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High-density single nucleotide polymorphism markers reveal the population structure of 2 local chicken genetic resources

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ABSTRACT Italy counts a large number of local chicken populations, some without a recognized genetic structure, such as Val Platani (**VPL**) and Cornuta (**COS**), which represent noteworthy local genetic resources. In this study, the genotype data of 34 COS and 42 VPL, obtained with the Affymetrix Axiom600K-Chicken Genotyping Array, were used with the aim to investigate the genetic diversity, the runs of homozygosity (**ROH**) pattern, as well as the population structure and relationship within the framework of other local Italian and commercial chickens. The genetic diversity indices, estimated using different approaches, displayed moderate levels of genetic diversity in both populations. The identified ROH hotspots harbored genes related to immune response and adaptation to local hot temperatures. The results on genetic relationship and population structure reported a clear clustering of the populations according to their geographic origin. The COS formed a nonoverlapping genomic cluster and clearly separated from the other populations, but showed evident proximity to the Siciliana breed (**SIC**). The VPL highlighted intermediate relationships between the COS-SIC group and the rest of the sample, but closer to the other Italian local chickens. Moreover, VPL showed a complex genomic structure, highlighting the presence of 2 subpopulations that match with the different source of the samples. The results obtained from the survey on genetic differentiation underline the hypothesis that Cornuta is a population with a defined genetic structure. The substructure that characterizes the Val Platani chicken is probably the consequence of the combined effects of genetic drift, small population size, reproductive isolation, and inbreeding. These findings contribute to the understanding of genetic diversity and population structure, and represent a starting point for designing programs to monitor and safeguard these local genetic resources, in order to define a possible official recognition program as breeds.

Key words: SNP, genetic diversity, local population, inbreeding, conservation

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INTRODUCTION

Intensive breeding has led to important changes in the patterns of the genomic diversity and compromised the consideration and the survival of local chicken breeds (Lyimo et al., 2014). Indigenous chickens appear to be more genetically diverse than the commercial breeds, as they have been improved and established through a long breeding history, by processes remarkably different from those used for commercial breeds (Tadano et al., 2007; Nie et al., 2019). In fact, most local populations are the result of adaptation to a singular and sometimes harsh environment, and are expected to thrive and cope with the climate change effects more easily than their modern counterparts that struggle to survive in similar conditions. Moreover, these local populations secured several genetic variants, such as those controlling feather color and comb types (Dorshorst et al., 2015). Therefore, the conservation of local populations is crucial to satisfy future unanticipated breeding and productive demands (Khanyile et al., 2015; Chen et al., 2019; Zhang et al., 2020), and represent a socioeconomic, cultural, and ecological value.

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In Italy, the of biodiversity loss in the poultry sector is a highly topical issue. In fact, in the context of agricultural policies focused on the conservation of livestock species, plans for the safeguarding of local poultry breeds have been launched in the last 20 yr, initially at a regional level and now concerning national policies (Cendron et al., 2020). Today, several populations are still reared locally by smallholders in extensive production systems, and some of which do not have a defined genetic structure, such as Cornuta di Sicilia (also known as Cornuta di Caltanissetta) and Val Platani (also known as Valplatani) chicken populations. The exact origin of these populations is unknown. Both represent local genetic resources historically present in the rural areas of the Sicily region (South Italy), but not officially recognized as breeds. The 2 populations have evolved over the centuries by natural adaptation, and are rated for egg deposition, tolerance to diseases, and their good adaptation to the local environment. The Cornuta is also reared as ornamental chicken, due to its duplex-comb phenotype, which corresponds to a 2-pronged horn or V-shaped comb that is restricted to the posterior portion of the comb developing region (Dorshorst et al., 2015). To date, no official data are available on morphological and productive characteristics of these populations.

The evaluation of local genetic resources involves records of phenotypes, the investigation of their breeding history, as well as the study of genetic variability both within and between populations (Wimmers et al., 2000). Population genetic analyses can help explain the evolutionary history of these animals, clarify the origin and differentiation of populations, and assist in genetic breeding (Elbeltagy et al., 2016; Fleming et al., 2016; Cendron et al., 2020; Evelyne et al., 2022).

The increasing use of high-throughput DNA analysis, such as single nucleotide polymorphism (**SNP**) microarrays and genomic sequencing, has enabled accurate assessment of chicken population genomics (Khanyile et al., 2015; Chen et al., 2019; Nie et al., 2019; Cendron et al., 2020; Malomane et al., 2019; Mastrangelo et al., 2020; Yuan et al., 2022). In this study, we assessed the genetic variability and population structure of Cornuta di Sicilia and Val Platani chicken populations using 600K SNP microarray data and compared them with other Italian local and commercial breeds.

MATERIALS AND METHODS

Sampling, Genotyping, and Quality Control

A total of 76 chickens from several farms located in Sicily were sampled to capture the representative genetic diversity within populations. Samples consisted of 34 Cornuta di Sicilia (**COS**, 7 males and 27 females) and 42 Val Platani (**VPL**, 9 males and 33 females) animals. Individual blood samples (2 mL) were collected in Vacutainers tubes containing EDTA as an anticoagulant from brachial wing vein. Animals were chosen on the basis of the information provided by farmers in order to collect unrelated individuals. Sampling was performed in accordance to the European rules (Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009). The animal study protocol was approved by the Bioethics Committee of the University of Palermo: protocol code UNPA-CLE-98597.

