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## Genomic variability of Cirneco dell'Etna and the genetic distance with other dog breeds

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### ABSTRACT

Cirneco dell'Etna is an old Italian breed of scent hunting dogs. Commonly used genomic measures such as heterozygosity, fixation indexes, and runs of homozygosity can help to improve knowledge about its genetic diversity. This study aimed to: (i) investigate Cirneco's genomic background, (ii) quantify its genomic inbreeding, and (iii) detect genomic regions differentiating the Cirneco's two allowed coat colours, self-coloured fawn and tan and white. Canine 230K SNP BeadChips was used to investigate 24 Cirneco (19 self-coloured fawn, and 5 tan and white) and other 106 dogs from eight phylogenetically and historically related breeds. The genetic distance, ancestry, and relationship among breeds were explored by multidimensional scaling, Reynolds distances, phylogenetic tree, and admixture analysis. The genomic inbreeding ( $F_{ROH}$ ) was calculated for each breed. Averaged Wright's fixation index  $\bar{F}_{ST}$  was used to identify the genes that most differentiated the two groups of Cirneco. All analyses highlighted that Segugio Italiano and Kelb tal Fenek are the closest breeds to Cirneco. Within the breed, tan and white subjects showed a more heterogeneous genetic background and a lower inbreeding in comparison with self-coloured fawn ones, even though more than half of the latter presented a superimposable admixture. The gene that most differentiated these two groups is Microphthalmia-Associated Transcription Factor (*MITF*), previously associated with white spotting in other breeds. Given the small size of the Cirneco population and its open registry, its management should carefully combine morphological and genealogical evaluations with genetic tools to identify the best breeders while maintaining an acceptable genetic pool.

### HIGHLIGHTS

- The genomic analysis demonstrated that Segugio Italiano and Kelb tal Fenek are genetically related to the Cirneco.
- The *MITF* gene is responsible for white blazing in Cirneco as in many other dog breeds.
- Genomic tools should be integrated with phenotypic and genealogical evaluations in the management of Italian autochthonous dog breeds to safeguard their welfare and biodiversity.

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Italian dog breed; SNPs; genomic inbreeding; genetic biodiversity; autochthonous breeds

## Introduction

The Cirneco dell'Etna (Cirneco for conciseness) is the oldest of the 16 Italian dog breeds officially recognised by the National Agency of the Italian Kennel Club (ENCI), with its breed standard definition dating back to 1939 (Tricomi and Moore 2016). This Cirneco breed is also recognised internationally by the

Federation Cynologique Internationale (FCI) with breed standard number 199, classified in Group 5 – Section 7 (Spitz and primitive types - Primitive type Hunting Dogs) with working trial in Italy (FCI 2020). Currently, the distribution of this breed is very limited, with only 15 officially registered breeders in Italy, who together enrol on average 130 puppies per year to the Register of Italian Origin (ROI) and the Register Additional

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 Supplemental data for this article can be accessed [here](#).

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**Figure 1.** Dogs of the past and Cirneco dell'Etna of the present. (a) 'Tomb owner's son hunting wild animals', a detail from the tomb of Khnumhotep II at Beni Hassan (1918–1884 B.C.E) (Woods et al. 2018). A hunting dog, resembling Cirneco dell'Etna or Kelb tal Fennek, is grasping an oryx; at the top right corner another dog, characterised by a white and black coat, is represented. (b) A detail from the roman mosaic 'Triumph of Neptune and the Four Seasons', from La Chebba, Tunisia, mid III Century A.D. (Bardo National Museum). A dog similar to Cirneco dell'Etna accompanies Spring. (c) A detail from a mosaic of the IV century A.D., in Villa Romana del Casale, Piazza Armerina (Sicily). A hunting scene is represented, with two dogs chasing a fox. (d) Photograph of a self-coloured fawn Cirneco dell'Etna (courtesy of breeders). (e) Photograph of a self-coloured fawn with white markings Cirneco dell'Etna (courtesy of breeders). (f) Photograph of a tan and white Cirneco dell'Etna (courtesy of breeders).

Recognised (RSR, used in case of unknown or incomplete genealogy) (ENCI 2020).

The Cirneco is a Mediterranean type dog described as being eclectic and highly appreciated as a hunting dog. It traces its presence since ancient times in Sicily (Italy), probably descending from the hunting dog of

Pharaohs period of ancient Egypt (Figure 1(a)). Hypothetically these dogs could have been dispersed from Egypt and spread across the Mediterranean basin by the Phoenicians during their explorative journeys (Figure 1(b); Fiorone 1950). Archaeological artefacts excavated in Sicily depicting images similar to the

Cirneco include coins, incisions, and mosaics dated centuries before Christ (Figure 1(c); Tricomi and Moore 2016).

The Cirneco breed is defined as a medium sized dog put together in an elegant and slender shape, being compact and strong built, having a fine coat and upright ears. The height at the withers ranges from 46 to 50 cm in males (body weight 10–13 kg) and from 44 to 48 cm in females (body weight 8–11 kg) (FCI 2020). It presents vitreous and dense texture hair, smooth on head, ears and legs and semi-long (2.5 cm approximately) sleek and fitting on the body and tail. It is described as a scent hunting dog that uses his heightened sense of smell to locate and pursue wild rabbit and other small furry and feathered preys (ENCI 2020). The breed being gentle-mannered, affectionate, eager and sprightly in action, can also be appreciated an excellent companion dog.

