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(Article begins on next page)

# 1    **Feral pigeon populations: their gene pool and links with local domestic breeds**

2    Dimitri Giunchi<sup>1,\*,\*\*</sup>, Nadia Mucci<sup>2,\*,\*\*</sup>, Daniele Bigi<sup>3</sup>, Chiara Mengoni<sup>2</sup>, N. Emilio Baldaccini<sup>1</sup>

3    <sup>1</sup> Dipartimento di Biologia, Università di Pisa, Via Volta 6, 56126 Pisa

4    <sup>2</sup>Area per la Genetica della Conservazione (BIO-CGE), Dipartimento per il monitoraggio e la tutela  
5    dell'ambiente e per la conservazione della biodiversità, Istituto Superiore per la Protezione e la  
6    Ricerca Ambientale (ISPRA), Via Cà Fornacetta, 9 40064 Ozzano dell'Emilia, Italy

7    <sup>3</sup> Department of Agricultural and Food Science (DISTAL), University of Bologna, Viale G. Fanin  
8    46, 40127 Bologna, Italy

9    \* Corresponding authors, email: dimitri.giunchi@unipi.it, nadia.mucci@isprambiente.it

10    \*\* D. Giunchi and N. Mucci contributed equally to the manuscript.

## 11    **Abstract**

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

28

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

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## 40    **Introduction**

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

50    The different ways pigeons established themselves in European and North-American urban habitats have  
51    been reviewed from a historical point of view by (Johnston and Janiga 1995; Haag-Wackernagel 1998;  
52    Baldaccini and Giunchi 2006). Given that nearly any domestic breed had (and still has) the potential to  
53    contribute to the feral gene pool, at least two main contributions to feral populations have been identified.  
54    Dovecotes had been rather widespread in several European countries until the 19<sup>th</sup> century [e.g. The  
55    British Isles (Ritchie 1920; Gompertz 1957); France (Van der Linden 1950); Italy (Giachetti 1894)].  
56    Pigeons breeding in dovecotes could revert to a free life in towns and sometimes they were even forced to  
57    leave their dovecotes e.g. during the French Revolution, when large dovecotes owned by aristocrats were  
58    destroyed (Van der Linden 1950). Urban dovecotes are now rare in Europe so they do not represent an  
59    important source of individuals for current feral populations. The main contribution to feral populations  
60    both in Europe and North America probably was and is still represented by homing pigeons that failed to  
61    return to their home loft (Goodwin 1960; Simms 1979; Stringham et al. 2012). In particular, Simms  
62    (1979) suggested that juveniles homing pigeons lost at the beginning of their training period constituted an  
63    important component of feral populations at least in the UK.

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

70    In recent years, domestic pigeons have been the subject of several genetic investigations, mainly  
71    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 (*Clid01*, *Clit13*, *Clid17*, *Clit17*, *Clid16*, *Clid19* - (Traxler et al. 2000);  
109 *Clid11*, *Clit47*, *Clit24*, *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 ( $N_a$ ), the number of effective ( $N_e$ ) and private alleles, and the expected ( $H_e$ )  
118 and observed ( $H_o$ ) heterozygosity were obtained using GenAlEx 6.503 (Peakall and Smouse 2006,  
119 2012; Smouse et al. 2015). Allelic richness ( $A_r$ ) 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  $F_{st}$  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  $F_{st}$   
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 ( $D_c$ )  
130 (Cavalli-Sforza and Edwards 1967) and the proportion of shared alleles ( $D_{ps}$ ) (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).  $D_a$  distance (Nei 1973) and  $F_{st}$  distance  
133 (Latter 1972) with sample size bias correction, Goldstein distance ( $\delta\mu$ )<sup>2</sup> (Goldstein et al. 1995) and

Shriver distance ( $D_{sw}$ ) (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  $\Delta K$  and Mean Likelihood (Janes et al. 2017). CLUMPP (Jakobsson and Rosenberg 2007) and DISTRICT (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  $F_{st}$  obtained by GenAlEx computation.

Genetic migration levels between populations was estimated using the function divMigrate of the DiveRsity R package (Sundqvist et al. 2016). Nei's  $G_{st}$  and filter\_threshold = 0.5 were set to plot the network and the relationships among populations.