DNA was extracted from blood using the commercial Illustra blood genomic Prep Mini Spin kit (GE Healthcare, Little Chalfont, UK).

Genotyping was performed using the 600K Affymetrix Axiom Chicken Genotyping Array (Affymetrix, Inc., Santa Clara, CA), which included 580,961 single nucleotide polymorphisms (**SNPs**) across the entire chicken genome. A commercial service provider performed the genotyping. The GRCg6a chicken assembly was used in this study as the reference genome, with markers located on chromosomes from 1 to 28. The software PLINK v. 1.9 (Chang et al., 2015) was used to perform filtering and quality control using the following criteria: a minor allele frequency ≥0.05, a genotype call rate for a SNP ≥0.95 and an individual call rate ≥0.90. A total of 451,258 informative polymorphic SNPs and 72 animals were kept after filtering.

Genetic Diversity Indices

PLINK 1.9 (Chang et al., 2015) was used to estimate the average minor allele frequency (**MAF**), observed (**Ho**), and expected (**He**) heterozygosity. Trends in historical effective population size (**Ne**) based on linkage disequilibrium (**LD**) were estimated by using the program SNeP v1.1 (Barbato et al., 2015).

We performed the runs of homozygosity (**ROH**) analysis by using PLINK v. 1.9 (Chang et al., 2015) to estimate the molecular inbreeding and the homozygosity pattern within population, using the parameters reported in Cendron et al. (2020). The mean number of ROH ($\mathbf{MN_{ROH}}$) and the average length of ROH ($\mathbf{AL_{ROH}}$) per animal were estimated. The ROH were classified in 5 groups based on the physical length: 2 to <4, 4 to <8, 8 to <12, 12 to <16, and >16 Mb. In order to infer the individual genomic inbreeding coefficient based on ROH ($\mathbf{F_{ROH}}$), the length of the genome covered by ROH was divided by the total autosomal genome length covered by the SNP array (944,270 kb). To identify the genomic regions that were most commonly associated with ROH over individuals in each population, we calculated the percentage of SNPs occurrence in ROH by counting the number of times a SNP was detected in those ROH. The top 0.999 SNPs of the percentile distribution were selected, and adjacent SNPs over this threshold were merged into genomic regions named ROH islands. Genomic coordinates for all identified selected regions were used to annotate genes that were either entirely or partially included within each selected region using the Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_000002315.6) provided by NCBI. Finally, to investigate the biological function of each annotated

gene within ROH islands, we conducted an extensive accurate literature search.

Genetic Relationship and Population Structure

The raw data of the 2 chicken populations were merged with the genotype data of 23 Italian local and 4 commercial populations (Table S1) retrieved from a previous study (Cendron et al., 2020), obtaining a final dataset consisting of 27 populations, 668 individuals, and 419,475 SNPs. This dataset included the genotype data of other additional individuals of the 2 populations here analyzed, sampled in other farms ($N = 22$ Valplatani and $N = 20$ Cornuta di Caltanissetta) (Cendron et al., 2020), and used for comparison with the current sampling. The SNP data were filtered to remove SNPs in high linkage disequilibrium (**LD**) ($r^2 > 0.2$) by using the −indep-pairwise (50 10 0.2) function in PLINK v. 1.9 (Chang et al., 2015), generating a pruned dataset of 79,193 SNPs. Finally, with the aim of investigating in detail the relationship among Sicilian chickens, a reduced dataset was also created using the Siciliana breed and the genotype data of the Val Platani and Cornuta populations generated within the frame of this study, together with the data derived from the aforementioned study (Cendron et al., 2020).

The genetic relationships among populations were estimated using the multidimensional scaling (**MDS**) approach based on the identity-by-state (**IBS**) matrix of genetic distances calculated by PLINK v. 1.9 (Chang et al., 2015) and plotted in the R environment (R Development Core Team, 2020). ARLEQUIN v. 3.5 software (Excoffier and Lischer, 2010) was used to estimate Reynolds genetic distances, visualized by the neighbor-net tree using SPLITSTREE v. 4.14.8 (Huson and Bryant, 2006). ARLEQUIN v 3.5 was also used to estimate population relatedness using pairwise estimates of F_{ST}. An additional Neighbor Joining (**NJ**) tree was constructed based on individual allele-sharing distances (**ASD**) (−distance 1-IBS in PLINK) and visualized using SPLITSTREE v. 4.14.8 (Huson and Bryant, 2006). Patterns of ancestry and admixture were examined by using the model-based clustering algorithm implemented in the ADMIXTURE software v1.3.0 (Alexander et al., 2009), applying the default settings at different K values ($K = 2-27$). The most likely number of ancestral genomic clusters was estimated following the cross-validation procedure. The results were plotted using the membercoef.circos function in the R package BITE (Milanesi et al., 2017). Finally, patterns of migration events were investigated using the TREEMIX software v. 1.13 (Pickrell and Pritchard, 2012). For this analysis, we used the genotype data of *Gallus gallus gallus* (**GGg**, $n = 20$) as outgroup, taken from the AVIANDIV collection (https://aviandiv.fli.de/) (Malomane et al., 2019). Five independent iterations were performed allowing migration events to range between 1 and 10, while the covariance matrix was estimated using 500 contiguous SNPs per block. The most supported number of migration edges was assessed using the linear method as implemented in the R package OptM (Fitak, 2018).