The Cirnecos have always been greatly sought-after for their highly developed hunting skills. In the 70's the selling price of a 40–50-day old puppy was 100,000–150,000 Italian lire, corresponding to 30% of the average monthly wage at the time (~500,000 lire; AAVV 1979). Today, the Cirneco dogs are very popular in demand across Europe and Russia.

Phenotypic characteristics, particularly coat colouring patterns, are not just fundamental traits for defining canine breeds, but also implications for determining a dogs' economic worth. The Cirneco breed standard allows for a fawn coat colour with shades from dark to light and all its dilutions (Figure 1(d,e); Migneco 1897), and for a tan coat with white markings: a white blaze or mark on head/chest/feet/point of tail/belly, and eventually a white collar too, although not appreciated (Figure 1(f)). The tan coat with mixture of slightly lighter and darker hairs is also admissible. Conversely, coat colours of solid brown, black or brindle, black or brown patches or black or brown hairs represent definite disqualifying faults. As of 2016, solid white coat and white coat with orange patches are being exempted due to the lack of registrations of dogs with these colours (FCI 2020).

Intensive selection for traits such as coat colour is crucial to ensure the future propagation of breeds. However, severe selection pressure can also lead to increased inbreeding occurrence in small population clusters (Wiener et al. 2017; McGreevy et al. 2018; Navas et al. 2020). The joint application of reliable pedigree and the genetic evaluation of diversity are a central stepping stone for the development of conservation programs aimed at preventing inbreeding depression and also managing gene flow that can

cause undesired phenotypes. A focussed breeding strategy complimented with a reliable pedigree verification system is essential for the successful implementation of a well-defined breeding programme, especially if its effectiveness is boosted by the use of genomic technologies such as SNP arrays (Kang et al. 2009; Vonholdt et al. 2010; Bai et al. 2015). This tool is now broadly adopted to assess the genetic diversity and is the tool of choice in several studies on breeds of domestic dogs, including the Italian autochthonous ones (Morin et al. 2004; Quignon et al. 2007; Talenti et al. 2018; Yang et al. 2019). The mechanisms for the expression of pigmentation are extremely complex; the study in dogs of genomic information coupled with pedigree reconstruction is still revealing new mechanisms, as recently stated by Dreger et al. (2020).

This study focuses on the genetic characterisation of the Cirneco breed, both within population with respect to the different coat colours, and in comparison with other breeds assumed to be closely related from a historical, geographical or phenotypical perspective. In particular, the aims of this study were: (i) to investigate the Cirneco's genomic background and its admixture with other related breeds, (ii) to estimate the genomic inbreeding and outbreeding, and (iii) to explore the genomic regions that differentiate white-blazed Cirneco from the rest.

## Materials and methods

### Samples

The samples of Cirneco dell'Etna ( $n=24$ , CIRN) were divided in two groups according to coat colour, namely 19 self-coloured fawn (SF) and five tan and white (TW). The following breeds were chosen for comparative purposes: the Basenji ( $n=10$ , BSJI) and Kelb tal Fenek, better known as Pharaoh Hound ( $n=15$ , KETF), being two primitive sighthound-type dogs, similar to the Cirneco in appearance and use, and also sharing a common history with it (Palamidessi 1963; Tricomi and Moore 2016); the Whippet ( $n=10$ , WHIP) and Italian Greyhound ( $n=20$ , IGIT), belonging to the sighthounds group; the Beagle ( $n=10$ , BEAG), Bracco Italiano ( $n=9$ , BRAC), Segugio Italiano a Pelo Forte ( $n=16$ , SIFP), and Segugio Italiano a Pelo Raso ( $n=16$ , SIFR), on the basis that there is historical evidence of being bred together with Cirneco, for example for hunting purposes (AAVV 1979). All the sampled adhered to their respective breed standard—a detailed description of which can be found on FCI website (<http://www.fci.be/en/>) or, in the case of KETF, on the dedicated website (<http://www.ketf.it/>).

kelb-tal-fenek.com/kelbtalfenek.htm). All the samples were taken randomly from dogs belonging to different breeders and with as much as a high degree of unrelatedness as possible

Data on the number of annual registered dogs with ENCI are available to the general public on ENCI website (<https://www.enci.it/>).

Except for the dogs used for the comparison, which come from previous studies (Parker et al. 2010; Talenti et al. 2018), the blood samples were obtained in accordance with the ethical committee statement of the University of Messina number 040/2020. DNA was extracted with DNeasy Blood & Tissue Kit (QIAGEN®, Hilden, Germany), according to the recommended manufacturer's protocol. The DNA samples were evaluated in terms of quality and concentration with NanoDrop 1000 spectrophotometer (Thermo Scientific®, Waltham, MA, USA) and then genotyped in outsourcing using Canine 230K SNP BeadChips on an iScan System (Illumina®, San Diego, CA, USA).

### Data processing and filtering

Quality control using PLINK 1.9 (Purcell et al. 2007), was applied to raw genotype data in order to exclude individuals with call rates <95% or directly related to more than one individual in the sample (according to Mendelian errors analysis) and SNPs with call rates <95% or with a minor allele frequency (MAF) <1%. Only markers on autosomes were retained. This new subset was used for further analyses.