156

#### *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 2, see Bigi et al. 2016 for further details).  $D_c$ ,  $D_{ps}$ ,  $D_a$ ,  $F_{st}$ ,  $(\delta\mu)^2$  and  $D_{sw}$  were computed as 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 20<sup>th</sup> 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  $6.2 \pm 0.5$  SE (F\_VE) to  $8.5 \pm 0.9$  (F\_RE) with an average value of  $7.6 \pm 0.8$ . The average number of effective alleles decreased to  $4.4 \pm 0.5$ . Mean values of expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity were  $0.718 \pm 0.062$  and  $0.726 \pm 0.050$ , 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.  $F_{st}$  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  $Da$ ,  $F_{st}$ , 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 ( $D_c$ ), proportion of shared



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

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

No defined genetic structure was identified by both multivariate and Bayesian analyses (Fig. 3a-c). DAPC analysis showed an admixed pattern in Central Italy, whereas samples from Sassari (F\_SS), Bolzano (F\_BZ), and Venice (F\_VE) clustered separately from the others. The population from Treviso (F\_TV) plotted in an intermediate position between the remaining populations and F\_VE.

Both Mean Likelihood and  $\Delta K$  obtained from STRUCTURE HARVESTER identified the best grouping at  $K = 4$ . At  $K = 2$  Bolzano and Sassari populations split from the remaining populations.  $K = 3$  permitted an additional distinction of ferals from Treviso and Venice. At the best value  $K = 4$ , ferals from Bolzano (F\_BZ), Venice (F\_VE), and Sassari (F\_SS) were associated to different clusters with high  $q$  individual membership values, while samples from F\_PV, F\_RE, F\_MO, F\_PI, and F\_LE remained not differentiated from each other and showed retracts of admixture with F\_BZ, F\_VE, and F\_SS. The genetic composition of F\_TV was mainly associated with F\_VE but showed traces of admixtures with the former five populations (Fig. 3c).

No genetic components shared between Bolzano (F\_BZ), Sassari (F\_SS) and the other feral populations was estimated by the divMigrate function (Fig. 6). Gene flow towards Treviso (F\_TV) from Venice (F\_VE) and Pavia (F\_PV) resulted well supported (asymmetric values = 0.8 and =0.52, respectively), as well as that from Pavia (F\_PV) to Reggio Emilia (F\_RE). Populations from Modena (F\_MO), Reggio Emilia (F\_RE), Leghorn (F\_LE) and Pisa (F\_PI) resulted to be quite interconnected. The highest values of gene flow (0.94 and 1.0) were reported between Modena (F\_MO) and Reggio Emilia (F\_RE).

#### *Relationship between feral pigeons and domestic breeds*

Phylogenetic trees built using *Fst*, *Da* and  $(\delta\mu)^2$  distances did not show any significant structure (ESM 4) and feral groups and the domestic lineages considered in the analysis were differently associated depending on the distance computation considered. As in the previous section, Cavalli-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  $\Delta K$   
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)

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

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

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

287 The level of admixture of the remaining populations (F\_PI, F\_LE, F\_MO, F\_RE and F\_PV) and the high  
288 level of estimated gene flow among them as well as between F\_PV and F\_TV are quite difficult to explain  
289 considering the above mentioned low rate of dispersal among cities (Johnston and Janiga 1995; Hetmanski  
290 2007; Jacob et al. 2015). and the inability of feral pigeons to undergo long flights, as experimentally  
291 demonstrated by Chelazzi and Pineschi (1974) and by Edrich and Keeton (1977). However, it is not to be

292 excluded that any dispersal event could occur between very close cities (e.g. Modena and Reggio Emilia).  
293 Actually, the migration events described by DivMigrate are estimated from the allele frequencies retrieved  
294 inside the populations and could be interpreted as common genetic components rather than a real gene  
295 flow. The inclusion in the analysis of domestic samples confirms this hypothesis. Indeed, in these feral  
296 populations it is possible to identify a significant component belonging to the domestic breeds considered  
297 in this study. In particular, the Racing Homer and Piacentino components largely dominates the gene pool  
298 of these populations. These domestic components are still detectable in F\_SS, F\_BZ, F\_VE and F\_TV, but  
299 at negligible percentages. This pattern can be explained by considering the long-lasting and still ongoing  
300 tradition of keeping and selecting pigeon breeds especially in Emilia-Romagna and Lombardy (Ghigi  
301 1950; McNeillie 1976). In particular, the Racing Homer component is quite evident mainly in populations  
302 located in areas where Racing Homer breeding and racing is widespread (i.e. Emilia-Romagna and  
303 Lombardy, see Figure 1), while it is almost absent where those activities are missing (i.e. Bolzano and  
304 Sardinia). Our data only partially confirm the hypothesis by Stringham et al. (2012, but see also Goodwin  
305 1960; Simms 1979) that Racing Homers constitute the most important component of feral pigeon  
306 populations. Indeed, other breeds, such as Piacentino, can be dominant or co-dominant in the feral gene  
307 pool, as observed for example in F\_PI, F\_PV and F\_RE.

