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 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, almost all of which are not shared by other feral populations and by the considered domestic breeds. Our results further emphasize the complex origin of feral populations which can be properly investigated only by considering the pool of domestic pigeons bred in the considered area and their past and present distribution.

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

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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; 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 two 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). 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), and to Central (Pisa, Livorno) and Northeastern Italy (Venezia, Treviso, Bolzano), where racing pigeon breeding is rather less common (Fig. 1) and to Sardinia (Sassari) where racing pigeon breeding is not present. 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.

Material 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

104 Italian law on animal welfare.

105

106 *DNA extraction and microsatellite genotyping*

107 DNA was extracted using the ZR Genomic DNA II kit (Zymo Research) and amplified at 12 loci
108 microsatellite (*Cliμd01*, *Cliμt13*, *Cliμd17*, *CliμT17*, *Cliμd16*, *Cliμd19* - (Traxler et al. 2000);
109 *CliuD11*, *CliuT47*, *CliuT24*, *UUCli10*, *UUCli13*, *UUCli14n*, *UUCli12*, *UUCli08* - (Stringham et al.
110 2012). Two independent replicates were performed in 8 µl of volume containing 0.5 U of Hot Start
111 Taq polymerase (Qiagen), 0.18 µM of each primer and 0.04 mg of Bovine Serum Albumin Fraction
112 V (Roche), with the following thermal protocol: (94 °C x 5'), 10 cycles at (94 °C x 40'') (55 °C-
113 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
114 extension at 72 °C for 10'.

115

116 *Data analysis*

117 The number of alleles (*Na*), the number of effective (*Ne*) and private alleles, and the expected (*He*)
118 and observed (*Ho*) heterozygosity were obtained using GenAlEx 6.503 (Peakall and Smouse 2006,
119 2012; Smouse et al. 2015). Allelic richness (*Ar*) was computed in Fstat to minimize the effect of a
120 different sample size (Goudet 2001). Departure from Hardy-Weinberg equilibrium was estimated
121 with the exact test in Genepop on the web (Raymond and Rousset 1995)
122 (http://genepop.curtin.edu.au/) using 100 batches and 1000 iterations per batch.

123 Pairwise *Fst* computation and AMOVA test (Excoffier et al. 1992) were performed in Genetix
124 4.05.02 (Belkhir et al. 2002) and GenAlEx 6.503, respectively, to evaluate the significance of
125 genetic differentiation between groups. The matrix of P-values corresponding to each pairwise *Fst*
126 computation was adjusted using the Bonferroni correction (Sokal and Rohlf 1995) for multiple
127 comparisons (nominal level for multiple tests = 0.05).

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

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

137 Clustering was computed using a Bayesian model in STRUCTURE 2.3.4 (Pritchard et al. 2000;
138 Falush et al. 2003; Hubisz et al. 2009) with no admixture and independent allele frequencies models.
139 The USEPOPINFO selection flag column was considered = 0. A total of five independent runs for a
140 number of subpopulations (K) from 1 to 10, was run with a burn-in period of 30,000 followed by
141 300,000 MCMC repetitions. STRUCTURE HARVESTER on the web (Dent and von Holdt 2012)
142 was used to process the data and identify the best number of clusters according to both ΔK and
143 Mean Likelihood (Janes et al. 2017). CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT
144 (Rosenberg 2004) were used respectively to merge the five independent results for each K and to
145 display the final data. A Discriminant Analysis of Principal Components (DAPC) that is not
146 affected by Hardy-Weinberg disequilibrium was also performed to verify the substructure of feral
147 populations using the Adegenet package (Jombart 2008, Jombart and Ahmed 2011) for R software
148 (R Core Team 2019). A total of 80 PCs and four discriminant functions were retained to draw the
149 plot.

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

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

156

157 *Relationship between feral pigeons and domestic breeds*

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

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

After the preliminary results, five breeds (Florentine, Italian Owl, Italian Owl Rondone, Triganino Schietto and Triganino Gazzo) that did not show any genetic relationship with feral pigeon populations (ESM 1) were excluded from the analysis. The Florentine disappeared in Italy at the beginning of the last century (Giachetti 1894) and it has been reintroduced only recently, while the outdoor breeding of Italian Owls and of Triganino Schietto and Gazzo was interrupted from the first half of 20th century (Vaccari and Zambon 2014). Therefore only a scarce contribution of these five breeds to the present-day Italian feral populations can be expected.

Results

Genetic variability of feral populations

The descriptive statistics for the genotypes are listed in Table 3. The allelic number ranged from 6.2 ± 0.5 SE (F_VE) to 8.5 ± 0.9 (F_RE) with an average value of 7.6 ± 0.8. The average number of effective alleles decreased to 4.4 ±0.5. Mean values of expected (*He*) and observed (*Ho*) heterozygosity were 0.718 ± 0.062 and 0.726 ± 0.050, respectively. Allelic richness was computed on a minimum value of 14 samples and ranged from 5.6 (F_VE) to 7.6 (F_LE) with an average value of 6.9. A total of eight different private alleles were detected in Sardinian ferals even if with a low frequency (< 10%).

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

Phylogenetic trees built using *Da*, *Fst*, and $(\delta\mu)^2$ distances did not show any significant structure (Fig. 2). Ferals from F_LE, F_PI, F_PV, F_MO and F_RE clustered together in all the trees, but the associated bootstrap values were so low as to make the topology not significant. Acceptable bootstrap values were obtained only for the group constituted by F_VE and F_TV. Additional results from other distance computations [Cavalli-Sforza chord distance (*Dc*), proportion of shared

196 alleles (*Dps*) and Shriver distance (*Dsw*)] were not shown as they did not produce a different
197 structure.

198 Mantel test evidenced a positive correlation between the genetic and geographic distances (r = 0.50;
199 P = 0.03, ESM 3a); this correlation remained almost unchanged (r = 0.46; P = 0.01) when the
200 Sardinian sample was removed from the analysis (ESM 3b).

201 No defined genetic structure was identified by both multiv47ariate and Bayesian analyses (Fig. 3a-
202 c). DAPC analysis showed an admixed pattern in Central Italy, whereas samples from Sassari
203 (F_SS), Bolzano (F_BZ), and Venice (F_VE) clustered separately from the others. The population
204 from Treviso (F_TV) plotted in an intermediate position between the remaining populations and
205 F_VE.

206 Both Mean Likelihood and ΔK obtained from STRUCTURE HARVESTER identified the best
207 grouping at K = 4. At K = 2 Bolzano and Sassari populations split from the remaining populations.
208 K = 3 permitted an additional distinction of ferals from Treviso and Venice. At the best value K = 4,
209 ferals from Bolzano (F_BZ), Venice (F_VE), and Sassari (F_SS) were associated to different
210 clusters with high q individual membership values, while samples from F_PV, F_RE, F_MO, F_PI,
211 and F_LE remained not differentiated from each other and showed retraces of admixture with F_BZ,
212 F_VE, and F_SS. The genetic composition of F_TV was mainly associated with F_VE but showed
213 traces of admixtures with the former five populations (Fig. 3c).