### Population structure

To depict the genetic structure of the selected individuals, a multidimensional scaling (MDS) analysis was conducted with PLINK 1.9. In order to explore the short-term divergence between the breeds, Reynolds distances (Reynolds et al. 1983) were calculated with an in-house script. Their representation as phylogenetic tree was realised with PHYLIP software package (Felsenstein 1989) and FigTree 1.4.4 software (Rambaut 2018). ADMIXTURE 1.3 (Alexander and Lange 2011) was used to investigate the admixture of each breed, using a number of different genetic clusters ( $K$ ) ranging from 2 to 11. The  $K$  with the lowest cross-validation value (cv-value) was regarded as the best fit model. The individuals' probability of assignment to each  $K$  group (Q-values) was analysed.

### Inbreeding and genetic diversity

For all the breeds, PLINK 1.9 software was used to calculate the expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), and Wright's fixation index ( $F_{IS}$ ), defined as the correlation between homologous alleles within individuals with reference to the local population (Wright 1951; Nei 1978).

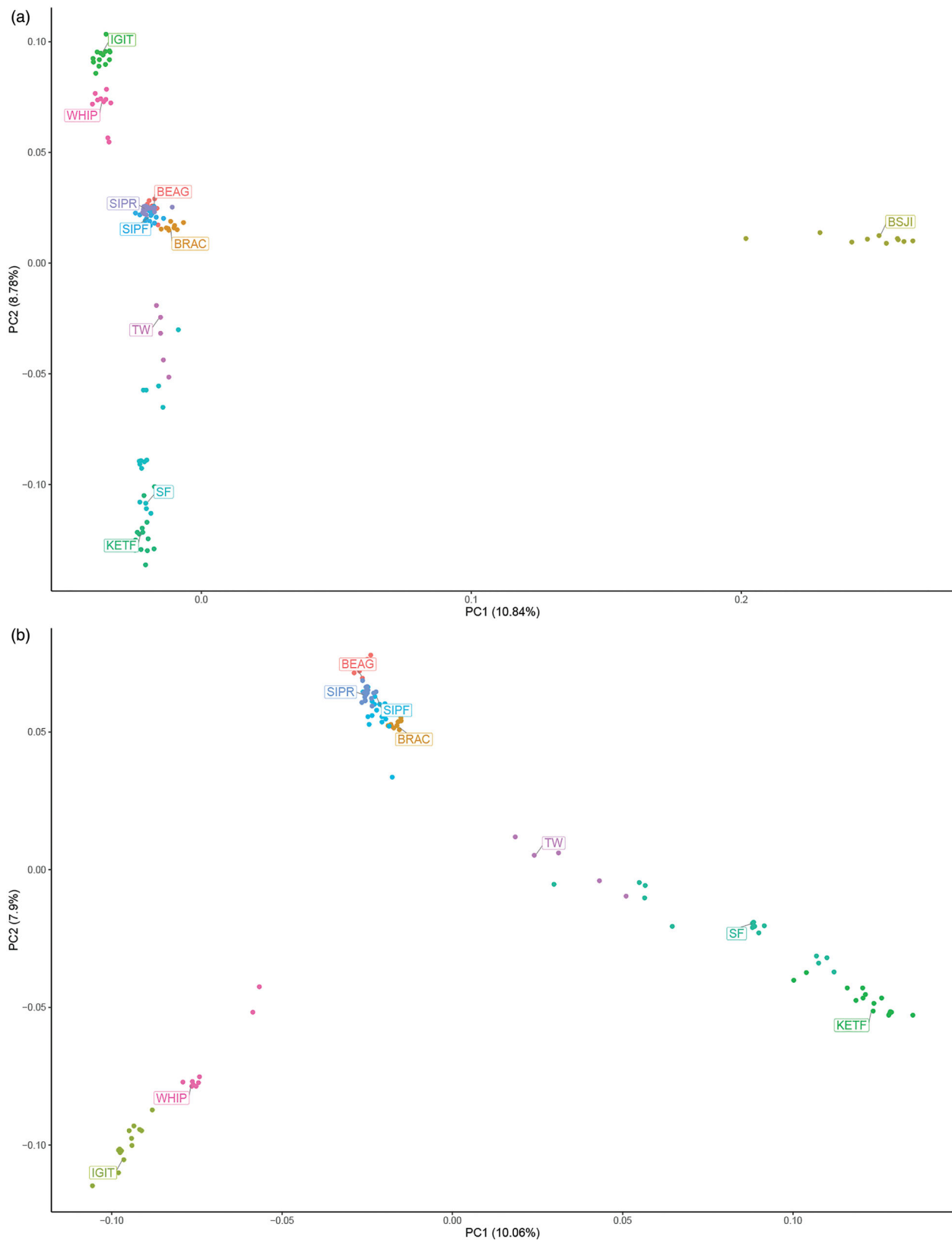
Runs of homozygosity (ROH) were investigated using a sliding window approach in PLINK 1.9 software. The sliding window was 50-SNPs long and contained  $\leq 5$  missing genotypes and no heterozygous SNPs. The criteria used to describe a ROH were: (i)  $\geq 50$  consecutive homozygous SNPs, (ii) a length  $\geq 1$  Mb, (iii) a density  $\geq$  one SNP per 50 Kb, and (iv) a gap between two consecutive SNPs  $\leq 100$  Kb. The ROH-based inbreeding coefficient ( $F_{ROH}$ ) was calculated for each individual animal dividing the total length of all ROH in its genome by the length of the autosomal genome covered by SNPs on the chip (McQuillan et al. 2008; Sams and Boyko 2019). The  $F_{ROH}$  for five different ROH length classes: from 1 to 2 Mb ( $1 < ROH < 2$ ), from 2 to 4 Mb ( $2 < ROH < 4$ ), from 4 to 8 Mb ( $4 < ROH < 8$ ), from 8 to 16 Mb ( $8 < ROH < 16$ ) and over 16 Mb ( $ROH > 16$ ) were also estimated. The number of generations for inbreeding events can be estimated on the basis of ROH length: ROH that originated recently are longer due to the smaller probability of being broken by recombination events, whilst more ancient ones tend to be shorter. In particular,  $F_{ROH}$  are expected to correspond to the ancestral population dating 50 ( $1 < ROH < 2$ ), 20 ( $2 < ROH < 4$ ), 12.5 ( $4 < ROH < 8$ ), 6 ( $8 < ROH < 16$ ) and 3 ( $ROH > 16$ ) generations ago (Howrigan et al. 2011).

