular, the Racing Homer and Piacentino components largely dominates the gene pool of these populations. These domestic components are still detectable in F_Sassari, F_Bolzano, F_Venice and F_Treviso, but at negligible percentages. This pattern can be explained by considering the long-lasting and still ongoing tradition of keeping and selecting pigeon breeds especially in Emilia-Romagna and Lombardy (Ghigi, 1950; McNeillie, 1976). In particular, the Racing Homer component is quite evident mainly in populations located in areas where Racing Homer breeding and racing is widespread (i.e. Emilia-Romagna and Lombardy, see Figure 1), while it is almost absent where those activities are missing (i.e. Bolzano and Sardinia). Our data only partially confirm the hypothesis by Stringham et al. (2012, but see also Goodwin, 1960; Simms, 1979) that Racing Homers constitute the most important component of feral pigeon populations. Indeed, other breeds, such as Piacentino, can be dominant or co-dominant in the feral gene pool, as observed for example in F_Pisa, F_Pavia and F_ReggioE.

Interestingly the five breeds excluded from the analysis (Florentine, Italian Owl, Italian Owl Rondone, Triganino Schietto and Triganino Gazzo; ESM 1) either disappeared from Italy at the beginning of the last century (Giachetti, 1894) and were reintroduced only recently (Florentine) or were not bred outdoor from the first half of 20th century (Italian Owls and Triganino Schietto and Gazzo; Vaccari and Zambon, 2014). Therefore only a scarce contribution of these breeds to the present-day Italian feral populations was expected.

Our results further emphasize the complex origin of feral populations and suggest a past and probably ongoing flow of domestic pigeons into feral populations in areas surrounded by a high number of pigeon fanciers. This seems to be confirmed by the difference observed between F_Venice and F_Treviso. These

populations form a fairly separated cluster, which probably indicates that they share a common origin. However, being located in a lagoon, Venice has no pigeon fanciers nearby and thus the genetic contribution of the domestic breeds considered in this study to its feral population is very low. On the other hand, Treviso is surrounded by farms that probably hosted and still host pigeon dovecotes, which could increase the likelihood of exchanges between domestic and feral pigeons (see below). It should be noted that it is rather impossible to have detailed information regarding the actual distribution of pigeon breeding around a given city and for this reason the above pattern is characterized by some unexplained variability that might be related to the scale of our analysis.

The mechanisms leading to the admixture between domestic and feral pigeons have probably been and still are both pigeon racing and feral pigeon foraging behaviour. As observed by Goodwin (1960) and Simms (1979) pigeon races are sources of numbers of lost Racing Homers that flock together with ferals. Furthermore, the daily foraging flights of ferals towards the surrounding crop fields (Johnston and Janiga, 1995; Giunchi et al., 2012) may encourage farm dovecote individuals to join them. As a partial support to this hypothesis, it should be noted that birds living in cities mostly surrounded by an unsuitable foraging habitat show both less propensity to perform foraging flight [i.e. Bolzano (Baldaccini et al., 2015) and Venice (Soldatini et al., 2006)] and a less relevant component of the studied domestic breeds in their gene pool.

Recent data on the monk parakeet *Myiopsitta monachus* and ring-necked parakeet *Psittacula krameri* suggest that a high degree of admixture is not directly related to invasive success in an urban habitat and does not prevent the possibility of rapid adaptation to the urban environment (Edelaar et al., 2015; Le Gros et al., 2016). In this regard, it would be interesting to use different markers (e.g. Single Nucleotide Polymorphisms (SNPs) already tested in American feral pigeons - Carlen and Munshi-South, 2020) to deepen the relationships between domestic and feral pigeons belonging to the same area. This approach would be helpful, in particular, to test whether the populations with a higher degree of admixtures actually show higher frequencies of phenotypic characters belonging to domestic breeds or whether the domestic phenotypes are quickly counter-selected by the urban environment (see Johnston and Janiga, 1995; Sol, 2008).