308 Our results further emphasize the complex origin of feral populations and suggest a past and probably  
309 ongoing flow of domestic pigeons into feral populations in areas surrounded by a high number of pigeon  
310 fanciers. This seems to be confirmed by the difference observed between F\_VE and F\_TV. These  
311 populations form a fairly separated cluster, which probably indicates that they share a common origin.  
312 However, being located in a lagoon, Venice has no pigeon fanciers nearby and thus the genetic  
313 contribution of the domestic breeds considered in this study to its feral population is very low. On the other  
314 hand, Treviso is surrounded by farms that probably hosted and still host pigeon dovecotes, which  
315 facilitates the urban drift of domestic birds. It should be noted that it is rather impossible to have detailed  
316 information regarding the actual distribution of pigeon breeding around a given city and for this reason the  
317 above pattern is characterized by some unexplained variability that might be related to the scale of our  
318 analysis.

319 The mechanisms leading to the admixture between domestic and feral pigeons have probably been and  
320 still are both pigeon racing and feral pigeon foraging behaviour. As observed by Goodwin (1960) and  
321 Simms (1979) pigeon races are sources of numbers of lost Racing Homers that flock together with ferals.  
322 Furthermore, the daily foraging flights of ferals towards the surrounding crop fields (Johnston and Janiga  
323 1995; Giunchi et al. 2012) may encourage farm dovecote individuals to join them. As a partial support to  
324 this hypothesis, it should be noted that, birds living in cities mostly surrounded by an unsuitable foraging

325 habitat show both less propensity to perform foraging flight [i.e. Bolzano (Baldaccini et al. 2015) and  
326 Venice (Soldatini et al. 2006)] and a less relevant component of the studied domestic breeds in their gene  
327 pool.

328 Recent data on the monk parakeet *Myiopsitta monachus* and ring-necked parakeet *Psittacula krameri*  
329 suggest that a high degree of admixture is not directly related to invasive success in an urban habitat and  
330 does not prevent the possibility of rapid adaptation to the urban environment (Edelaar et al. 2015; Le Gros  
331 et al. 2016). In this regard, it would be interesting to use a genomic approach in order to test whether the  
332 populations with a higher degree of admixture, actually show higher frequencies of phenotypic characters  
333 belonging to domestic breeds or whether the domestic phenotypes are quickly counter-selected by the  
334 urban environment (see Johnston and Janiga 1995; Sol 2008)

335 As observed above, feral pigeons from Bolzano, Venice/Treviso and Sassari were characterized by unique  
336 genetic components, that are mainly not shared by other feral populations. Considering the geographic  
337 position of those populations, it can be hypothesized that these unique components belong to domestic  
338 breeds not originated and/or reared in Italy (e.g. central-european breeds for Bolzano, eastern breeds for  
339 Venice/Treviso). Concerning birds from Sassari, it should be noted that Sardinia still hosts wild  
340 populations of rock doves (Ragionieri et al. 1991; Johnston 1992; Johnston and Janiga 1995). Johnston  
341 and Janiga (1995) indicated that when wild and feral pigeons live in sympatry it is likely that they  
342 interbreed, thus it can be hypothesized that wild pigeons may have contributed to F\_SS. In this regard, it is  
343 interesting to observe that, contrary to Jacob et al. (2015) and Biala et al. (2015), we found eight private  
344 alleles in feral samples, all belonging to the Sardinian population. This result could be explained by  
345 considering at least three possible factors: a) the effect of genetic drift, being Sardinian populations  
346 relatively isolated (see above); b) the effect of domestic breeds not included in our sample and not  
347 affecting other feral populations or c) the gene flow between wild rock doves and feral pigeons as  
348 hypothesized by Ragionieri et al. (1991). Our data do not allow to discriminate among these effects so this  
349 topic deserves further investigations.

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

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

366

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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  $\Delta K$  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  $\Delta K$  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

410 **Table 1** Geographic origin of feral samples.