### Selection signature analyses

Averaged Wright's fixation index ( $\bar{F}_{ST}$ ) was determined for the CIRN genomes by averaging five adjacent SNPs values to reduce the effect of outlier values and provide a better estimate of regions of interest (Onzima et al. 2018). This analysis was performed to investigate genetic diversity, based on allele frequency differences, between SF and TW groups (Holsinger and Weir 2009). A  $\bar{F}_{ST}$  of 0.6 was considered as threshold and SNPs with higher values (0.0001%) were mapped to the reference genome assembly CanFam3.1 (Hoeppner et al. 2014).

### Results

Registrations of dogs with ENCI over the last 10 years have shown that four breeds had an annual number



**Figure 2.** Multidimensional scaling analysis plot of the all the individuals (a) and excluding Basenji (b). Each dot corresponds to a different individual. Each colour corresponds to a different breed. BEAG: Beagle; BRAC: Bracco Italiano; BSJI: Basenji; TW: Tan and white Cirneco dell'Etna; SF: Self-coloured fawn Cirneco dell'Etna; IGIT: Italian Greyhound; KETF: Kelb tal Fenek (Pharaoh Hound); SIPF: Segugio Italiano a Pelo Forte; SIPR: Segugio Italiano a Pelo Raso; WHIP: Whippet; PC: principal component.

**Table 1.** Reynolds distances between dog breeds.

	TW	SF	BEAG	BRAC	BSJI	IGIT	KETF	SIPF	SIPR	WHIP
TW		0.172	0.235	0.242	0.320	0.241	0.214	0.178	0.188	0.251
SF			0.246	0.252	0.327	0.250	0.200	0.192	0.200	0.263
BEAG				0.270	0.346	0.269	0.269	0.207	0.214	0.279
BRAC					0.348	0.274	0.271	0.218	0.225	0.286
BSJI						0.250	0.200	0.192	0.200	0.263
IGIT							0.272	0.220	0.227	0.257
KETF								0.220	0.227	0.280
SIPF									0.110	0.234
SIPR										0.241
WHIP										

Reynold distances here reported were obtained from 100 bootstraps; all the standard deviations were  $<0.001$ . The values obtained including and excluding BSJI were superimposable. TW: Tan and white Cirneco dell'Etna; SF: Self-coloured fawn Cirneco dell'Etna; BEAG: Beagle; BRAC: Bracco Italiano; BSJI: Basenji; IGIT: Italian Greyhound; KETF: Kelb tal Fenek (Pharaoh Hound); SIPF: Segugio Italiano a Pelo Forte; SIPR: Segugio Italiano a Pelo Raso; WHIP: Whippet.

of registrations  $<500$ , three breeds between 500 and 2000, and one (SIPR) increased by almost 4000 per year (Figure S1). CIRN in particular had only 1249 dogs registered within the same ten-year period. BSJI stands out as the least popular breed in Italy among the ones considered in this study. No data were available for KETF breed.

Quality control led to the exclusion of four CIRN samples (one for low call rate and three due to relatedness) and six IGIT (for relatedness), leaving 15 CIRN in group SF and five in group TW, and 119,883 SNPs.

Consistently with previous studies (Parker et al. 2004), our population analyses highlighted how the BSJI was an outlier, both explaining most of the variability along the first MDS component axis (Figure 2(a)), and presenting the highest Reynolds distances with all other breeds ( $m = 0.335$ ) (Table 1). Removing BSJI from the analysis, the MDS plot managed to separate and distinguish sighthounds (IGIT and WHIP), scent hounds (BEAG, SIPR, SIPF and BRAC) and primitive dogs (CIRN and KETF; Figure 2(b)). It is worth mentioning that, even though the two coat colourings were certainly part of the same genetic pool, the TW animals were closer to scent hounds whilst the majority of SF was proximal to KETF. The breed that was nearest to both TW and SF was SIPF (Reynolds distances 0.178 and 0.192 respectively), whilst the farthest was WHIP (Reynolds distances of 0.251 and 0.263). In the dendrogram (Figure 3), breeds were well distinguishable except for the presence of one SF in KETF and TW branches, of one TW in SF branch and of two SIPR in SIPF branch.