214 No genetic components shared between Bolzano (F_BZ), Sassari (F_SS) and the other feral
215 populations was estimated by the divMigrate function (Fig. 6). Gene flow towards Treviso (F_TV)
216 from Venice (F_VE) and Pavia (F_PV) resulted well supported (asymmetric values = 0.8 and =0.52,
217 respectively), as well as that from Pavia (F_PV) to Reggio Emilia (F_RE). Populations from
218 Modena (F_MO), Reggio Emilia (F_RE), Leghorn (F_LE) and Pisa (F_PI) resulted to be quite
219 interconnected. The highest values of gene flow (0.94 and 1.0) were reported between Modena
220 (F_MO) and Reggio Emilia (F_RE).

221

222 *Relationship between feral pigeons and domestic breeds*

223 Phylogenetic trees built using *Fst*, D*a* and $(\delta\mu)^2$ distances did no show any significant structure
224 (ESM 4) and feral groups and the domestic lineages considered in the analysis were differently
225 associated depending on the distance computation considered. As in the previous section, Cavalli-
226 Sforza chord distance (*Dc*), the proportion of shared alleles (*Dps*) and Shriver distance (*Dsw*) were

227 not informative and were not shown.

228 The DAPC of the nine feral populations and the six Italian breeds did not reveal a sharp separation
229 between the two groups (Fig. 4a). With the exception of domestic SB and RM, all the other breeds
230 overlap with feral populations in the plot. F_VE and F_SS did not show any tracks of an origin
231 from the considered domestic lineages because in the plots they did not overlap with any of them.
232 The Bayesian analysis partially confirmed the results of DAPC and the *q* individual membership
233 values identified a relationship between domestic and local feral pigeons (Fig. 4c and 5). The ΔK
234 computation in STRUCTURE HARVESTER identified 8 main informative clusters (Fig. 4b). The
235 main splits at K = 2, 4 and 6 were also plotted in Figure 4c to describe the main similarities among
236 groups. Interestingly, the first split (K = 2) does not occur between feral and domestic pigeons but
237 between Homers (RH and IH) and the other groups. At K = 4, Runt (RN) and Sottobanca (SB)
238 separated from other domestic lineages while ferals from Sassari (F_SS) separated from peninsular
239 populations. At K = 6, ferals from Sassari, Bolzano (F_BZ), Venice (F_VE) and Treviso (F_TV)
240 separated from the other populations while Romagnolo (RM) splits from Homers. The evaluation of
241 the estimated membership coefficient for each population *(q)* at K = 8 identified the predominance
242 of the domestic component characterizing the Homers (light blue bar in Fig. 5) in feral populations
243 from Lombardy, Emilia-Romagna and Tuscany, particularly in F_RE, F_MO, and F_LE. Rather
244 high percentages of domestic Piacentino (PC) and lower values of domestic Sottobanca (SB) and
245 Runt (RN) were found in all populations, including F_BZ and F_SS, although the latter populations
246 were characterized by over 60% of a unique private component. Ferals from Venice (F_VE)
247 showed a high percentage of a unique genetic component, that was also detected in the other feral
248 populations but that was almost irrelevant in domestic lineages. As evidenced in Figure 5, the
249 population of Venice did not show relevant traces of admixture with the considered domestic
250 populations.

251

252 **Discussion**

253 This paper represents one of the few studies dealing with the genetic structure of feral pigeon
254 populations. Up to now, only one study was specifically focused on clarifying the pattern of genetic
255 differentiation of these birds between urban areas (Jacob et al. 2015). Furthermore, while it is largely
256 accepted that feral pigeons originated from domestic breeds (see for instance, Johnston and Janiga 1995),
257 this study is the first one that systematically investigates the affinities between feral pigeon populations
258 and the domestic breeds commonly reared in the same area. To our knowledge only Biala et al. (2015)

tried to quantify the gene flow between several domestic breeds and feral pigeons. However, while Biala et al. (2015) used only one feral group, composed of birds sampled in different towns, we sampled a significant number of true feral populations in order to investigate the contribution of domestic breeds to their gene pool and how this contribution varies among populations located in different areas.

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

As observed by Jacob et al. (2015), also in our samples the genetic distance between feral populations was correlated with their geographic distance, both considering all samples or only the peninsular ones. This result further confirms that the exchange rate of individuals among cities is relatively rare (Johnston and Janiga 1995; Hetmanski 2007). It should be noted, however, that the observed pattern of geographic differentiation is rather complex and only partially explained by the geographic distance between populations. Indeed, while the present data do not support a well defined genetic structure, it is interesting to observe that Sardinia and North-eastern populations (F_BZ, F_VE and F_TV to a lesser extent) tended to cluster separately and showed a null or rather low gene flow with the remaining populations. On the contrary, populations belonging to Tuscany, Emilia-Romagna and Lombardy (F_PI, F_LE, F_MO, F_RE and F_PV) showed a high level of admixtures almost independent of their geographic distance. The genetic distinction of populations like F_BZ, F_SS and F_VE, can be attributed to their relative geographic isolation. Actually, Bolzano is located in an Alpine valley surrounded by habitats mostly unsuitable for pigeons, while Sardinia is an island and it is known that pigeons do not like to fly over large water bodies (Wagner et al. 1972). The effect of the surrounding water should probably be taken into account also for Venice: for instance, as reported by (Soldatini et al. 2006), the number of Venetian pigeons involved in foraging flights outside the city is very low considering the size of the population, which possibly confirm the low propensity of these pigeons to fly over the lagoon and thus also to disperse inland. The relatively high gene flow from F_VE to F_TV estimated by divMigrate can be interpreted both considering the relatively short distance between the two cities, but also the likely common origin of the two populations.

The level of admixture of the remaining populations (F_PI, F_LE, F_MO, F_RE and F_PV) and the high level of estimated gene flow among them as well as between F_PV and F_TV are quite difficult to explain considering the above mentioned low rate of dispersal among cities (Johnston and Janiga 1995; Hetmanski 2007; Jacob et al. 2015). and the inability of feral pigeons to undergo long flights, as experimentally demonstrated by Chelazzi and Pineschi (1974) and by Edrich and Keeton (1977). However, it is not to be

excluded that any dispersal event could occur between very close cities (e.g. Modena and Reggio Emilia). Actually, the migration events described by DivMigrate are estimated from the allele frequencies retrieved inside the populations and could be interpreted as common genetic components rather than a real gene flow. 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_SS, F_BZ, F_VE and F_TV, 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_PI, F_PV and F_RE.

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_VE and F_TV. 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 facilitates the urban drift of domestic birds. 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 a use genomic approach in order 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, that are mainly not shared by other feral populations. 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_SS. 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 relationships between feral pigeon populations and domestic breeds, shedding 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.