411

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

417

418

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

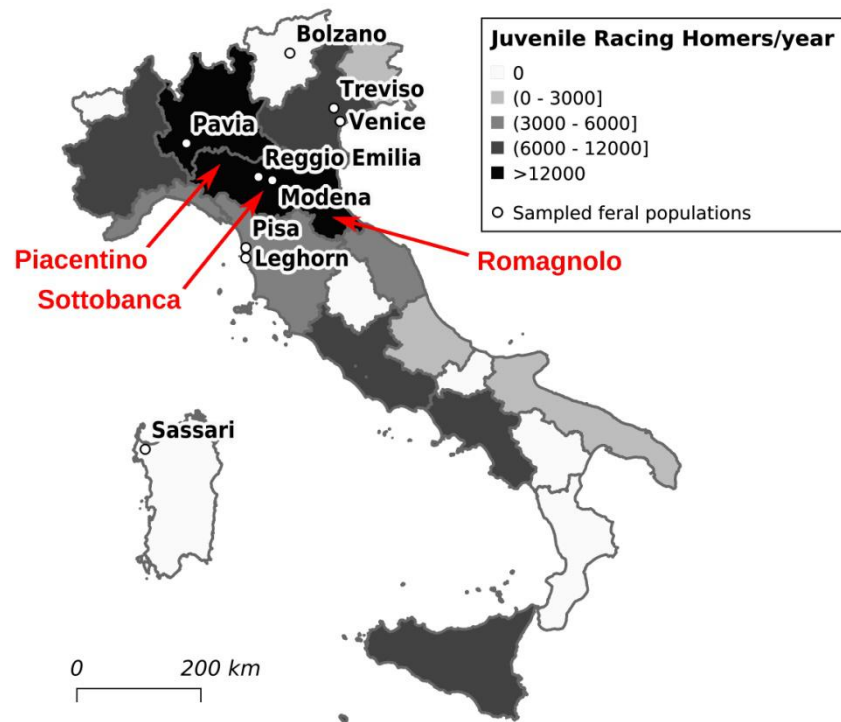
Domestic Breed	Acronim	No. samples
<b>Italian Owl</b>	<b>IO1</b>	<b>29</b>
<b>Italian Owl Rondone</b>	<b>IO2</b>	<b>20</b>
<b>Florentine</b>	<b>FL</b>	<b>20</b>
Piacentino	PC	25
Romagnol	RM	20
Runt	RN	19
Sottobanca	SB	26
<b>Triganino Schietto</b>	<b>TM1</b>	<b>26</b>
<b>Triganino Gazzo</b>	<b>TM2</b>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