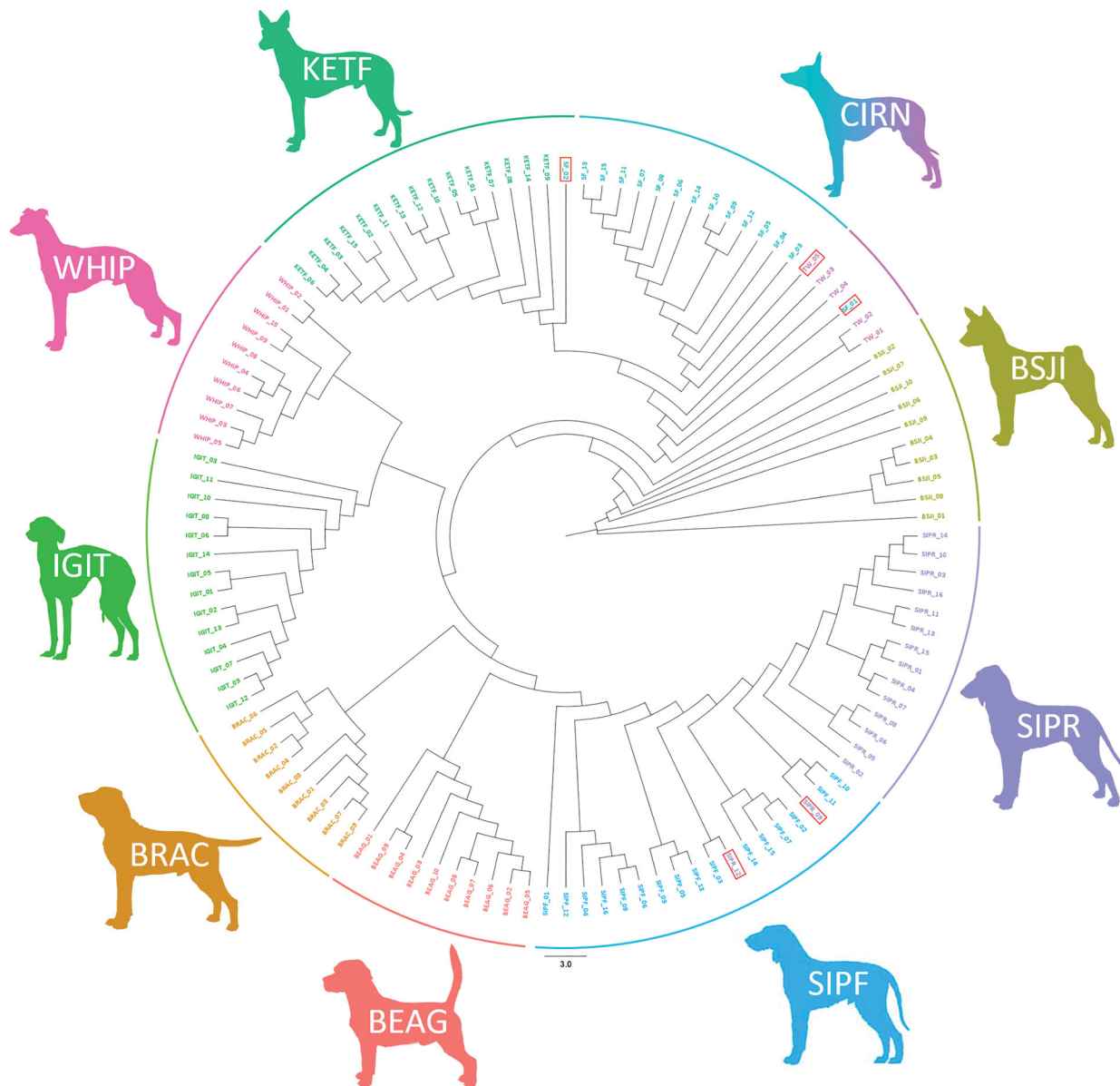
ADMIXTURE analysis (Figure 4) firstly isolated BSJI from all the other breeds ( $K = 2$ ); then KETF and CIRN became identifiable ( $K = 3$ ); the following analysis

divided the two sight hounds, WHIP and IGIT, from the others ( $K = 4$ ).

The best fitted admixture model was identified at  $K = 7$ , with a cv-value = 0.606. The model with  $K = 8$  had a very similar cv-value (0.608), and differed in that it distinguished BRAC and BEAG; in order to better identify the contribution to all the officially recognised breeds, this latter result will be presented and discussed. SIPF and SIPR, only recognised as distinct breeds since 1989, shared the same admixture, a finding consistent with previous studies (Talenti et al. 2018; Pallotti et al. 2017). With regard to CIRN, it was observed that SF group had a significantly higher membership coefficient (i.e., Q-value) for its own  $K$  ( $0.781 \pm 0.256$ , ranging from 0.219 to 1.000) than TW group ( $0.337 \pm 0.103$ , ranging from 0.220 to 0.478), with seven of the SF having membership coefficient approaching 1 and the remaining major membership coefficients that can be traced to KETF ( $0.121 \pm 0.036$ ) and the sum of SIPF and SIPR ( $0.201 \pm 0.093$ ).

Values of expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), and Fixation Index ( $F_{IS}$ ) of all the breeds considered in this study are shown in Supplementary Materials (Table S1). The two groups of CIRN had the highest differences between  $H_o$  and  $H_e$ : 0.017 for TW and  $-0.012$  for SF. An  $H_o$  greater than  $H_e$  was also observed in BRAC (0.013) and, to a minor extent in IGIT (0.003).