As observed above, feral pigeons from Bolzano, Venice/Treviso and Sassari were characterized by unique genetic components, mostly not shared by the other feral populations investigated. Considering the geographic position of those populations, it can be hypothesized that these unique components belong to domestic breeds not originated and/or reared in Italy (e.g. central-european breeds for Bolzano, eastern breeds for Venice/Treviso). Concerning birds from Sassari, it should be noted that Sardinia still hosts wild populations of rock doves (Ragionieri et al., 1991; Johnston, 1992; Johnston and Janiga, 1995). Johnston

and Janiga (1995) indicated that when wild and feral pigeons live in sympatry it is likely that they interbreed, thus it can be hypothesized that wild pigeons may have contributed to F_Sassari. In this regard, it is interesting to observe that, contrary to Jacob et al. (2015) and Biala et al. (2015), we found eight private alleles in feral samples, all belonging to the Sardinian population. This result could be explained by considering at least three possible factors: a) the effect of genetic drift, being Sardinian populations relatively isolated (see above); b) the effect of domestic breeds not included in our sample and not affecting other feral populations or c) the gene flow between wild rock doves and feral pigeons as hypothesized by Ragionieri et al. (1991). Our data do not allow to discriminate among these effects so this topic deserves further investigations.

Our data emphasize the critical role of the sampling protocol when studying the relationship between feral pigeons and domestic breeds. Indeed, papers studying this topic often did not consider single populations of feral pigeons, but mixed together pigeons sampled in different cities (see e.g. Biala et al., 2015; Shao et al., 2019). Moreover the domestic breeds included in the analysis were selected without taking into consideration the local tradition of pigeon breeding in the areas where feral pigeons were sampled. This probably explains some of the inconsistencies in the results obtained for instance by Stringham et al. (2012) and Shao et al. (2019), as the former suggested a strong relationship between Racing Homers and feral pigeons, which is not evident in the latter study.

To conclude, our data provide the first detailed analysis of the variability of the relationships between feral pigeon populations and domestic breeds, shedding further light on the way feral populations originated and are maintained. Our results emphasize the complexity of the feral gene pool whose composition shows high spatial variability possibly depending on both ecological and anthropic factors. In particular, factors such as geographic isolation of feral populations along with the prevalent farming activities and the local diversity of domestic pigeon breeds seem to play a central role in this regard. Further studies are needed in order to investigate the role of wild rock dove on Italian feral gene pool and in particular on Sardinian feral populations.

Declaration of Competing Interest

The authors of this study had no financial or competing interest

Acknowledgments

We thank local regional authorities for providing permits to sample feral pigeons across Italy. We

also thanks Andrea Barbon, Pierluigi Berra, Danilo Pisu, Cecilia Soldatini and the staff of the wildlife recovery centres of the following cities for their collaboration in collecting samples: Livorno (Gianluca Bedini, Renato Ceccherelli, Nicola Maggi), Modena (Piero Milani), Reggio-Emilia (Sergio Mazzali), Sassari (Marco Muzzeddu). Thanks are due also to the Federazione Colombofila Italiana and to the Federazione Italiana Allevatori Colombi (FIAC) for samples of domestic breeds and for data regarding the rearing activity of Racing Homers in Italy. Wendy Doherty edited the language of the manuscript. The comments of four anonymous reviewers greatly improved previous drafts of this manuscript.

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534

535 **Figure captions**

536 **Fig. 1.** Sampling localities of feral pigeons (white dots), areas of origin of some of the Italian
537 domestic breeds considered in the analysis (arrows) and average number of juvenile Racing Homers
538 reared in the different Italian regions in the period 2013-2018 (data provided by the Federazione
539 Colombofila Italiana). Italian Racing Homer and Runt are not reported in the map because their
540 origin can not be ascribed to a well defined geographical area, even though the selection of the Runt
541 breed probably started in central Italy (Bigi et al. 2016).

542 **Fig. 2.** Results of the Discriminant Analysis of Principal Components (DAPC) and of Bayesian
543 computations on feral pigeon samples. **(a)** DAPC evidenced a clear differentiation of F_Bolzano,
544 F_Sassari, and F_Venice from the individuals of the remaining populations which plotted together
545 with the exception of Treviso whose position was intermediate between F_Venice and F_Pavia. **(b)**
546 The ΔK and Mean Likelihood computations suggested that four clusters represented the best
547 genetic subdivision of the sampling. **(c)** Bayesian analysis assigned F_Bolzano, F_Venice, and
548 F_sassari to unique and distinctive populations. The differently colored bars in F_Treviso, F_Pavia,
549 F_Modena, F_ReggioE, F_Leghorn, and F_Pisa describe admixed genetic compositions within
550 these populations. The barplot at K = 2 and K=3 allows tracking the genetic components and the
551 main subdivisions among groups.