421

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

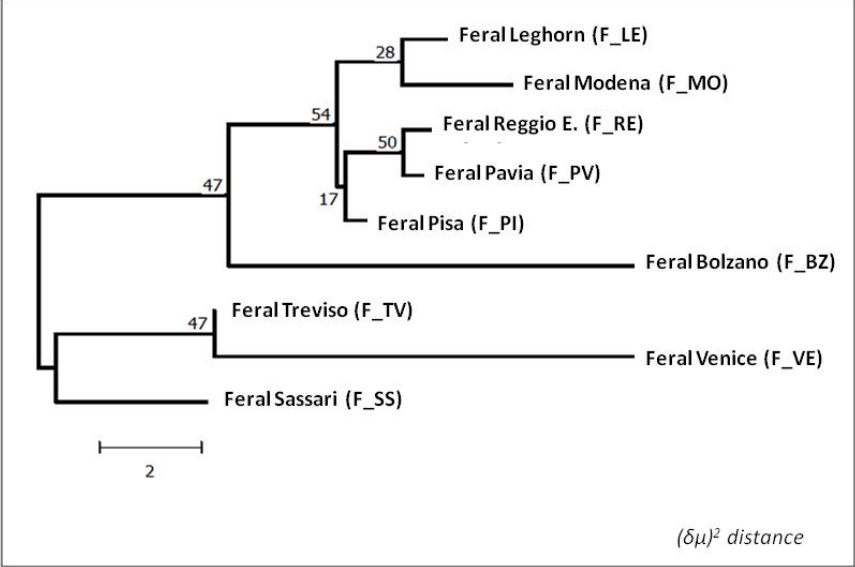
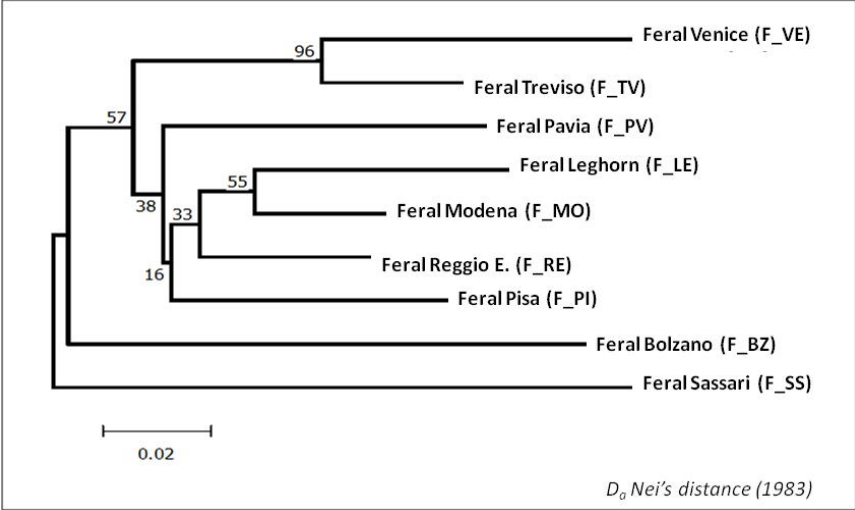
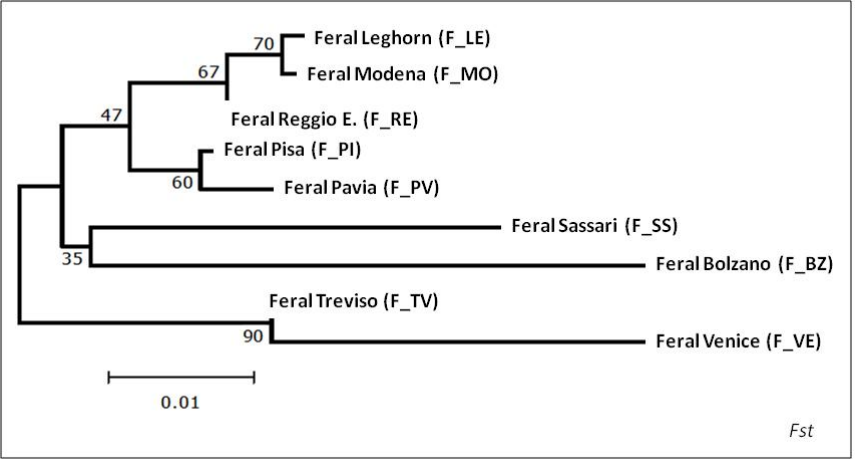
Population	<i>Na</i>	<i>Ne</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>
F_BZ	6.9 $\pm$ 0.7	3.9 $\pm$ 0.3	6.2	0.762 $\pm$ 0.037	0.727 $\pm$ 0.027
F_TV	7.8 $\pm$ 0.9	4.6 $\pm$ 0.6	6.9	0.705 $\pm$ 0.040	0.735 $\pm$ 0.036
F_VE	6.2 $\pm$ 0.5	3.4 $\pm$ 0.4	5.6	0.639 $\pm$ 0.065	0.651 $\pm$ 0.052
F_PV	7.9 $\pm$ 0.7	4.6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