In order to investigate and confirm both the differences between TW and SF and among all the breeds included in this study, genomic inbreeding, as a measure of the level of homozygosity that individuals have within a population ( $F_{ROH}$ ), was calculated for all the individuals and the mean value of those belonging to the same breed was considered as the breed's  $F_{ROH}$  (Supplementary Materials, Table S2). As reported in Figure 5(a),  $F_{ROH}$  ranged from 0.099 (SIPR) to 0.252 (WHIP) and had an average of  $0.182 \pm 0.065$ . The  $F_{ROH}$  of the SF group (0.195) was almost twice that of the TW group (0.100). This pattern was observed in all the classes of ROH length. The  $F_{ROH}$  referring to the long ROH ( $>16$  Mb), which indicates recent inbreeding or the recurrent use of popular sires, was the lowest in all the breeds. This was to be expected given the high number of markers contained by the SNP chip. The two highest values were associated with the BSJI and SF breeds, both of which belong to small populations base. The boxplot (Figure 5(b)) showed that, although there were no outliers, CIRN  $F_{ROH}$  had a high variability, especially when compared with other breeds such as BEAG and BRAC. Only one subject from the TW



**Figure 3.** Phylogenetic tree of the dogs included in this study. The relationships among the different breeds were calculated on the basis of Reynolds' distances. Each line indicates the most represented breed; individuals falling under a line of another breed are framed in red. BEAG: Beagle; BRAC: Bracco Italiano; BSJI: Basenji; TW: Tan and white Cirneco dell'Etna; SF: Self-coloured fawn Cirneco dell'Etna; IGIT: Italian Greyhound; KETF: Kelb tal Fenek (Pharaoh Hound); SIPF: Segugio Italiano a Pelo Forte; SIPR: Segugio Italiano a Pelo Raso; WHIP: Whippet.

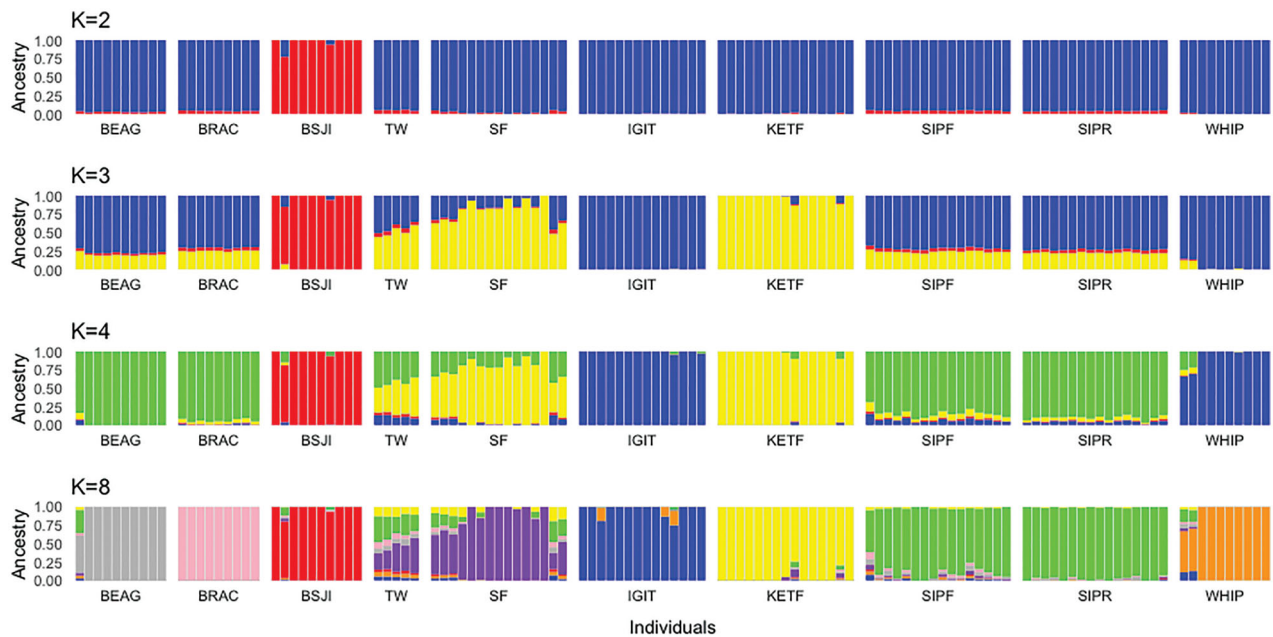
group had a  $F_{ROH}$  similar to the SF group, whilst the others were lower.

The analysis of the  $\bar{F}_{ST}$  was used to compare the two groups of CIRN, SF and TW. The genes associated with SNPs characterised by a  $\bar{F}_{ST} \geq 0.600$  (top 0.0001%) are shown in Table 2 and Figure S2. The highest  $\bar{F}_{ST}$  value (0.764) was associated with a SNP located on Microphthalmia-Associated Transcription Factor (*MITF*) gene. ROH including this gene were present in nine (60%) subjects of SF group and only in one (20%) of TW group.