RESULTS

Genetic Diversity Indices

The genetic diversity indices, estimated using different approaches, were adopted to identify the levels of variability in 2 local chicken populations. Descriptive statistics are reported in Table 1. The results displayed moderate levels of genetic diversity in both populations. VPL showed the highest Ho, He, and MAF values and the lowest average F_{ROH}. The individual F_{ROH} values within populations varied from 0.005 to 0.432 and from 0.093 to 0.642 in VPL and COS, respectively. A continuous decline in Ne was found across generations for both populations (Figure S1). Based on the genomic data, the Ne value at the most recent generation (the 13th) was 39 and 77 for COS and VPL, respectively.

ROH and ROH Islands

We detected a total of 2,911 and 1,090 segments in COS and VPL, respectively. The mean number of ROH (MN_{ROH}) ranged from 16 (VPL) to 86 (COS), whereas the average length (AL_{ROH}) ranged from 3.38 Mb (COS) to 3.75 Mb (VPL). For both populations, the majority of ROH segments (76 and 72% for COS and VPL, respectively) were shorter than 4 Mb in length, while only the 2% (COS) and 3% (VPL) of segments were longer than 16 Mb.

We also investigated the ROH islands using the top 0.999 SNPs in ROH of the percentile distribution within each population, and thus identifying different thresholds in the 2 populations (0.381 and 0.794 for VPL and COS, respectively). Figure 1A and B showed the Manhattan plots of SNPs in ROH occurrence in VPL and COS, respectively. Although the frequency of SNPs in the ROH was relatively balanced and the signals were moderate in height, we found several outstanding peaks with a high percentage of ROH, especially in the COS population. Table 2 showed the genomic coordinates of

Table 1. Genetic diversity indices for Cornuta (COS) and Val Platani (VPL) populations.

Population	Ho ± SD	He ± SD	MAF ± SD	Ne	F_{ROH} ± SD
VPL	0.362 ± 0.150	0.368 ± 0.124	0.279 ± 0.131	77	0.103 ± 0.126
COS	0.253 ± 0.211	0.240 ± 0.188	0.259 ± 0.132	39	0.307 ± 0.174

Abbreviations: F_{ROH}, inbreeding coefficient based on runs of homozygosity; He, expected heterozygosity; Ho, observed heterozygosity; MAF, average minor allele frequency; Ne, effective population size relating to the 13th generation; SD, standard deviation.