424



425  
426 **Figure 1**  
427



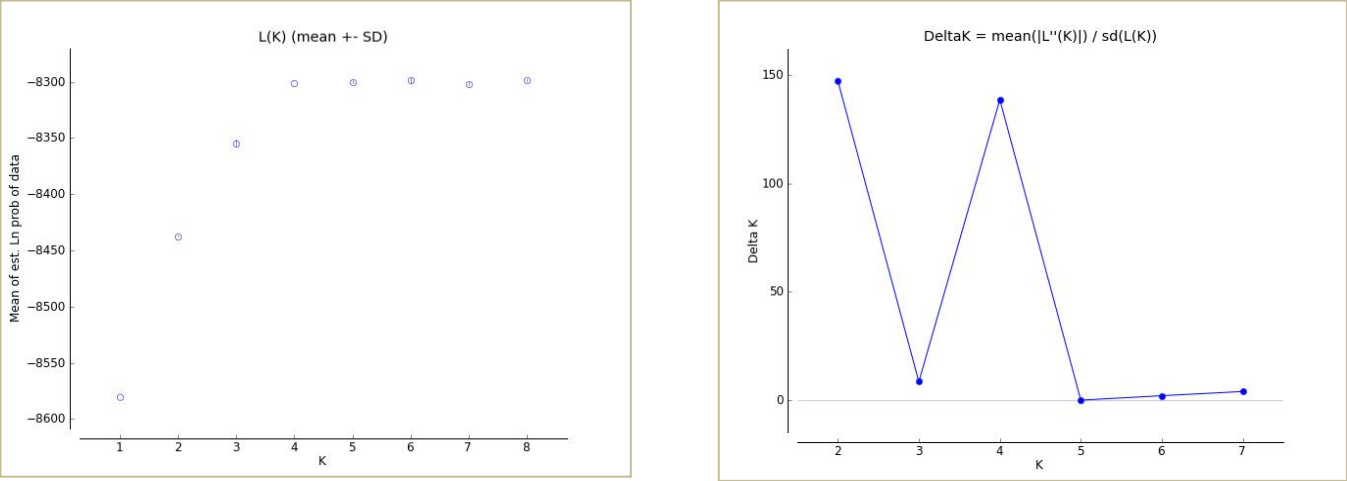


429 Figure 2

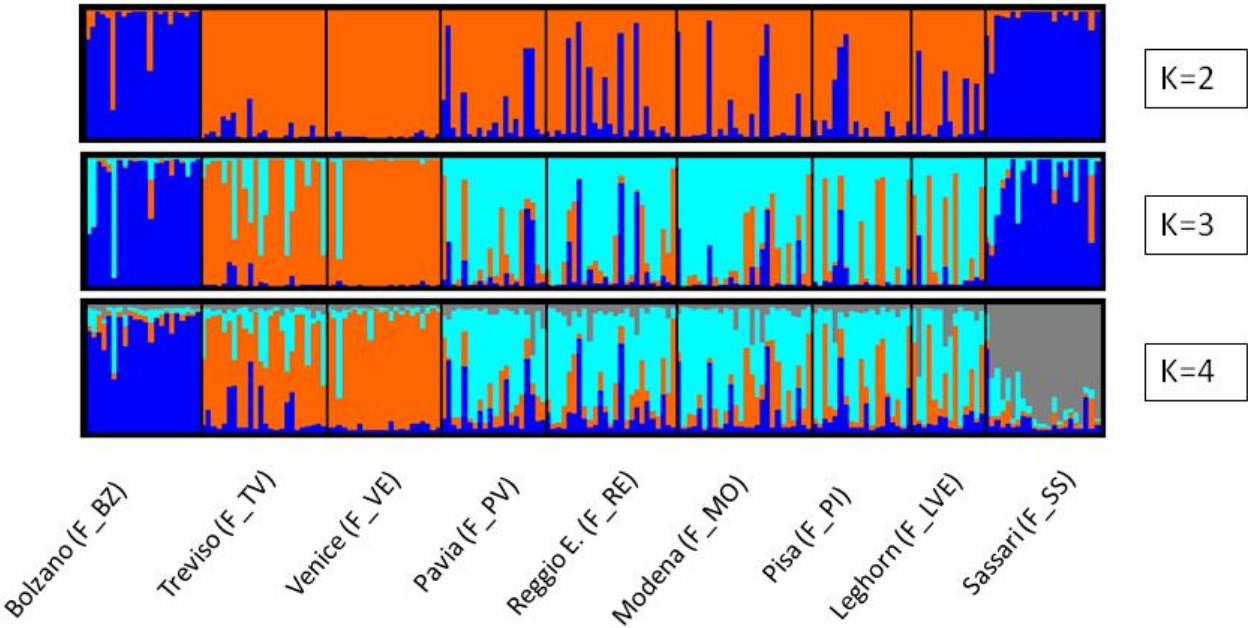
(a)