References

Baldaccini NE, Giunchi D (2006) Le popolazioni urbane di colombo: considerazioni sulla loro genesi e sulle metodologie di gestione. Biologia Ambientale 20:125–141

Baldaccini NE, Giunchi D, Mongini E, Ragionieri L (2000) Foraging flights of wild rock doves (Columba l. livia): a spatio-temporal analysis. Italian Journal of Zoology 67:371–377

Baldaccini NE, Lombardo D, Mongini E, Giunchi D (2015) I colombi della città di Bolzano: interventi di gestione e caratteristiche della popolazione. Biologia Ambientale 29:9-20

Ballarini G, Baldaccini NE, Pezza F (1989) Colombi in città. Aspetti biologici, sanitari e giuridici. Metodologie di controllo. Istituto Nazionale di Biologia della Selvaggina, Bologna

Belkhir K, Borsa P, Chikhi L, et al (2002) GENETIX 4.05, Windows TM Software for Population Genetics. Laboratory Genome, Populations, Interactions. Universite de Montpellier II, Montpellier, France.(in French)

Biala A, Dybus A, Pawlina E, Proskura WS (2015) Genetic diversity in eight pure breeds and urban form of domestic pigeon (Columba livia var. domestica) based on seven microsatellite loci. Journal of Animal and Plant Sciences 25:1741–1745

Bigi D, Mucci N, Mengoni C, et al (2016) Genetic investigation of Italian domestic pigeons increases knowledge about the long-bred history of *Columba livia* (Aves: Columbidae). Italian Journal of Zoology 83:173–182

Bowcock AM, Ruiz-Linares A, Tomfohrde J, et al (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455–457

Cavalli-Sforza LL, Edwards AW (1967) Phylogenetic analysis. Models and estimation procedures. American journal of human genetics 19:233–257

Chelazzi G, Pineschi F (1974) Recoveries after displacement in pigeon belonging to an urban population. Monitore Zoologico Italiano (NS) 8:151–157

Dent E.A., von Holdt B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation of

Genetic Resources 4:359-361

Dieringer D, Schlotterer C (2003) Microsatellite analyzer (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3:167–169

Domyan ET, Shapiro MD (2017) Pigeonetics takes flight: Evolution, development, and genetics of intraspecific variation. Developmental Biology 427:241–250

Edelaar P, Roques S, Hobson EA, Gonçalves da Silva A, Avery ML, Russello MA, Senar JC, Wright TF, Carrete M, Tella JL (2015) Shared genetic diversity across the global invasive range of the monk parakeet suggests a common restricted geographic origin and the possibility of convergent selection. Molecular Ecology 24:2164–2176

Edrich W, Keeton WT (1977) A comparison of homing behavior in feral and homing pigeons. Zeitschrift für Tierpsychologie 44:389–401

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. Genetics 131:479–491

Falush D, Stephens M, Pritchard J (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Ghigi A (1950) Piccioni Domestici e Colombicoltura. REDA, Roma

Giachetti GC (1894) Monografia dei piccioni domestici. Tip. degli Operai, Milano

Giunchi D, Albores-Barajas Y, Baldaccini NE, Vanni L, Soldatini C (2012) Feral Pigeons: Problems, Dynamics and Control Methods. In: Soloneski S, Larramendy ML (eds) Integrated Pest Management and Pest Control – Current and Future Tactics. InTech, Rijeka, pp 215–240

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463–471

Gompertz T (1957) Some Observations on the feral pigeon in London (With Addendum: Two cases of polyneuritis in Feral Pigeons. *Derek Goodwin*). Bird Study 4:2–13

Goodwin D (1960) Comparative ecology of feral pigeons in inner London. British Birds 53:201–212

Goudet J (2001) FSTAT, a program to estimate and test gene diversity and fixation indices (version 2.9. 3). http://www2. unil. ch/popgen/softwares/fstat. htm

Haag-Wackernagel D (1998) Die Taube: Vom heiligen Vogel der Liebesgöttin zur Strassentaube. Schwabe, Basel

Hetmanski T (2007) Dispersion asymmetry within a feral pigeon *Columba livia* population. Acta Ornithologica 42:23–31

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322–1332

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23:254–267

Jacob G, Prévot-Julliard A-C, Baudry E (2015) The geographic scale of genetic differentiation in the feral pigeon (*Columba livia*): implications for management. Biological Invasions 17:23–29

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bionformatics 23:1801–1806

Janes K, Jasmine,Miller J, Dupuis J, René M, Gorrell J, Cullingham C, Andrew R (2017) The K=2 conundrum. Molecular Ecology 26:3594-3602

Johnston RF (1992) Evolution in the rock dove: skeletal morphology. The Auk 109:530–542

Johnston RF (1994) Geographic variation of size in feral pigeons. The Auk 111:398–404

Johnston RF, Janiga M (1995) The Feral Pigeons. Oxford University Press, London

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405

Jombart T, Ahmed I (2011) Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070–3071

Latter BDH (1972) Selection in finite populations with multiple alleles. III. Genetic divergence with centripetal selection and mutation. Genetics 70:475–490

Le Gros A, Samadi S, Zuccon D, Cornette R, Braun MP, Senar JC, Clergeau P (2016) Rapid morphological changes, admixture and invasive success in populations of Ring-necked parakeets (*Psittacula krameri*) established in Europe. Biological Invasions 18:1581–1598

Lever C (1987) Naturalized birds of the world. Wiley, New York

McNeillie A (1976) Guide to the pigeons of the world. Elsevier-Phaidon, Oxford

Nei M (1973) Analysis of gene diversity in subdivided populations. PNAS 70:3321–3323

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research 6:288–295

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Pritchard JK, Stephens M, Peter D (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

Ragionieri L, Mongini E, Baldaccini NE (1991) Problemi di conservazione in una popolazione di colombo selvatico (*Columba livia livia* Gmelin) della Sardegna. Supplemento alle Ricerche di Biologia della Selvaggina 18:35–46

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249

Ritchie J (1920) The Influence of Man on Animal Life in Scotland: a Study of Fauna Evolution. Cambridge University Press, Cambridge

Rosenberg N (2004) DISTRUCT: a program for graphical display for population structure. Molecular Ecology Notes 4:137–138

Shao Y, Tian H-Y, Zhang J-J, et al (2019) Genomic and phenotypic analyses reveal mechanisms underlying homing ability in pigeon. Molecular Biology and Evolution

Shapiro MD, Kronenberg Z, Li C, Domyan ET, Pan H, Campbell M, Tan H, Huff CD, Hu H, Vickrey AI, Nielsen SC (2013) Genomic diversity and evolution of the head crest in the rock pigeon. Science 339:1063–1067

Shriver MD, Jin L, Boerwinkle E, Deka R, Ferrell RE, Chakraborty R (1995) A novel measure of genetic distance for highly polymorphic tandem repeat loci. Molecular Biology and Evolution 12:914–920

Simms E (1979) The public life of the street pigeon. Hutchinson, London

Smouse PE, Whitehead MR, Peakall R (2015) An informational diversity framework, illustrated with sexually deceptive orchids in early stages of speciation. Molecular Ecology Resources 15:1375–1384

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. W.H. Freeman and Company, New York

Sol D (2008) Artificial selection, naturalization, and fitness: Darwin's pigeons revisited. Biological Journal of the Linnean Society 93:657–665

Soldatini C, Mainardi D, Baldaccini EN, Giunchi D (2006) A temporal analysis of the foraging flights of feral pigeons (*Columba livia* f. *domestica*) from three Italian cities. Italian Journal of Zoology 73:83–92

Sossinka R (1982) Domestication in birds. Avian Biology 6:373–403

Stringham SA, Mulroy EE, Xing J, et al (2012) Divergence, convergence, and the ancestry of feral populations in the domestic rock pigeon. Current Biology 22:302–308

Sundqvist, L, Keenan K, Zackrisson M, Prodöhl P, Kleinhans D (2016) Directional genetic differentiationand relative migration. Ecology and Evolution 6:3461-3475.