552 **Fig. 3.** Directional relative migration estimated by the function divMigrate of the DiveRsity R
553 package (Sundqvist et al., 2016). The circles represent the considered feral populations while
554 arrows and numbers identify the direction and the value of migrations. Only significant asymmetric
555 links with values higher than 0.5 were plotted.

556 **Fig. 4.** Discriminant Analysis of Principal Components e Bayesian computations in feral and
557 domestic breeds. The computations involved only pigeon lineages that could have contributed to the
558 genetic composition of feral populations. **(a)** DAPC showed that Sottobanca and Romagnol
559 contributed marginally to the sampled feral populations, while Italian Beauty Homer, Racing
560 Homer, Piacentino and in part Runt breeds clustered together with ferals from Lombardy, Emilia-
561 Romagna and Tuscany. **(b)** The ΔK and Mean Likelihood computations suggested that 8 clusters
562 represented the best genetic substructure of the sampling. **(c)** Bayesian analysis evidenced a sharp
563 distinction between feral and domestic samples. At K = 2, the main difference was found between
564 Homers with other domestic and feral pigeons. At K = 4, 6 and 8 the most relevant subdivisions
565 were internal to both domestic and feral groups. Moreover, light blue, pink colored and light green
566 bars found in ferals at K = 8 suggested a probable origin of some individuals from Italian Beauty

567 Homer, Racing Homer, Piacentino and Runt breeds.

568 **Fig. 5.** Bar chart showing population membership (*q*) values at K = 8 (see Figure 4 for further
569 details). Admixed colored bars are representative of an admixed genetic composition and origin.
570 Identical colors indicate a common origin.

571 **Table 1.** Geographic origin of feral samples and domestic breeds considered in the study. The
572 breeds set off in bold were excluded from the analyses after preliminary investigations (see Material
573 and Methods). The domestic samples were already analysed in Bigi et al. (2016).

Sample	Region	Sample size
Feral populations		
F_Bolzano	Trentino-Alto Adige	22
F_Treviso	Veneto	24
F_Venice	Veneto	22
F_Pavia	Lombardy	20
F_ReggioE	Emilia-Romagna	25
F_Modena	Emilia-Romagna	26
F_Pisa	Tuscany	19
F_Leghorn	Tuscany	14
F_Sassari	Sardinia	22
Domestic breeds		
Italian Owl		**29**
Italian Owl Rondone		**20**
Florentine		**20**
Piacentino		25
Romagnol		20
Runt		19
Sottobanca		26
Triganino Schietto		**26**
Triganino Gazzo		**20**
Racing Homer		29
Italian Beauty Homer		16

574

575

576 **Table 2.** Variability indexes. Abbreviations: number of alleles (*Na*), number of effective alleles
577 (*Ne*), allelic richness (*Ar*), observed (*Ho*) and expected (*He*) heterozygosity. Except for *Ar*, values
578 are averages ± SE.

Population	***Na***	***Ne***	***Ar***	***Ho***	***He***
F_Bolzano	6.9 ± 0.7	3.9 ± 0.3	6.2	0.762 ± 0.037	0.727 ± 0.027
F_Treviso	7.8 ± 0.9	4.6 ± 0.6	6.9	0.705 ± 0.040	0.735 ± 0.036
F_Venice	6.2 ± 0.5	3.4 ± 0.4	5.6	0.639 ± 0.065	0.651 ± 0.052
F_Pavia	7.9 ± 0.7	4.6 ± 0.5	7.2	0.710 ± 0.065	0.743 ± 0.038
F_ReggioE	8.5 ± 0.9	4.5 ± 0.5	7.1	0.739 ± 0.048	0.736 ± 0.036
F_Modena	8.3 ± 1.1	4.5 ± 0.6	7.0	0.714 ± 0.056	0.724 ± 0.046
F_Pisa	8.1 ± 0.8	5.0 ± 0.6	7.5	0.802 ± 0.124	0.758 ± 0.036
F_Leghorn	7.6 ± 0.7	4.5 ± 0.6	7.6	0.708 ± 0.050	0.725 ± 0.044
F_Sassari	7.3 ± 0.8	4.6 ± 0.4	6.7	0.686 ± 0.069	0.733 ± 0.050
	7.6 ± 0.8	4.4 ± 0.5	6.9 ± 0.6	0.718 ± 0.062	0.726 ± 0.050