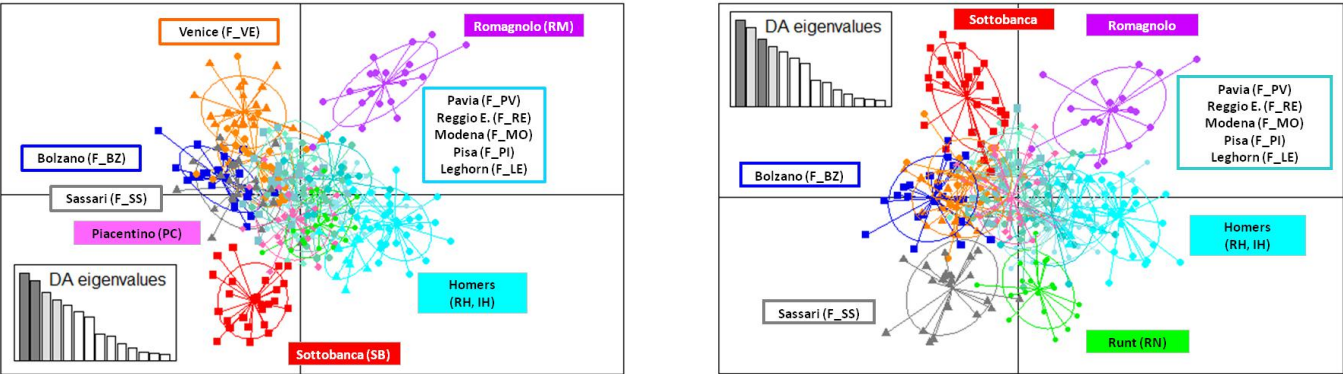
(b)



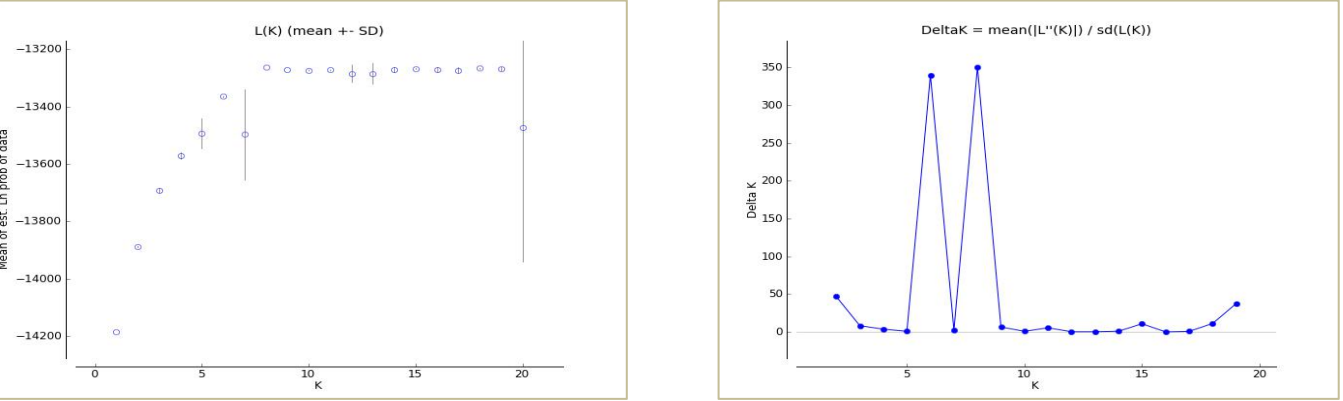
(c)



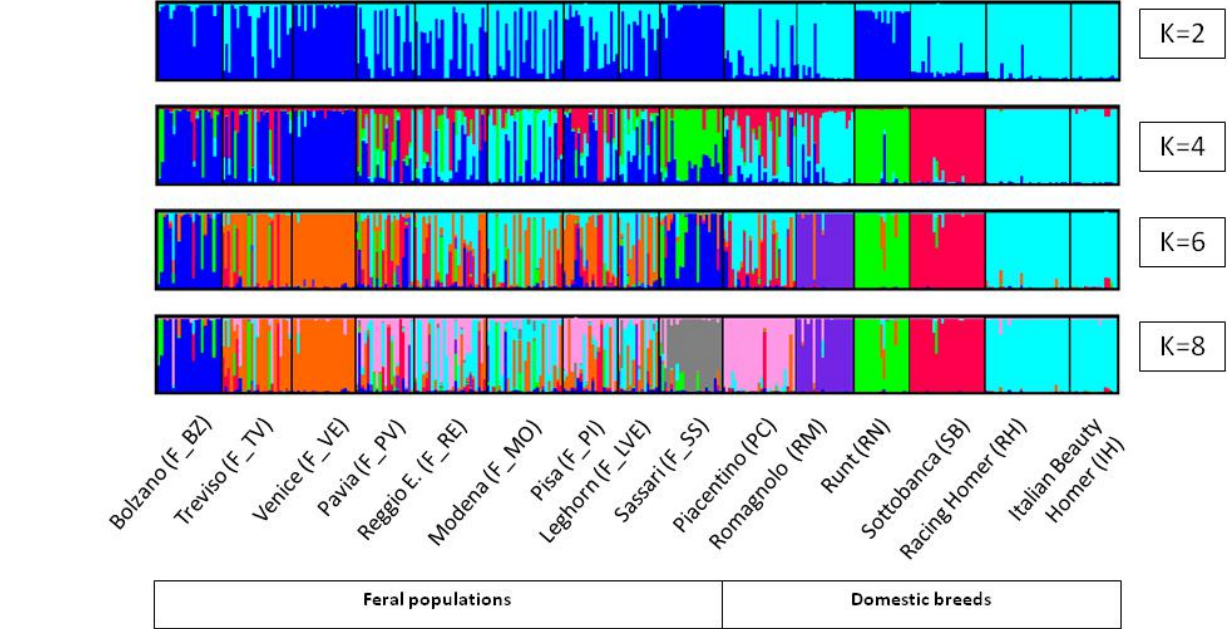
(a)