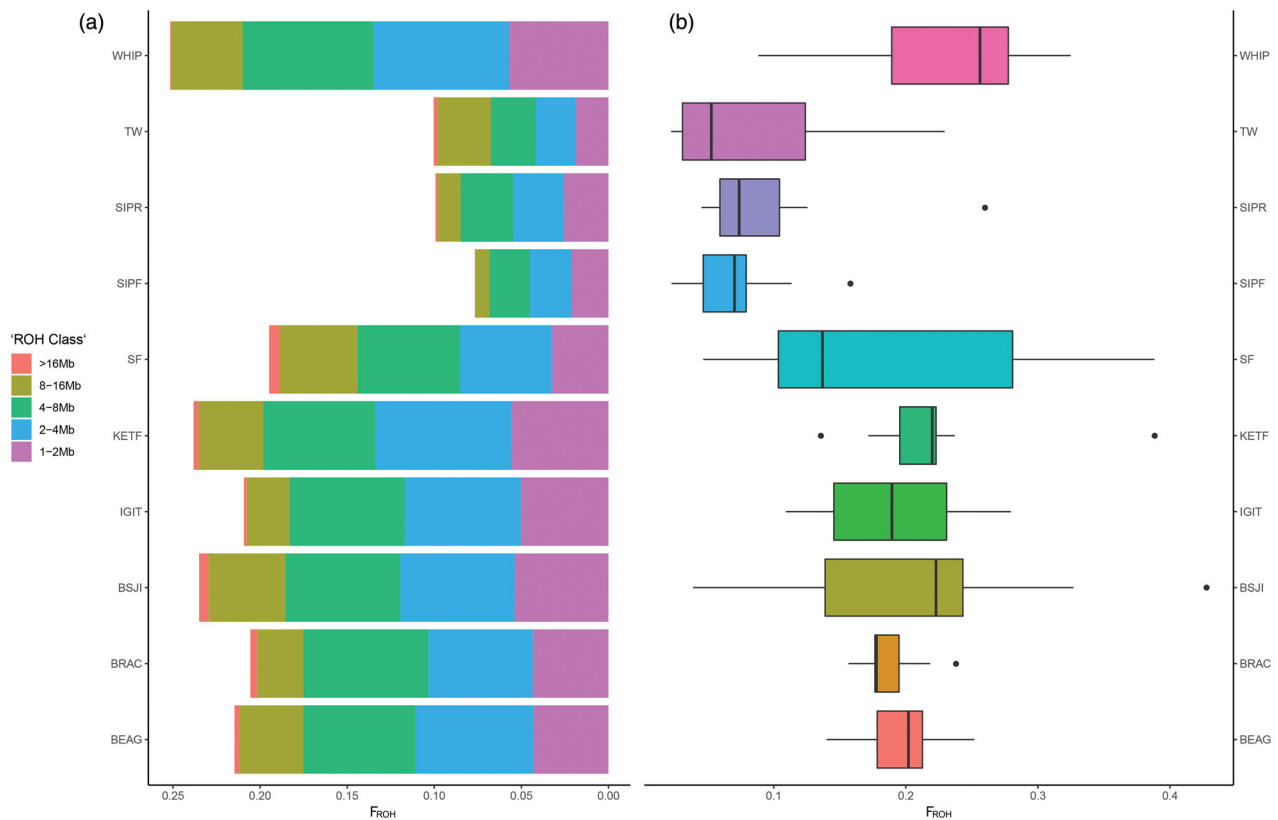
## Discussion

Previous studies (Talenti et al. 2018) highlighted how the Cirneco is a well distinguished breed from a genetic point of view, with measures (e.g., phylogenetic clustering, SNP-based inbreeding and parameters of homozygosity) consistent with other well-known and studied breeds. The present study puts under the spotlight the Cirneco and compares it with other breeds that might have influenced its evolution in its recent and old history. The genetic differences due to





**Figure 4.** ADMIXTURE analysis of the breeds included in this study. Each colour corresponds to a different cluster. BEAG: Beagle; BRAC: Bracco Italiano; BSJI: Basenji; TW: Tan and white Cirneco dell'Etna; SF: Self-coloured fawn Cirneco dell'Etna; IGIT: Italian Greyhound; KETF: Kelb tal Fenek (Pharaoh Hound); SIPP: Segugio Italiano a Pelo Forte; SIPR: Segugio Italiano a Pelo Raso; WHIP: Whippet.



**Figure 5.** Analysis of ROH-based inbreeding coefficient ( $F_{ROH}$ ). (a) Barplot of  $F_{ROH}$  calculated for different ROH length classes; (b) Boxplot of individual  $F_{ROH}$ . BEAG: Beagle; BRAC: Bracco Italiano; BSJI: Basenji; TW: Tan and white Cirneco dell'Etna; SF: Self-coloured fawn Cirneco dell'Etna; IGIT: Italian Greyhound; KETF: Kelb tal Fenek (Pharaoh Hound); SIPP: Segugio Italiano a Pelo Forte; SIPR: Segugio Italiano a Pelo Raso; WHIP: Whippet.

**Table 2.** Results of  $\bar{F}_{ST}$  analysis comparing Self-coloured fawn (SF) and Tan and white (TW) Cirnecos, showing the genes where SNPs with  $\bar{F}_{ST} > 0.6$  (top 0.0001%) localised.