579

Figure

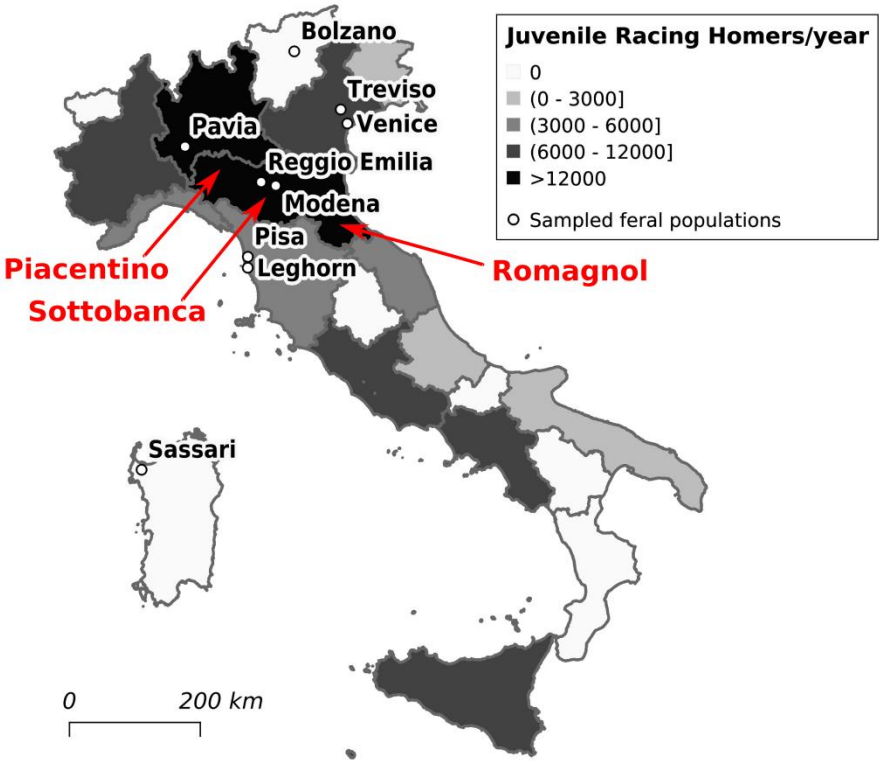
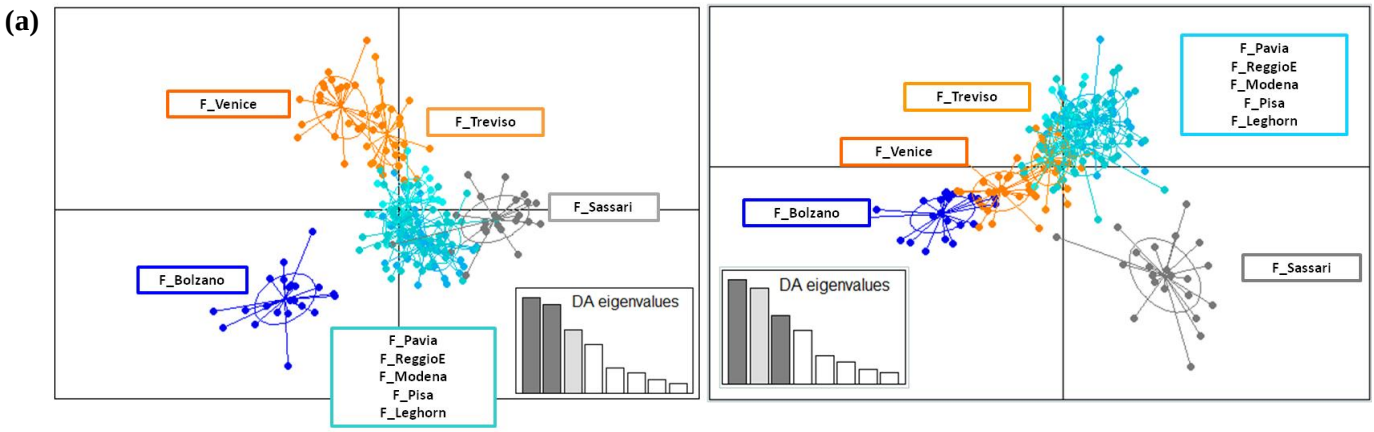
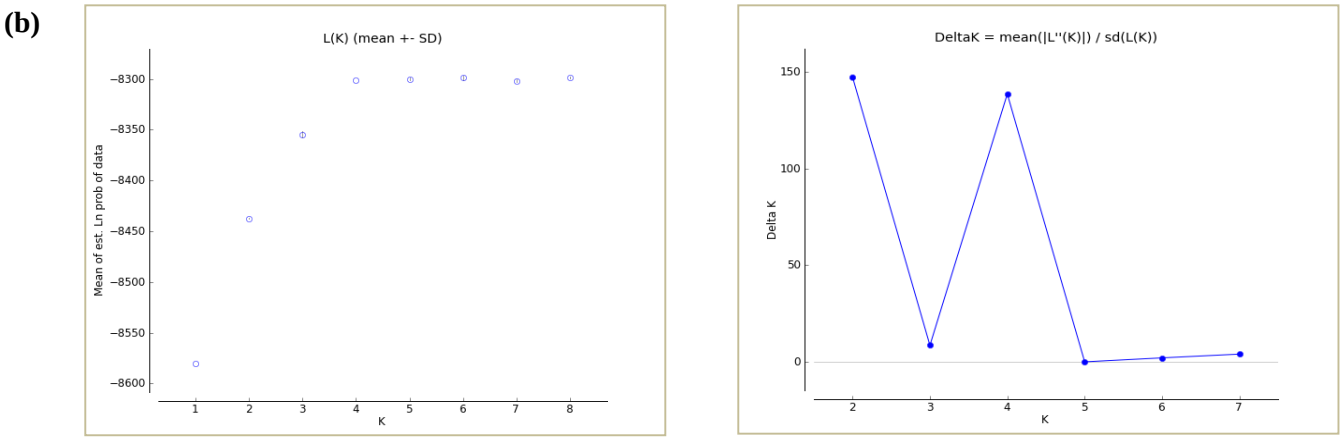