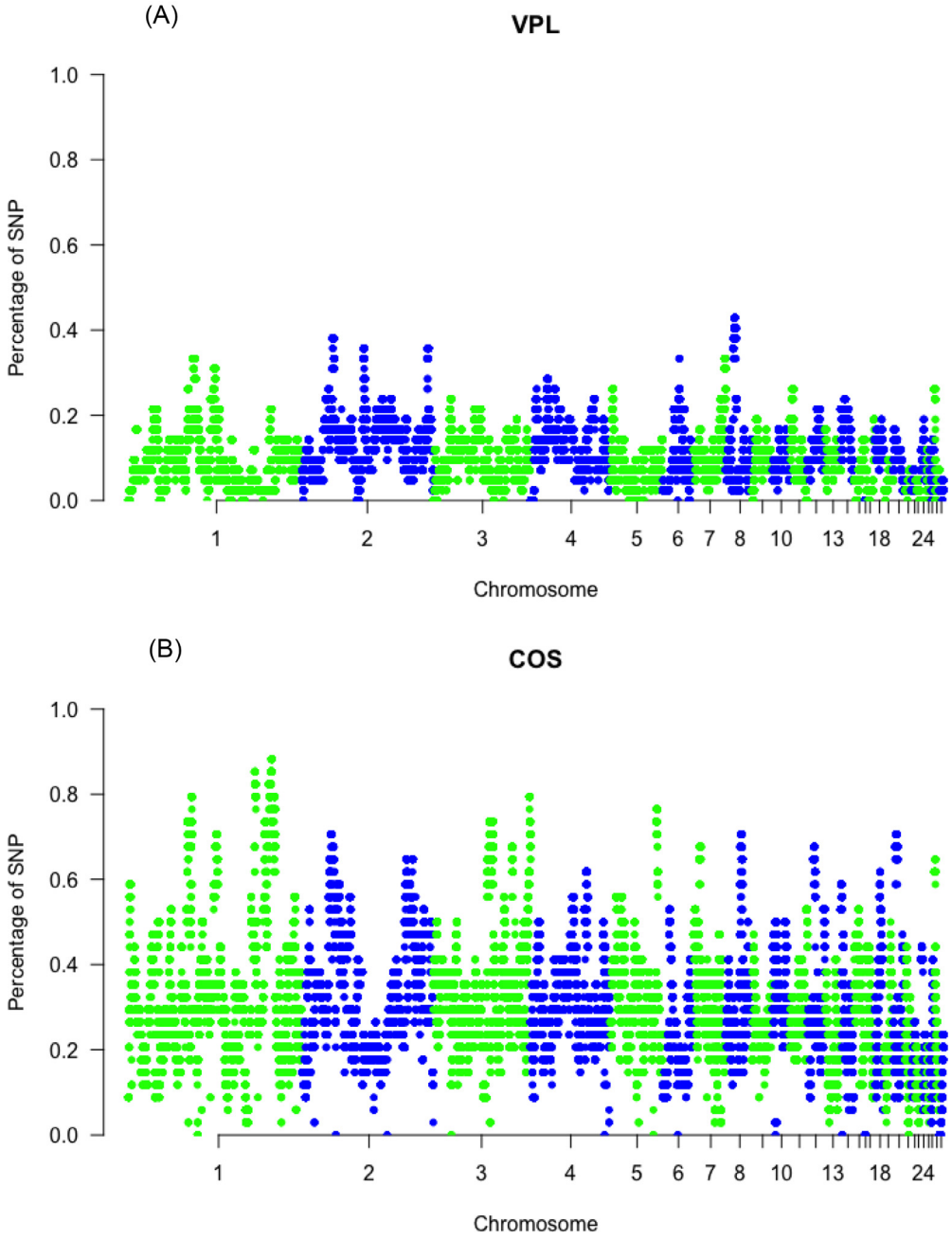

Figure 1. Manhattan plot of frequency (%) of a single nucleotide polymorphism (SNP) in run of homozygosity (ROH) islands in (A) Val Platani (VPL) and (B) Cornuta (COS) chicken populations.

the ROH islands, the number of SNPs per ROH, and the annotated genes. A total of 7 ROH islands were identified in the 2 chicken populations. The highest number of ROH islands was identified in COS including 5 regions containing 1,280 SNPs detected on 2 chromosomes (**GGA**) (GGA01 and GGA03). The VPL showed 2 islands containing a total of 1,217 markers on GGA02 and GGA08. Within the reported ROH islands, a total of 28 genes for COS and 24 genes for VPL were mapped.

Genetic Relationship and Population Structure

The MDS plot in Figure 2 showed the genetic relationship among the 27 Italian populations by plotting both individuals (Figure 2A) and centroids (Figure 2B) according to C1 and C2. The component C2 (9.1%) allowed us to separate the 3 Sicilian chickens, and in particular the Siciliana (**SIC**) breed and the COS from the other populations. The VPL was on the gradient between the 2 Sicilian populations (SIC and COS) and the other breeds involved in the study. With the aim of providing additional indications regarding breed relationships, we represented a neighbor-net graph per population based on Reynolds genetic distances (Figure 3). The graph showed a clear clusterization of populations that originated from the same geographic area. The VPL and COS populations departed from the same node in which SIC breed and COS showed a closer relationship. A branch with moderate length was observed for COS, whereas the longest one was found for other

Table 2. Runs of homozygosity island identified in Cornuta (COS) and Val Platani (VPL) populations.

Pop	GGA	Start	End	No. SNPs	Genes
COS	1	71,946,443	72,476,008	136	*CREBL2, GPR19, CDKN1AL, CDKN1B*
	1	144,148,005	145,223,027	409	*METTL21EP, ERCC5, BIVM, KDELC1, TEX30, METTL21C, TPP2, FGF14, ITGBL1, NALCN*
	1	160,360,225	163,478,002	507	*PCDH9, PCDH20*
	1	166,213,331	166,787,767	106	-
	3	109,602,219	109,946,808	122	*CLIC5, ENPP4, RCAN2, CYP39A1, TDRD6, PLA2G7, IMP3, ANKRD66, TAS2R7, MEP1A, ADGRF5, TNFRSF21*
VPL	2	34,736,309	36,687,450	626	*TBC1D5, SATB1, KCNH8, EFHB, RAB5A, PP2D1, KAT2B, SGO1, ENS-1, ZNF385D*
	8	9,161,725	12,046,076	591	*PLA2G4A, PTGS2, PDC, TPR, PRG4, HMCN1, IVNS1ABP, SWT1, TRMT1L, AMY1AP, AMY1A, RNPC3, COL11A1, OLFM3*

Abbreviation: GGA, *Gallus gallus* chromosome.

populations (the 2 Robusta breeds, SIC and Pepoi). The F_{ST} pairwise distances (Table S2) reported the highest values between Robusta Maculata and Siciliana breeds (0.600), whereas the lowest value was between VPL and Bianca di Saluzzo (0.102). Considering the 2 investigated populations, VPL showed the lowest average distance (0.196) toward the other chicken populations, whereas the COS showed the highest value (0.342).

The results of populations genomic structure obtained through the admixture analysis (Figure 4), reported the model-based clustering of individuals' genome into a predefined number of components. We reported K values from 2 to 15 in order to underline ancestral components shared among different Italian chicken populations. The model, assuming 2 ancestral populations ($K = 2$), separated the 3 Padovana breeds from all the rest. Two Sicilian chicken populations (SIC and COS) were the first to separate within the Italian dataset at $K = 3$ (yellow), followed by the 2 Polverara (**PPN** and **PPB**) breeds at $K = 4$ (green). From $K = 5$ and for subsequent K values, the VPL population started clustering apart from all other populations, and showed shared genomic components with other populations. Moreover, the results of the admixture analysis from $K = 7$, showed the presence of substructure for the VPL population. From $K > 16$ up to $K = 27$ (Figure S2) each population tended to show its own distinct cluster but with some exceptions; in fact, the VPL and COS and other Italian local breeds (Valdarnese, Modenese, and Bianca di Saluzzo) showed less distinct clusters than other breeds with a heterogeneous genetic structure and substructure evidence.