Tang Q, Low GW, Lim JY, Gwee CY, Rheindt FE (2018) Human activities and landscape features interact to closely define the distribution and dispersal of an urban commensal. Evolutionary Applications 11.9:1598–1608

Toschi A (1939) Ricerche ed osservazioni sul Colombo selvatico (*Columba livia* L.). Ricerche di Zoologia Applicata alla Caccia 13:1–124

Traxler B, Brem G, Müller M, Achmann R (2000) Polymorphic DNA microsatellites in the domestic pigeon, *Columba livia* var. *domestica*. Molecular Ecology 9:366–368

Vaccari A, Zambon F (2014) Storia del Triganino Modenese. Notiziario della Federazione Italiana Allevatori Colombi 14:5–11

Van der Linden CG (1950) Le Pigeon Voyager. Payot, Paris

Wagner G (1972) Topography and pigeon orientation. In: Galler SR, Schmidt-Koenig K, Jacobs GJ, Belleville RE (eds) Animal Orientation and Navigation SP-262. NASA, Washington, DC, pp 259–273

372 **Figure caption**

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

379 **Fig. 2.** Phylogenetic trees drawn using three different distance-based models: *Da*, *Fst,* and $(\delta\mu)^2$
380 distance. No significant topology was supported in any of the three phylogenetic trees. The unique
381 significant clustering was found between F_TV and F_VE.

382 **Fig. 3.** Results of the Discriminant Analysis of Principal Components (DAPC) and of Bayesian
383 computations on feral pigeon samples. **(a)** DAPC evidenced a clear differentiation of F_BZ, F_SS,
384 and F_VE, individuals from Central Italy plotted together, while the position of F_TV was
385 intermediate between F_VE and F_PV. **(b)** The ΔK and mean likelihood computations suggested
386 that 4 clusters represented the best genetic subdivision of the sampling. **(c)** Bayesian analysis
387 assigned F_BZ, F_VE, and F_SS to unique and distinctive populations. At K = 4, the different
388 colored bars in F_TV, F_PV, F_MO, F_RE, F_LE, and F_PI describe admixed genetic
389 compositions within these populations, thus evidencing a relevant genetic flow among them. The
390 barplot at K = 2 and K=3 allows tracking the genetic components and the main subdivisions among
391 groups.

392 **Fig. 4.** Discriminant Analysis of Principal Components e Bayesian computations in feral and
393 domestic breeds. The computations involved only pigeon lineages that could have contributed to the
394 genetic composition of feral populations. **(a)** DAPC showed that SB, and RM contributed
395 marginally to the sampled feral populations, while IH, RH, PC and in part RN clustered together
396 with ferals from Lombardy, Emilia-Romagna and Tuscany. **(b)** The ΔK and mean likelihood
397 computations suggested that 8 clusters represented the best genetic substructure of the sampling. **(c)**
398 Bayesian analysis evidenced a sharp distinction between feral and domestic samples. At K = 2, the
399 main difference was found between Homers with other domestic and feral pigeons. At K = 4, 6 and
400 8 the greater subdivisions were internal to both domestic and feral groups. Moreover, light blue,
401 pink colored and light green bars found in ferals at K = 8 suggested a probable origin of some
402 individuals from IH, RH, PC and RN breeds.

403 **Fig. 5.** Bar chart showing population membership (Q) values at K=8. Admixed colored bars are

404 representative of an admixed genetic composition and origin. Identical colors indicate a common
405 origin.

406 **Fig. 6.** Directional relative migration estimated by divMigrate in DivRsity. The circles represent the
407 feral populations from this study while arrows and numbers identify the direction and the value of
408 migrations. Only significant asymmetric links with values higher than 0.5 were plotted.

409

Table 1 Geographic origin of feral samples.

City	Acronim	Region	N. samples
Bolzano	F_BZ	Trentino-Alto Adige	22
Treviso	F_TV	Veneto	24
Venice	F_VE	Veneto	22
Pavia	F_PV	Lombardy	20
Reggio Emilia	F_RE	Emilia-Romagna	25
Modena	F_MO	Emilia-Romagna	26
Pisa	F_PI	Tuscany	19
Leghorn	F_LE	Tuscany	14
Sassari	F_SS	Sardinia	22

Table 2 Domestic breeds considered in the study. The breeds set off in bold (IO1, IO2, FL, TM1 and TM2) were excluded from the analyses after preliminary investigations (see Material and Methods)

Domestic Breed	Acronim	No. samples
Italian Owl	**IO1**	**29**
Italian Owl Rondone	**IO2**	**20**
Florentine	**FL**	**20**
Piacentino	PC	25
Romagnol	RM	20
Runt	RN	19
Sottobanca	SB	26
Triganino Schietto	**TM1**	**26**
Triganino Gazzo	**TM2**	**20**
Racing Homer	RH	29
Italian Beauty Homer	IH	16

Table 3 Variability indexes. Abbreviations: number of alleles (*Na*), number of effective alleles (*Ne*), allelic richness (*Ar*), observed (*Ho*) and expected (*He*) heterozygosity. Except for *Ar*, values are averages ± SE

Population	***Na***	***Ne***	***Ar***	***Ho***	***He***
F_BZ	6.9 ± 0.7	3.9 ± 0.3	6.2	0.762 ± 0.037	0.727 ± 0.027
F_TV	7.8 ± 0.9	4.6 ± 0.6	6.9	0.705 ± 0.040	0.735 ± 0.036
F_VE	6.2 ± 0.5	3.4 ± 0.4	5.6	0.639 ± 0.065	0.651 ± 0.052
F_PV	7.9 ± 0.7	4.6 ± 0.5	7.2	0.710 ± 0.065	0.743 ± 0.038
F_RE	8.5 ± 0.9	4.5 ± 0.5	7.1	0.739 ± 0.048	0.736 ± 0.036
F_MO	8.3 ± 1.1	4.5 ± 0.6	7.0	0.714 ± 0.056	0.724 ± 0.046
F_PI	8.1 ± 0.8	5.0 ± 0.6	7.5	0.802 ± 0.124	0.758 ± 0.036
F_LE	7.6 ± 0.7	4.5 ± 0.6	7.6	0.708 ± 0.050	0.725 ± 0.044
F_SS	7.3 ± 0.8	4.6 ± 0.4	6.7	0.686 ± 0.069	0.733 ± 0.050
Global Mean ± SE	7.6 ± 0.8	4.4 ± 0.5	6.9 ± 0.6	0.718 ± 0.062	0.726 ± 0.050