Figure 1


(a)
F_Venice
F_Treviso
F_Sassari
F_Bolzano
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
DA eigenvalues
F_Treviso
F_Venice
F_Bolzano
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Sassari
DA eigenvalues


(b)
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K

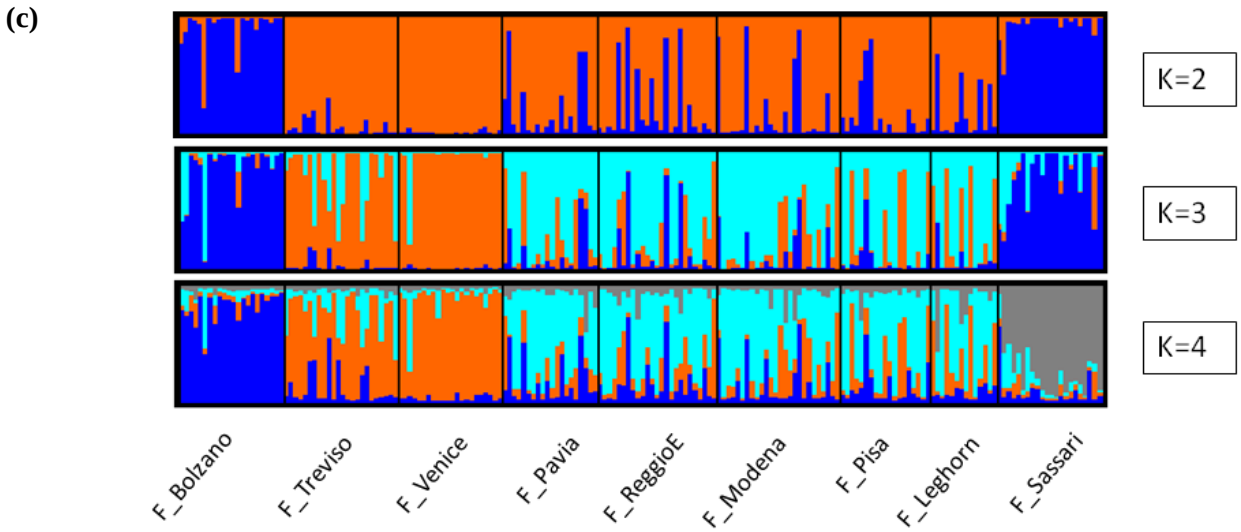

(c)
K=2
K=3
K=4
F_Bolzano
F_Treviso
F_Venice
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Sassari