MDS analysis was performed also on SIC, COS, and VPL populations to explore in detail the relatedness among Sicilian chickens (Figure S3), and to evaluate any differences between the individuals of COS and VPL sampled in this study and those present in the dataset of Cendron et al. (2020) (indicated with **COR** for Cornuta and **VLP** for Valplatani, respectively). The first 2 components clustered cohesively all the SIC's individuals, whereas the COS and VPL showed more dispersed groupings. The variability plotted in the metric space was particularly pronounced in Val Platani (VPL and VLP) that, according to the C1 component, showed

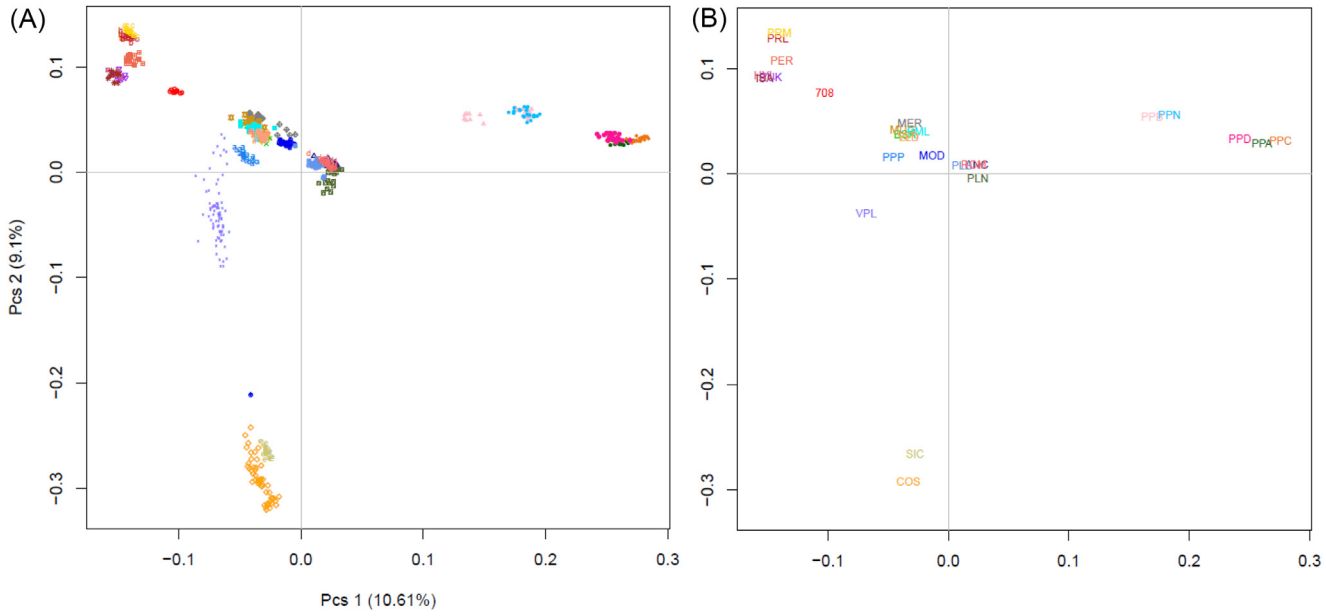

Figure 2. Genetic relatedness of chicken populations inferred by multidimensional scaling (MDS) approach and using (A) all of the individuals and (B) the breed-average coordinates of eigenvalues of C1 and C2. For a full definition of populations, see Table S1.