Gene ID	$\bar{F}_{ST}$	CFA	Start	End	Gene name
MITF	0.764	20	21621927	21870578	Microphthalmia-Associated Transcription Factor
CHL1	0.641	20	16792690	16984613	Cell Adhesion Molecule L1 Like
MROH8	0.632	24	25770731	25828080	Maestro Heat Like Repeat Family Member 8
LRCH1	0.623	22	4578733	4771753	Leucine Rich Repeats and Calponin Homology Domain Containing 1
NXN	0.613	9	44847558	44988906	Nucleoredoxin
ABLIM1	0.610	28	25260873	25454304	Actin Binding LIM Protein 1
DNM3	0.598	7	26574453	27024759	Dynamamin 3

CFA: canine chromosome.

the deliberate segregation according to coat-colour of the population are also highlighted, showing how genetic metrics such as inbreeding, breed composition and heterozygosity differ sensibly between self-coloured fawn (SF) group and the tan and white (TW) coated Cirnecos.

As a matter of fact, the TW present a more heterogeneous genetic background than that found in the SF individuals, with higher degree of admixing and dispersion in the MDS plot. In addition, they show a higher level of outbreeding, with inbreeding coefficients halved compared to SF, and an observed heterozygosity higher than the expected one. Nevertheless, more than half of the SF have a genetic background that is superimposable to TW subjects. A possible contribution to the higher diversity can come from past admixing event with KETF or, more recently, from another closely related scent hound, the Segugio Italiano (either SIPF and SIPR). The first hypothesis is consistent with the geographical proximity and economic exchange between Sicily and Malta (Abela 1647; Cassar 1996; Norwich 2006) the country of origin of KETF. On the other hand, the Segugio Italiano is also a popular dog breed in Sicily used for boar hunting, and may have been used occasionally to improve the Cirneco's hunting skills. It cannot be excluded that other breeds, not analysed in this study but geographically and phenotypically close to Cirneco, such as Podenco Ibicenco (Talenti et al. 2018), might have also had an influence on its genetic background.

When looking at genomic regions of putative selection in Cirneco, the gene that most differentiates the two groups (i.e., with a higher  $\bar{F}_{ST}$  value) is *MITF*, a gene associated with white spotting in several dog breeds (Rothschild et al. 2006; Karlsson et al. 2007; Baranowska Körberg et al. 2014). White spotting is diffused in many breeds, but its fixation is greater in Pointer, Setter, Spaniel and Terrier clades, probably selected for improving the visibility of dogs during the hunt (Dreger et al. 2019). Another identified gene that might be related to coat colour is *MROH8*, it being localised near to the Agouti Signalling Protein (*ASIP*) gene, which regulates the distribution of red and

black pigments (Kerns et al. 2004; Dreger et al. 2020). The present study is the first to give evidence of an association between *MITF* gene and the white blazing in the Cirneco breed. Additional studies involving more subjects and further independent genomic analyses might detect other regions that underwent selection in Cirneco of one or another coat colour.

Despite the limited number of breeds and individuals considered, this study offers a better understanding of one of the most ancient Italian breeds, not only from a population structure point of view, but also on the consequences of its selection management. The results also show how the low number of ultra-long runs of homozygosity suggests an appropriate management of the pedigree, and that the small population size is likely to be imputed for the high level of genetic inbreeding. Since a relevant part of the SF share their genetic background with TW and given the small size of this population, a selection based only on specific aesthetic (phenotypic) characteristics such as coat colour should be carefully evaluated in order to avoid the risks derived by potential inbreeding depression. In a recent study involving almost 12,000 dogs of 212 breeds, Dreger et al. (2019) demonstrated that many breeds carry alleles that might result in pure-bred dogs with non-compliant phenotypes. As a consequence, breed associations may need to revise their standards, focussing on reducing truly undesirable traits, whilst enhancing those caused by ancestral variants (Dreger et al. 2019). For example, since merle allele demonstrated to be necessary for producing the Harlequin coat colour, the American Kennel Club has recently admitted merle Great Danes (Dreger et al. 2019). Similar problems are shared by other domestic species too: it is worth mentioning that, since 2006 (<http://server01.anafi.it/DelibereDal1981/226.htm>), the current Herd Book of the Italian Friesian cattle breed provides for a section dedicated to the inclusion of the red and white subjects, which were traditionally culled out of the breeding programme. The public opinion is becoming more aware on animal welfare issues and the health problems caused by excessive

and too restrictive standard definition and selection (Farstad 2018).

## Conclusions

This study provides important new knowledge about the current genetic diversity and genomic structure of the Cirneco dog breed. Our analyses reveal that Segugio Italiano (SIPR and SIPF) and KETF are potentially the breeds that most likely had an influence on the Cirneco. Within this breed, TW animals exhibited a lower genomic inbreeding state and spread more widely across the MDS plot compared to SF. Moreover, their admixture is more heterogeneous, but about half of SF present a similar genetic background. The gene that most differentiates these two groups is *MITF*, already known for being responsible for white blazing in many other dog breeds.

These results strongly suggest that the management of small populations has to incorporate genetic tools to preserve both the morphology and the genetic pool by limiting potential inbreeding effects. This is particularly true for the Cirneco, where dogs conforming to the standard can be registered in the RSR additional registry even if their genealogy is unknown, despite the fact that a standard phenotype does not necessarily correspond to a pure genotype. Moreover, gaps in genetic investigation come with the risk of introgression of mutations linked to problems that go beyond the coat colour. Therefore, it is highly recommended that a Cirneco genomic database is developed and maintained as a valuable resource for safeguarding its health and biodiversity and to ensure a bright future to a dog that has a distant past.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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