Figure 2

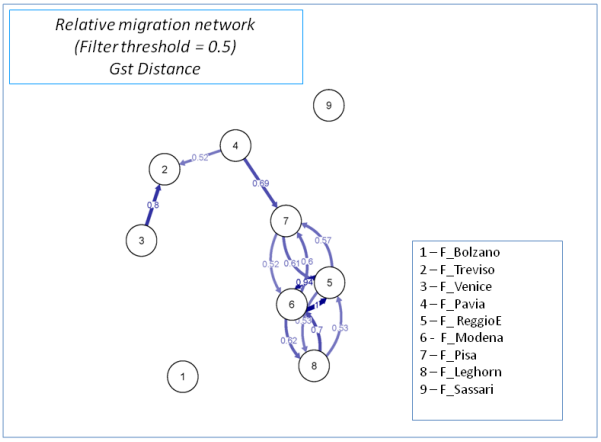

Relative migration network
(Filter threshold = 0.5)
Gst Distance
1 – F_Bolzano
2 – F_Treviso
3 – F_Venice
4 – F_Pavia
5 – F_ReggioE
6 - F_Modena
7 – F_Pisa
8 – F_Leghorn
9 – F_Sassari

Figure 3

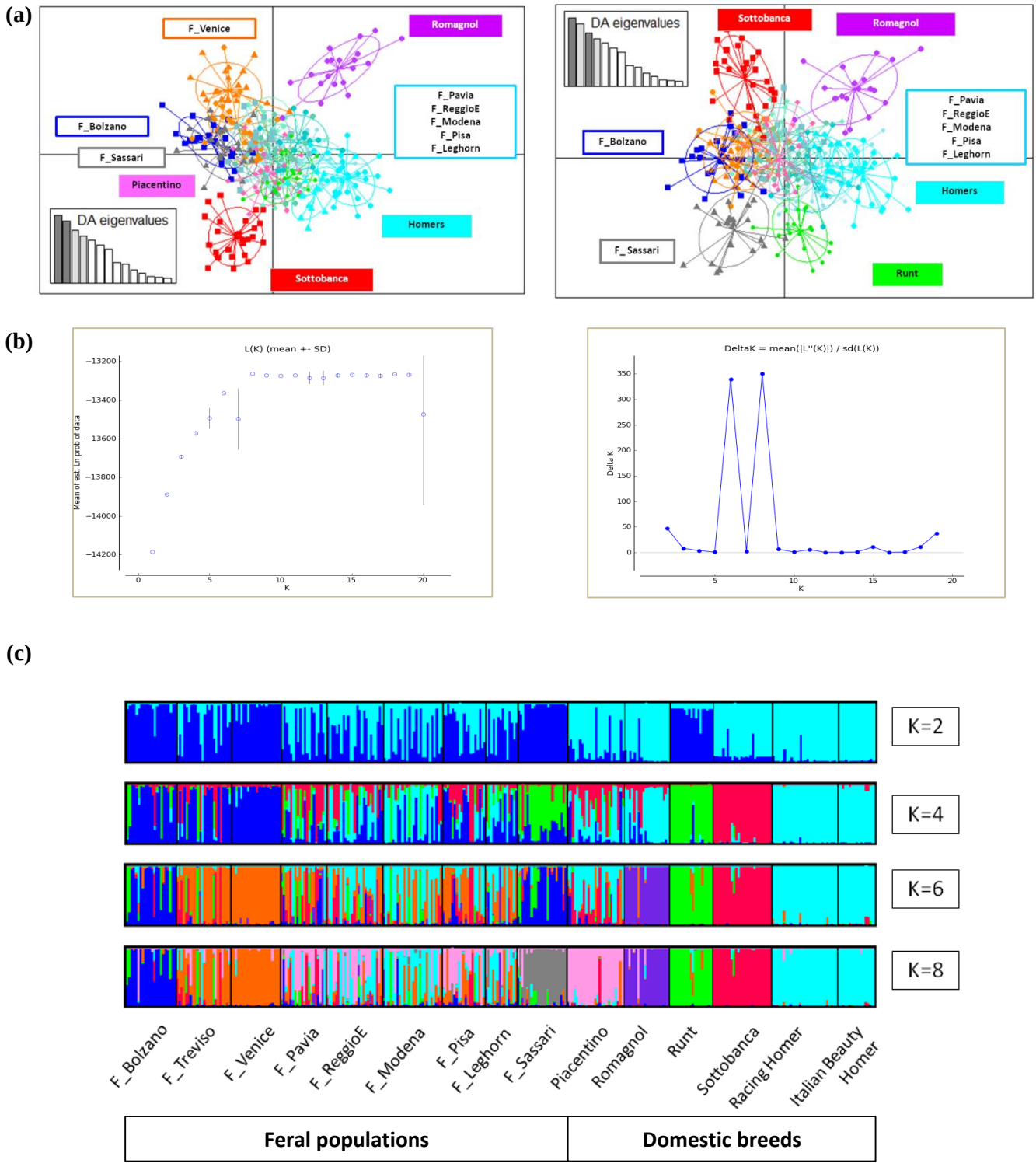

(a)
F_Venice
Romagnol
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Bolzano
F_Sassari
Piacentino
DA eigenvalues
Homers
Sottobanca
DA eigenvalues
Sottobanca
Romagnol
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Bolzano
Homers
F_ Sassari
Runt
(b)
L(K) (mean +- SD)
Mean of est. Ln prob of data