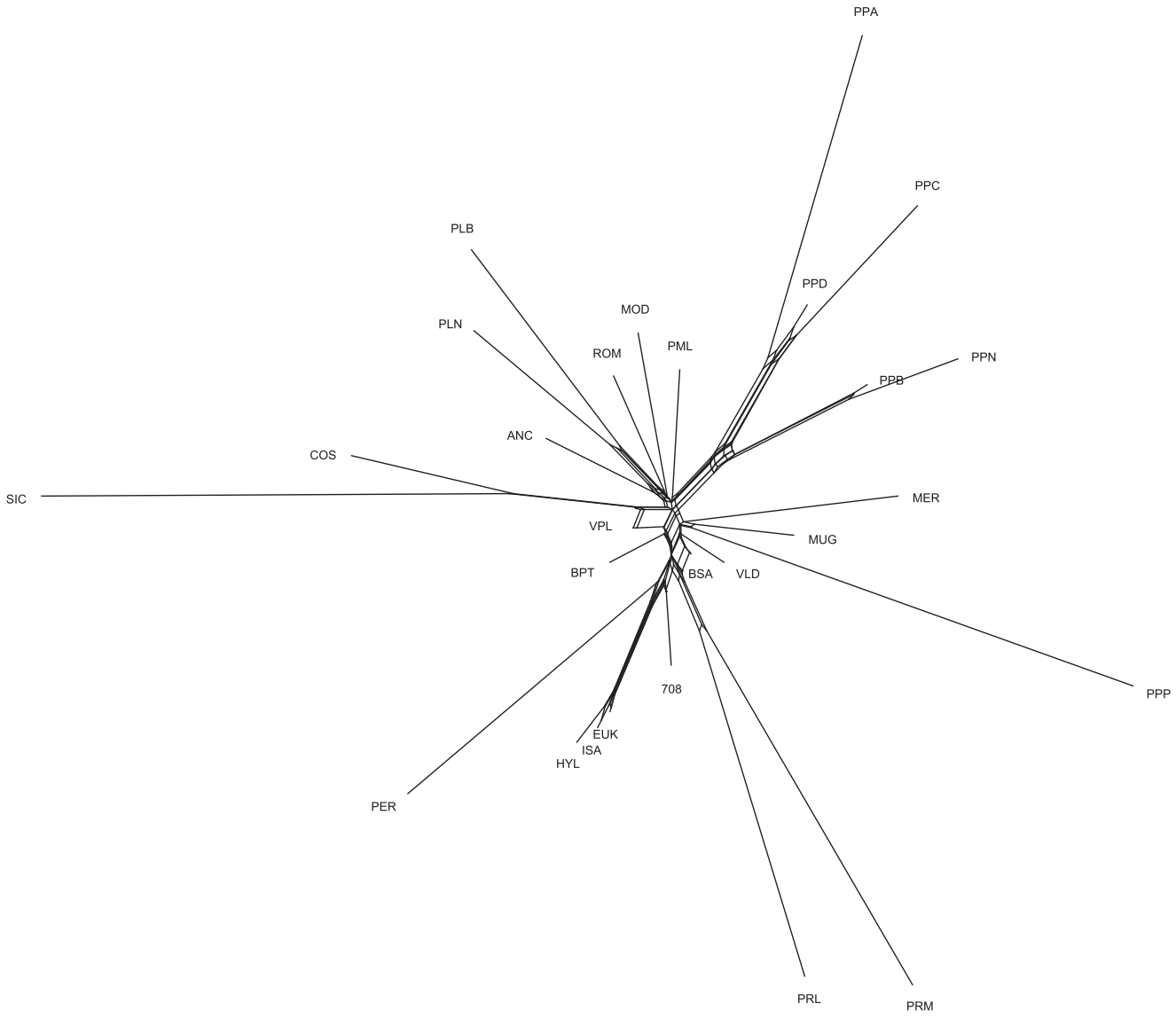

Figure 3. Neighbor-net graph based on Reynolds genetic distances of all chicken populations. For a full definition of populations, see Table S1.

the presence of substructure. In agreement with MDS outcomes, the NJ tree based on allele sharing distance (**ASD**) separated the individuals according to their population of origin (Figure S4). This clustering trend was confirmed by the results of the admixture analysis observed at $K = 3$ at which individuals of the VPL (this study) were distinct from those of VLP (Cendron et al., 2020) (Figure S5). In contrast, the 2 Cornuta's groups (COR and COS) shared a similar genetic background.

Finally, we used the TREEMIX to model both population splits and gene flow using the whole dataset. The graph showed a clear distribution of clusters according to the geographic origin and highlighted shared ancestral components among chicken populations (Figure 5). All the Sicilian populations were in a single clade, in which COS and COR, as well as VPL and VLP, showed common branches. The optM function supported only one migration event between the base of the branch that included the 2 Val Platani subgroups (VPL and VLP) and the base of the branch of 3 commercial stocks (ISA, EUK, and HYL) (Table S1).

DISCUSSION

Information about genetic diversity and population structure among native chicken ecotypes is of fundamental importance for genetic improvement, for understanding of environmental adaptation as well as for conservation and sustainable management programs (Psifidi et al., 2016; Malomane et al., 2019; Yuan et al., 2022). Therefore, the genomic characterization represents the prerequisite to plan breeding programs and conservation strategies, particularly for uncharacterized populations. In this study, we assessed the population structure of 2 local chickens and showed their genetic background and their relationships comparing them with other Italian breeds.

Genetic Diversity Indices

The diversity indices in the 2 local populations were quite similar to the range reported for Italian (Strillacci et al., 2017; Cendron et al., 2020), European (Malomane