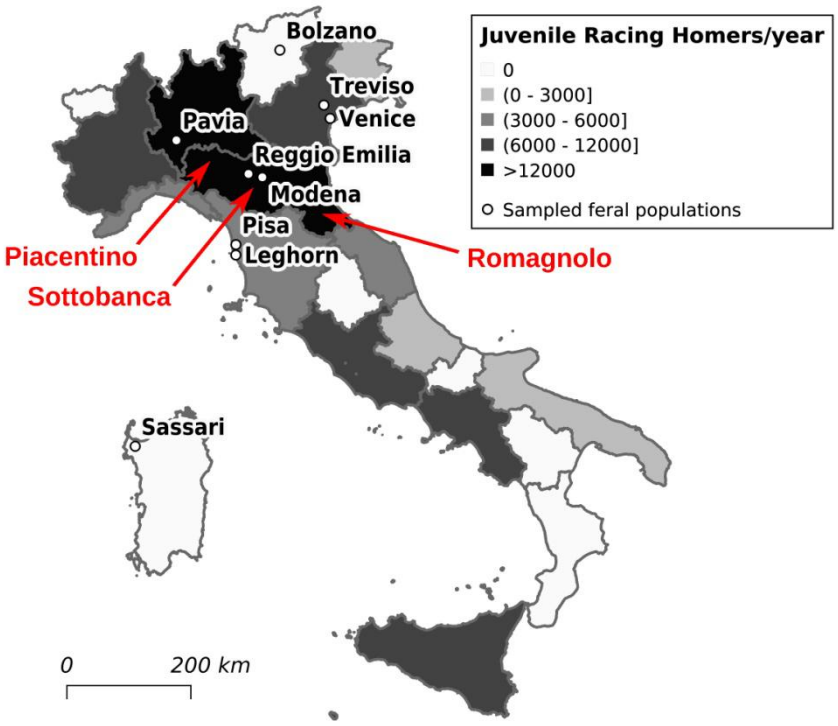
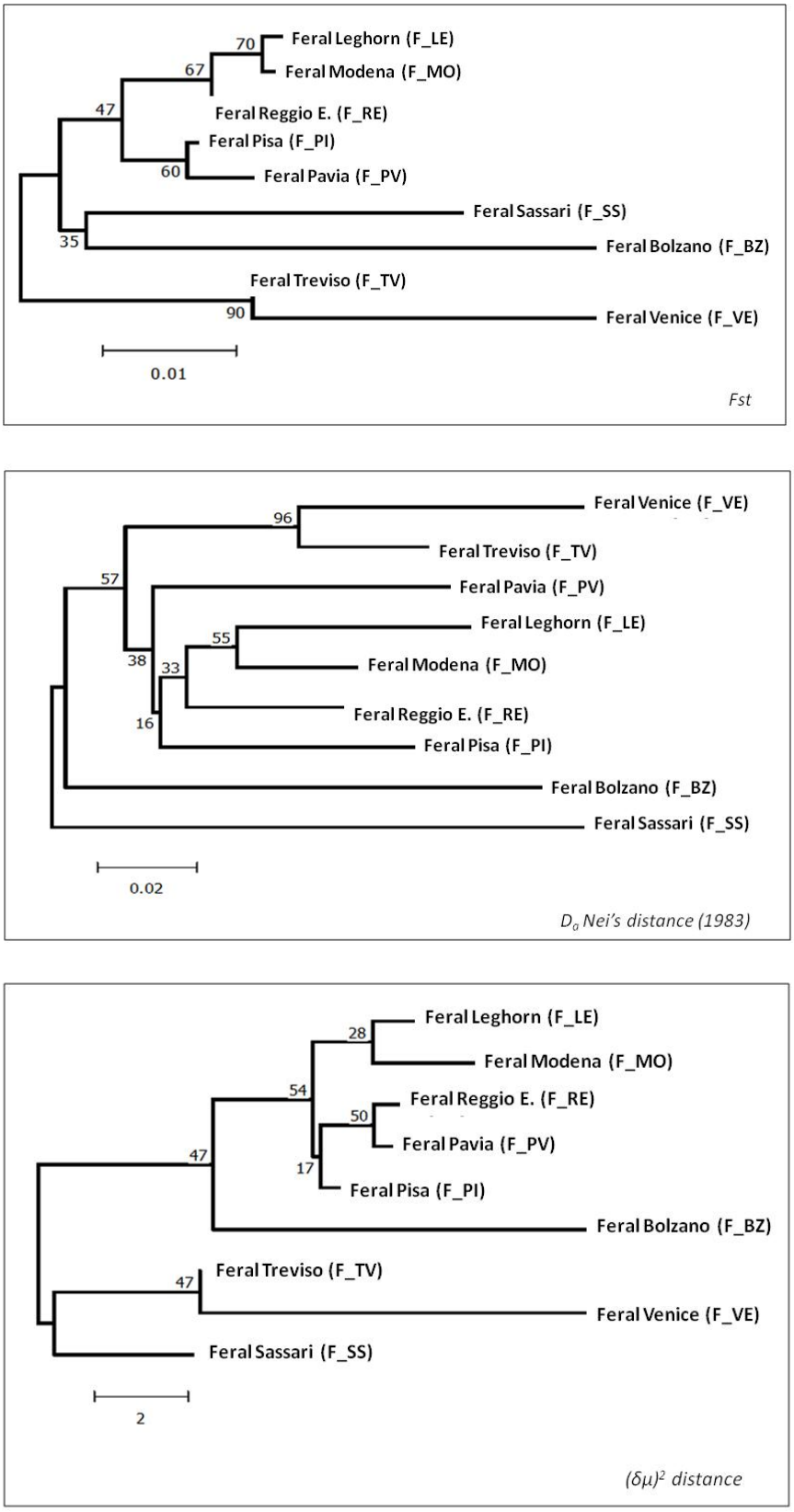

Juvenile Racing Homers/year
0
(0 - 3000]
(3000 - 6000]
(6000 - 12000]
>12000
Sampled feral populations
Bolzano
Treviso
Venice
Pavia
Reggio Emilia
Modena
Pisa
Leghorn
Piacentino
Sottobanca
Romagnolo
Sassari
0
200 km