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
(c)
K=2
K=4
K=6
K=8
F_Bolzano
F_Treviso
F_Venice
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Sassari
Piacentino
Romagnol
Runt
Sottobanca
Racing Homer
Italian Beauty Homer
Feral populations
Domestic breeds

Figure 4

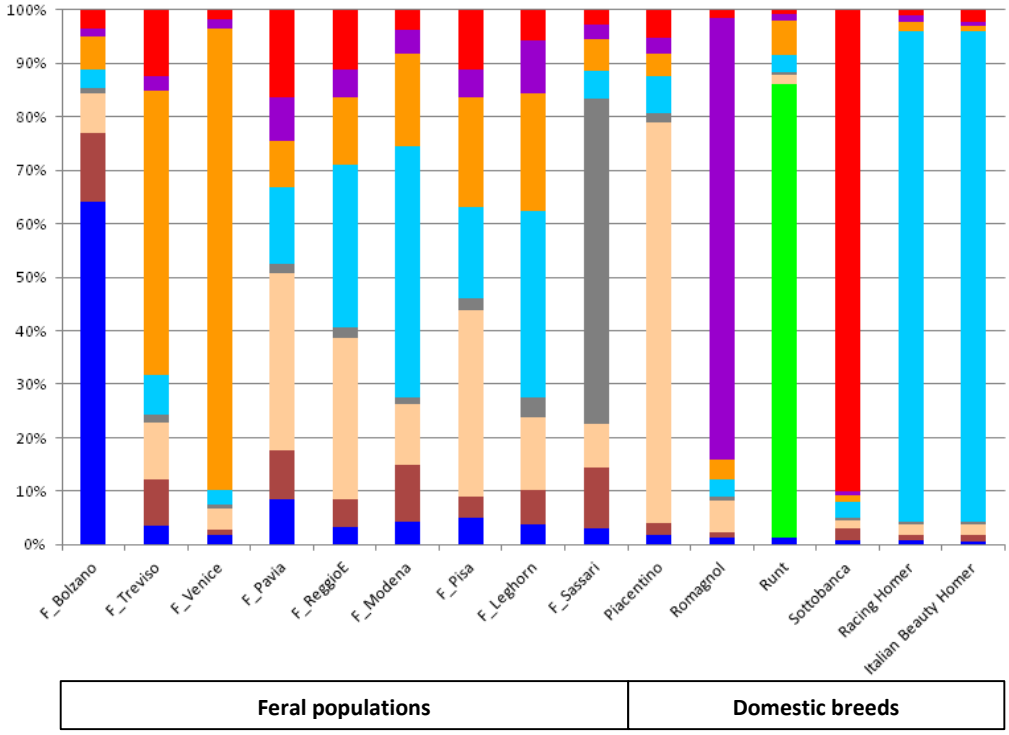

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
F_Bolzano
F_Treviso
F_Venice
F_Pavia
F_ReggioE
F_Modena
F_Pisa
F_Leghorn
F_Sassari
Piacentino
Romagnol
Runt
Sottobanca
Racing Homer
Italian Beauty Homer
Feral populations
Domestic breeds

Figure 5