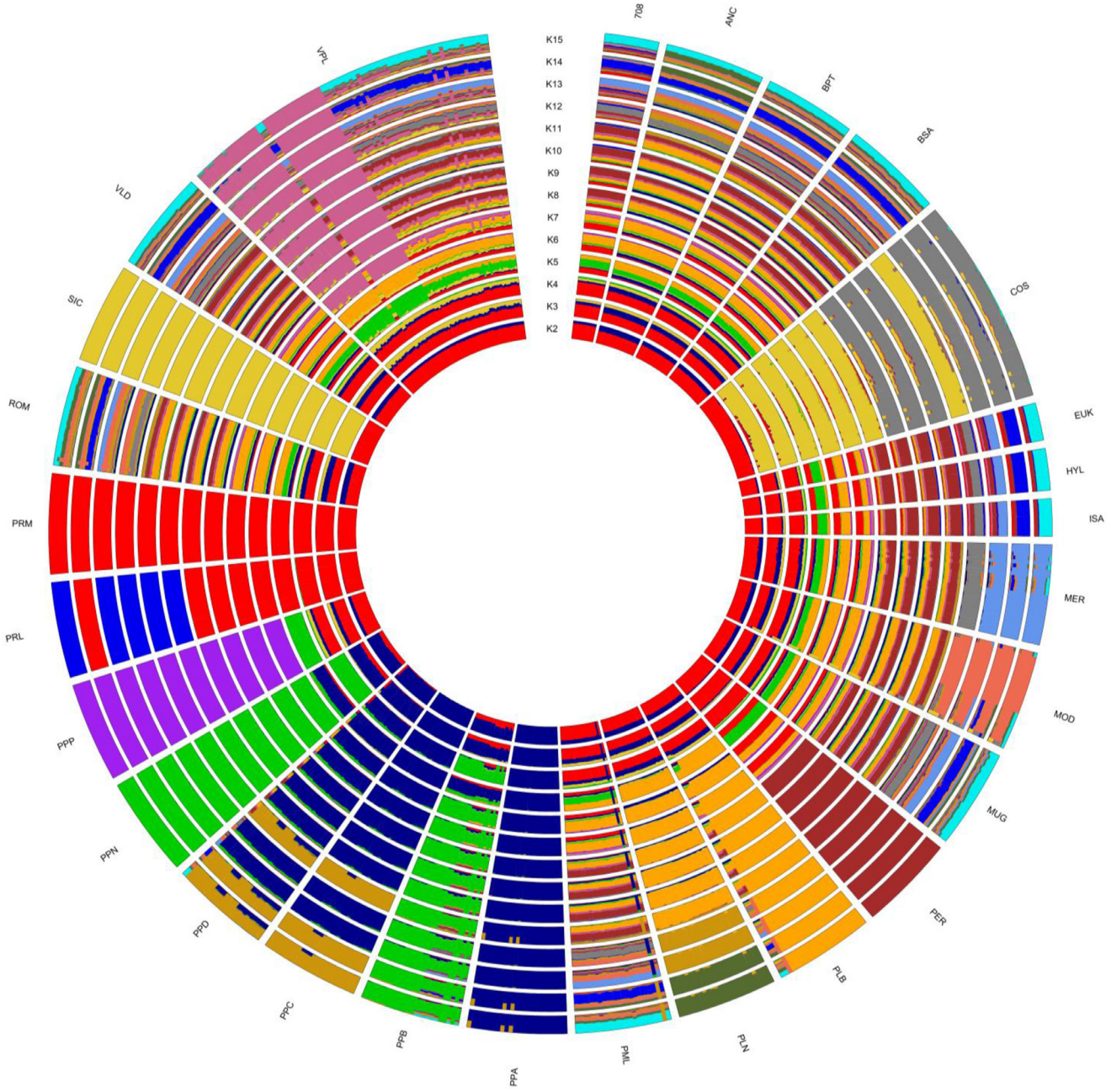

Figure 4. Model-based clustering of chicken populations from $K = 2$ to $K = 15$. For a full definition of populations, see Table S1.

et al., 2019), and other native chicken populations (Evelyne et al., 2022; Yuan et al., 2022; Wang et al., 2023). However, the values differed from the results of a previous study (Cendron et al., 2020), in which the authors reported lower values of heterozygosity and higher values of inbreeding for Cornuta di Sicilia and Val Platani populations. Although the results were obtained by using the same array, the differences between studies can be attributed to the use of different sampling strategy to sample individuals, which in our study belonged to a higher number of farms. The lowest genetic diversity reported in the previous study (Cendron et al., 2020) for these populations can be due to the strict use of line-breeding or the use of a few male chickens to select offspring. In both populations, observed heterozygosity (Ho) was either equal to or higher than expected heterozygosity (He), indicating that the diversity management has improved in recent years. The moderately high values of H_o and H_e reflected the high percentage of polymorphic SNPs in Val Platani. Analysis of trends in effective population size suggested a decrease in genetic variation over time in both populations. Other studies have also shown that the effective population size of local breeds is progressively shrinking (Khanyile et al., 2015; Zhang et al., 2020).

The ROH-based genomic inbreeding coefficients of the 2 Sicilian chickens were similar to the estimates in other local populations (Zhang et al., 2018; Yuan et al., 2022; Wang et al., 2023). Considering the pattern of ROH, the mean number and the average length of the segments identified in Cornuta and Val Platani were comparable to those reported in broiler chickens (Marchesi et al.,

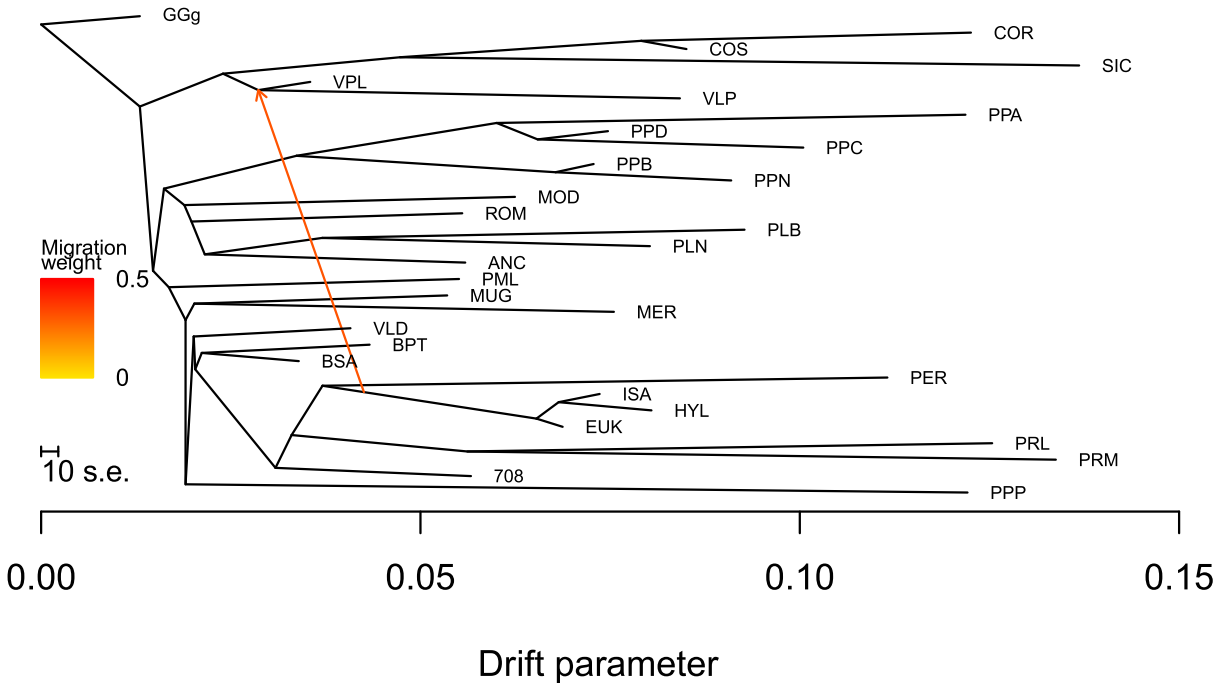

Figure 5. TREEMIX analysis with the most supported number of migration events ($m = 1$). For the full definition of populations, see Table S1.