Figure 1

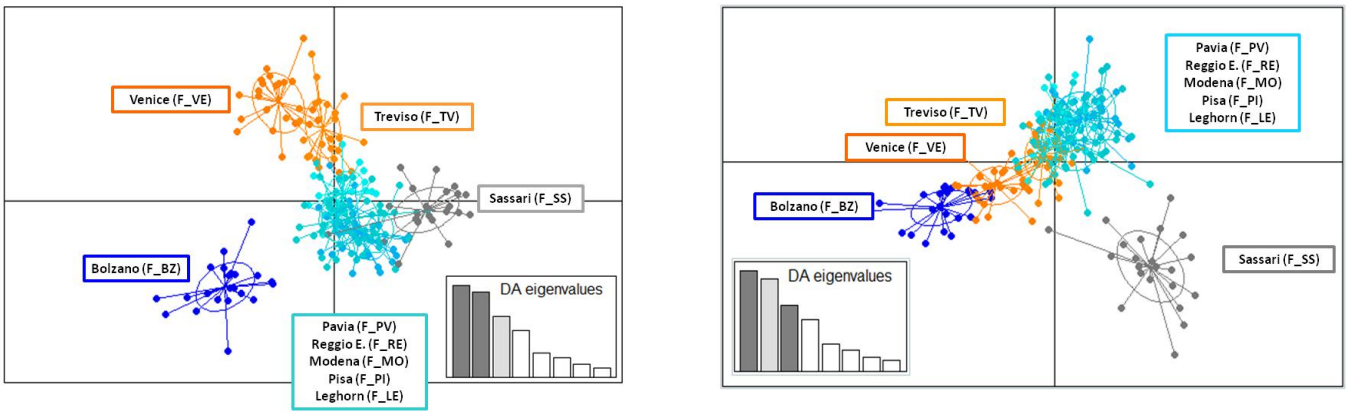
428



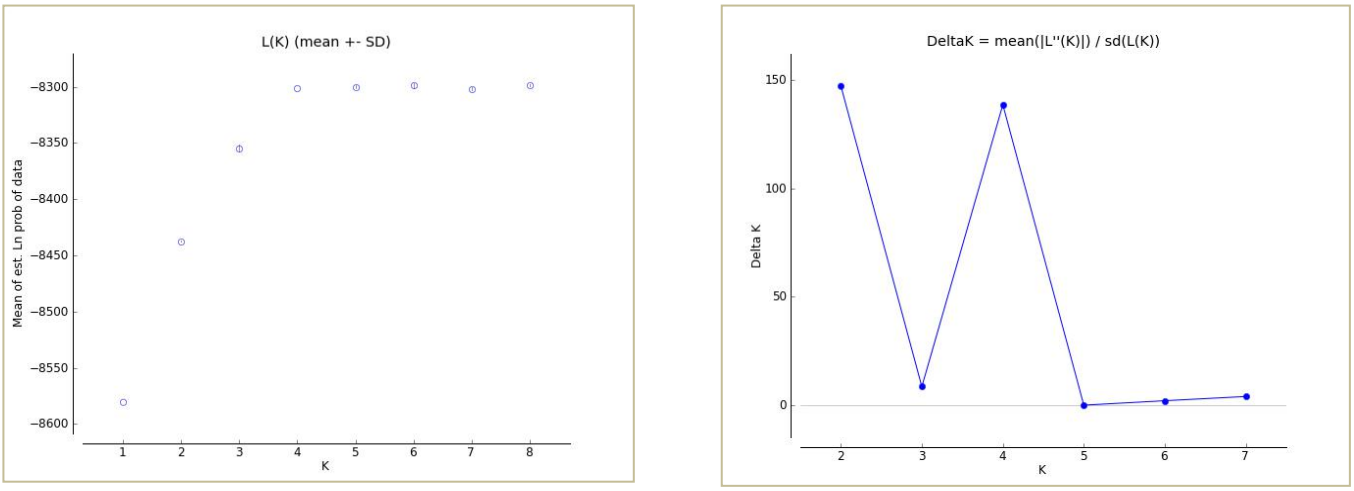
429 Figure 2

430

(a)


Venice (F_VE)
Treviso (F_TV)
Sassari (F_SS)
Bolzano (F_BZ)
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LE)
DA eigenvalues
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LE)
Treviso (F_TV)
Venice (F_VE)
Bolzano (F_BZ)
Sassari (F_SS)
DA eigenvalues

(b)


L(K) (mean +- SD)
Mean of est. Ln prob of data
−8300
−8350
−8400
−8450
−8500
−8550
−8600
1
2
3
4
5
6
7
8
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
150
100
50
0
2
3
4
5
6
7
K

(c)

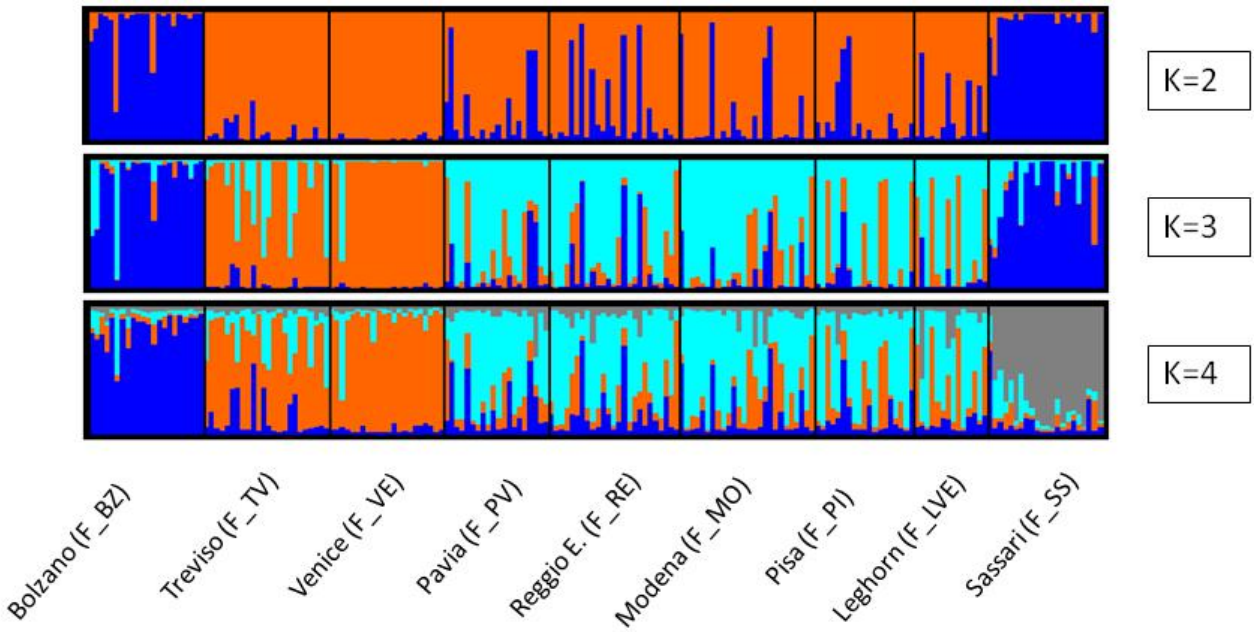
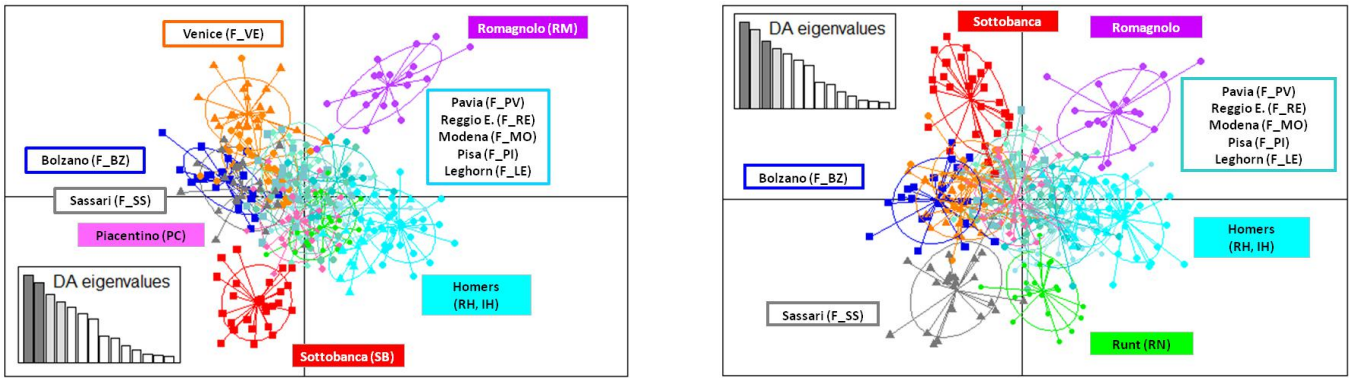
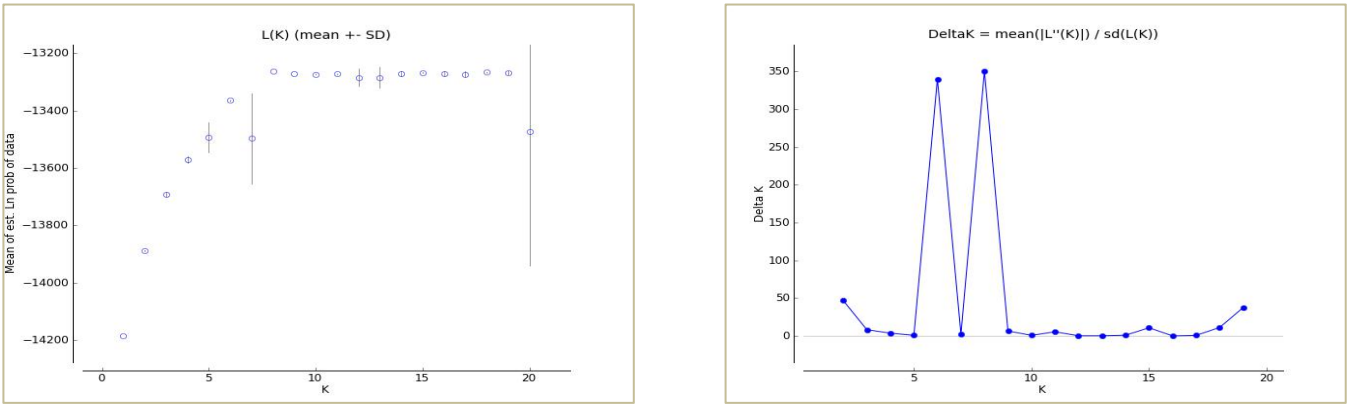