(b)



(c)



432 Figure 4

433

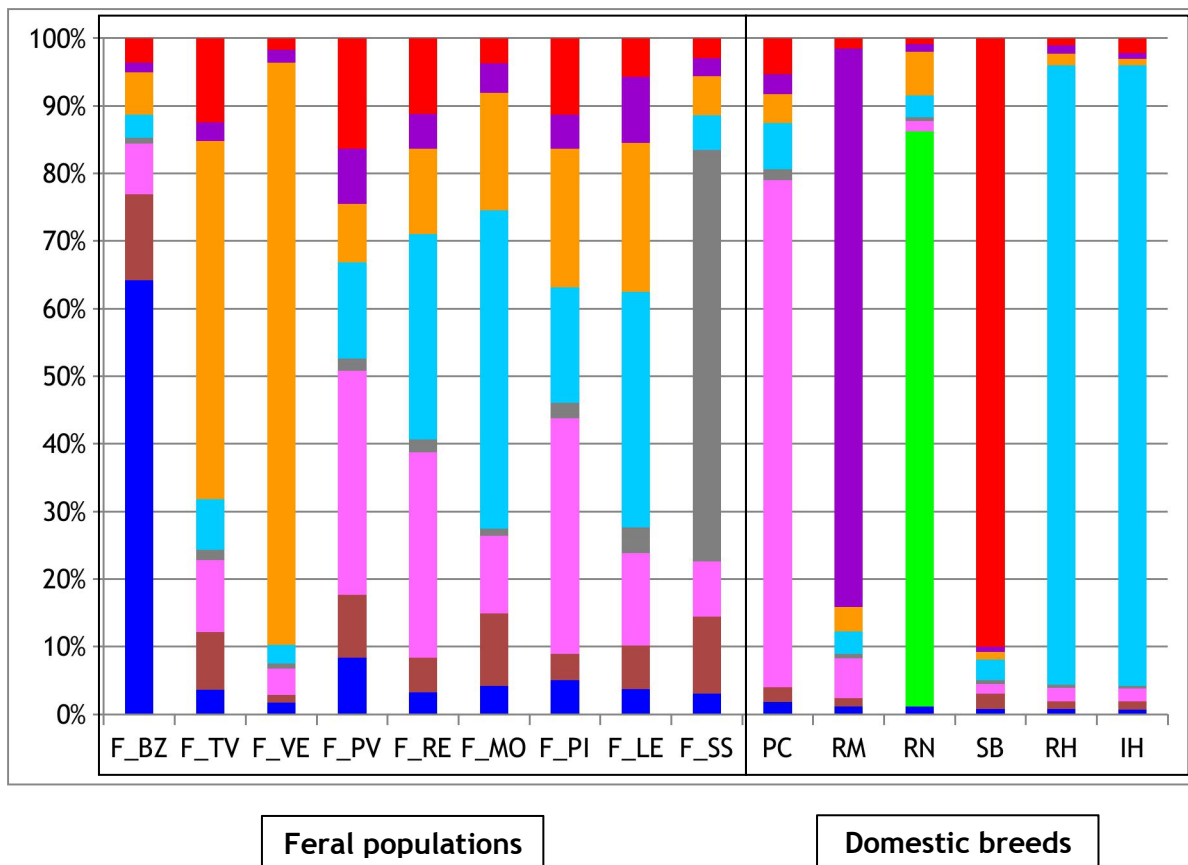


Figure 5