K=2
K=3
K=4
Bolzano (F_BZ)
Treviso (F_TV)
Venice (F_VE)
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LVE)
Sassari (F_SS)

Figure 3

(a)


Venice (F_VE)
Romagnolo (RM)
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LE)
Bolzano (F_BZ)
Sassari (F_SS)
Piacentino (PC)
DA eigenvalues
Homers
(RH, IH)
Sottobanca (SB)
DA eigenvalues
Sottobanca
Romagnolo
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LE)
Bolzano (F_BZ)
Homers
(RH, IH)
Sassari (F_SS)
Runt (RN)

(b)


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

(c)

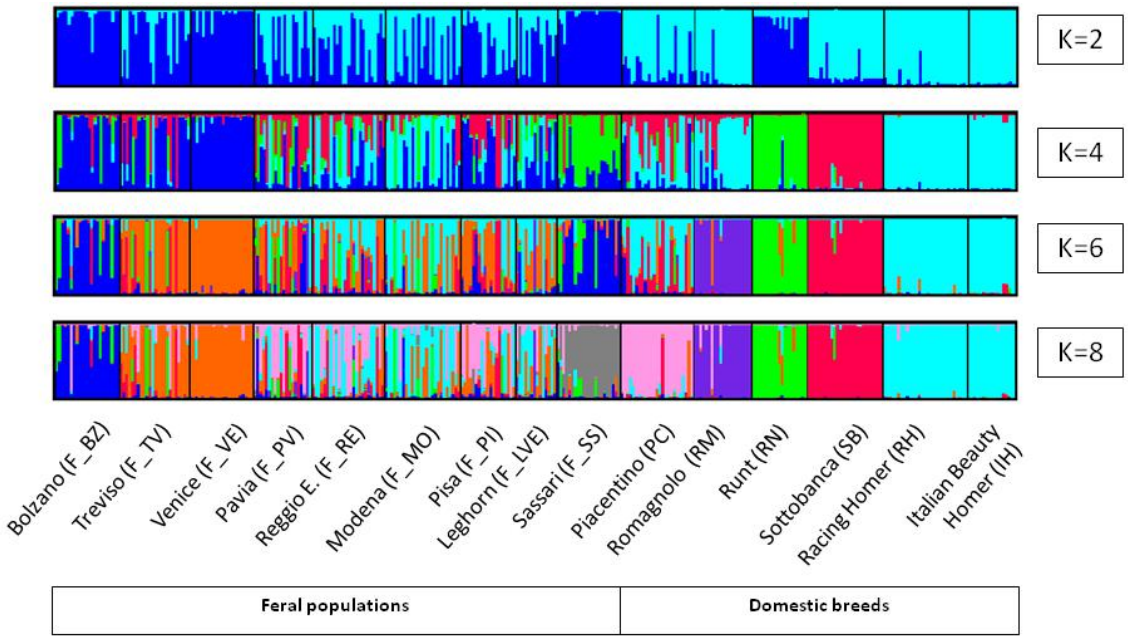

K=2
K=4
K=6
K=8
Bolzano (F_BZ)
Treviso (F_TV)
Venice (F_VE)
Pavia (F_PV)
Reggio E. (F_RE)
Modena (F_MO)
Pisa (F_PI)
Leghorn (F_LVE)
Sassari (F_SS)
Piacentino (PC)
Romagnolo (RM)
Runt (RN)
Sottobanca (SB)
Racing Homer (RH)
Italian Beauty Homer (IH)
Feral populations
Domestic breeds

Figure 4

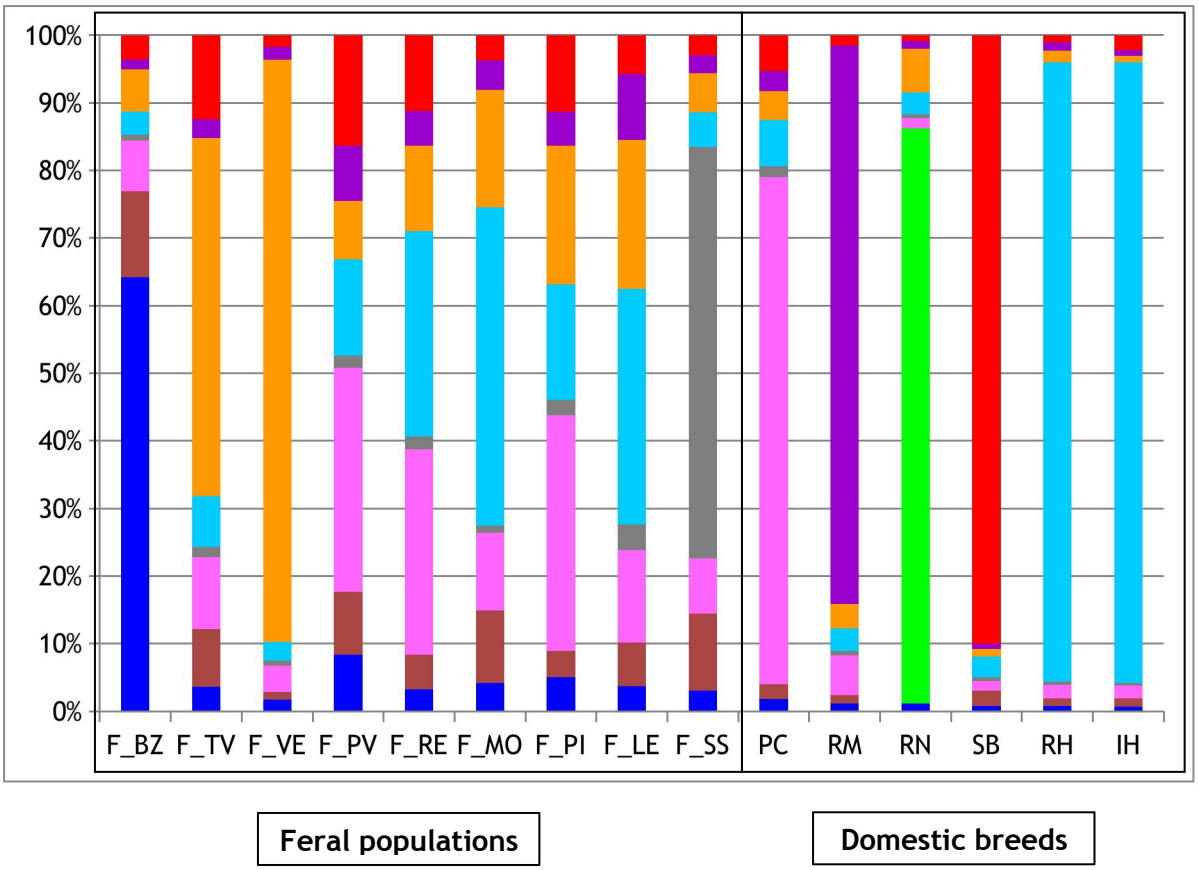

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
F_BZ
F_TV
F_VE
F_PV
F_RE
F_MO
F_PI
F_LE
F_SS
PC
RM
RN
SB
RH
IH
Feral populations
Domestic breeds

Figure 5

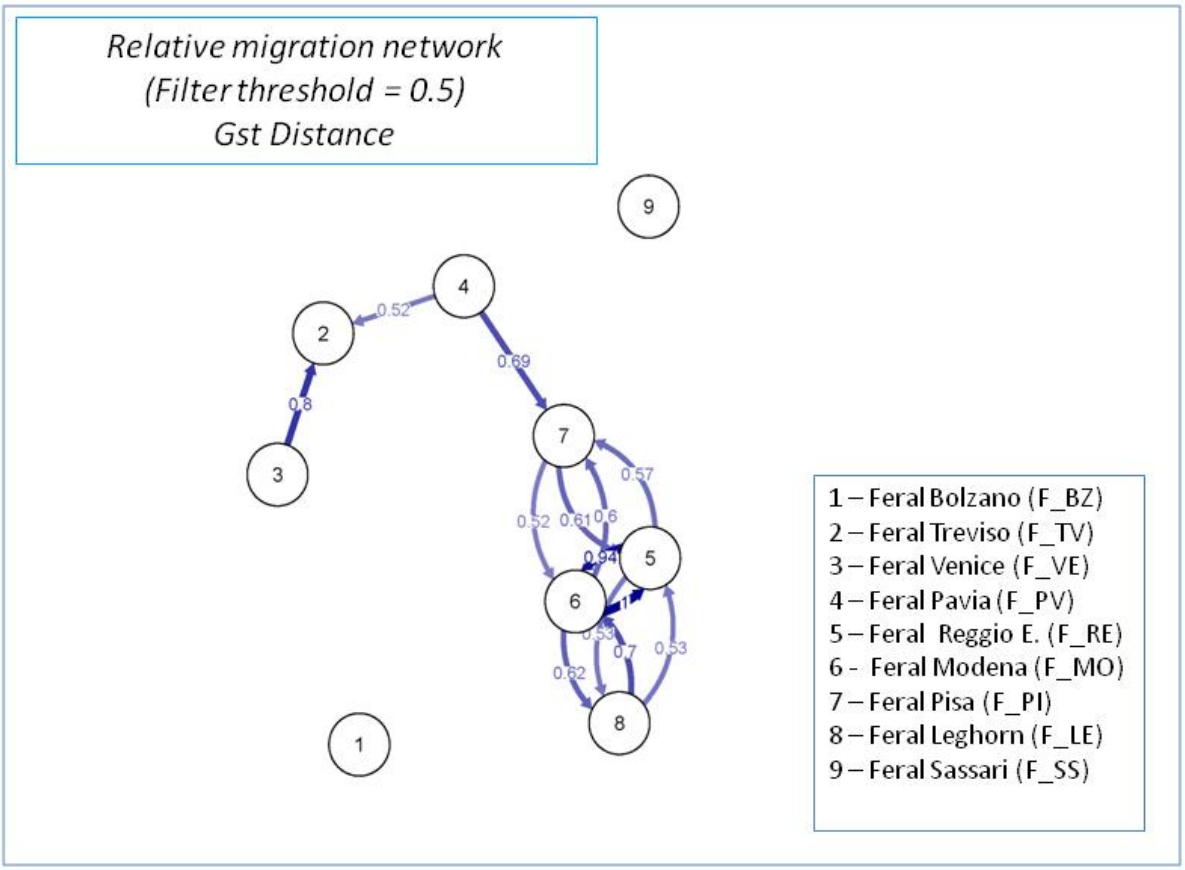

Relative migration network
(Filter threshold = 0.5)
Gst Distance
9
4
2
0.52
0.69
0.8
7
3
0.57
0.52
0.61
0.6
0.94
5
6
1
0.59
0.7
0.53
0.62
8
1
1 – Feral Bolzano (F_BZ)
2 – Feral Treviso (F_TV)
3 – Feral Venice (F_VE)
4 – Feral Pavia (F_PV)
5 – Feral Reggio E. (F_RE)
6 - Feral Modena (F_MO)
7 – Feral Pisa (F_PI)
8 – Feral Leghorn (F_LE)
9 – Feral Sassari (F_SS)

Figure 6