2018). We found differences in the total and mean number of ROH per individual in the 2 populations, whereas the average length of ROHs was similar. In general, short ROH segments (<4 Mb) were predominant in both populations, indicating low recent inbreeding in these chickens. In fact, the presence of a high percentage of short and medium ROH segments is indicative of relatedness dating back to ancient times (Howrigan et al., 2011). In a previous study, the same populations here analyzed, have shown a large mean portion of their genome covered by longer ROH (>30 Mb). In fact, the overlapping generations in smallholder farming systems promote mating of closely related chickens thereby increasing inbreeding levels. The relatively low proportion of genome covered by homozygous segments for the individuals here sampled, particularly in Val Platani, supports the effective genetic management aimed at avoiding mating between related animals, which is fundamental for the development of a conservation program.

ROH Islands

ROH islands might be indicative of genomic regions underwent natural and/or artificial selection (Mastrangelo et al., 2017). In chicken, several studies showed ROH regions harboring candidate genes associated with production traits, immune responses and environmental adaptation (Fleming et al., 2016; Strillacci et al., 2018; Yuan et al., 2022; Wang et al., 2023). Given the variable polymorphism content, the homozygosity threshold to call a ROH island was different between the 2 populations, as well as reported in other studies on chickens (Talebi et al., 2020).

The ROH islands identified in Cornuta mapped genes which play a key role in affecting growth (*METTL21C*) (Yang et al., 2019), fat deposition (*ITGBL1*) (D'Andre et al., 2013), and body weight (*PCDH9*) (He et al., 2022) in chicken, or involved in muscle development (*CREBL2*) (Hu et al., 2021) in duck. We also identified candidate genes related to adaptation to hot temperatures in chicken species, such as *FGF14* (Coble et al., 2014) and *NALCN* (Gu et al., 2020). The genes within the ROH island on GGA03 (*CLIC5, ENPP4, RCAN2, TDRD6, PLA2G7 IMP3, ANKRD66 MEP1A, ADGRF5*) overlapped with an island reported in Italian turkey (Bernini et al., 2021). Among these, we identified genes involved in immune responses, such as *PLA2G7* (Abasht et al., 2019) or reported as differentially expressed in dwarf and normal chickens (*ENPP4*) (Ye et al., 2014). Moreover, this island mapped *TAS2R7*, a gene that influence the sense of bitter taste (Su et al., 2016), playing a critical role in animal feeding as it can help to avoid intake of toxic and harmful substances. In Val Platani, some genes within the 2 detected ROH islands overlapped with homozygous regions reported in chicken (Strillacci et al., 2018; Cendron et al., 2020). We identified genes associated with adaptation and survival in hot conditions, such as *TRMT1L* (Walugembe et al., 2019), *TBC1D5* (Fleming et al., 2017), and *PTGS2* (Zhao et al., 2022), as well as genes associated with immune (*KCNH8*) (He et al., 2015) and inflammatory response (*SATB1*) (Zhang et al., 2016). It is also interesting to note within the ROH island on GGA08 mapped candidate genes related to reproductive physiology in avian species (*PTGS2* and *PLA2G4*) (Bernini et al., 2021), or associated with the regulation of growth and body size (*COL11A1*) (Wang et al., 2017) and feed intake efficiency (*AMY1A*) (Zhang et al., 2021).

Several factors could have led to the identification of these selection signals. These local populations have been mainly reared as backyard chickens as they are more resistant to diseases and viruses, compared with commercial chickens. In order to adapt these conditions, selective sweep might have occurred in genomic regions related to immune responses and local adaptation. The results suggests that the genes included in these regions may be under selection because they play an important role in the process of adaptation to heat stress, and therefore may point to a selection signature typical of populations reared in southern Italy that is characterized by high summer temperatures.

Genetic Relationship and Population Structure

With the aim of understanding the genetic relationships among and within populations, different approaches have been carried out. In general, the results highlighted a genetic pattern of the Italian local chickens according to their genetic and geographic origin, and showed that all individuals clustered within their own population. The north-south geographic distribution of the genetic diversity was highlighted by both the first 2 dimensions of the MDS plot and the neighbor-net, confirming previous studies on Italian chicken (Cendron et al., 2020), but also on other livestock species, such as Italian cattle (Mastrangelo et al., 2018) and sheep breeds (Ciani et al., 2014). Such a partition was also supported by the TREEMIX and neighbor-net, which indicated an agreement between clustering and geographic origin.

The Siciliana and Cornuta showed an evident differentiation from the other chicken populations. In particular, the Cornuta formed a nonoverlapping cluster, showed a close relationship with Siciliana breed, and it was clearly separated from the other populations, which agrees with the result claimed by Cendron et al. (2020). In confirmation of this, no migration event was detected between Cornuta and the other chicken population involved in the study. Previous studies also showed the Siciliana breed clearly differentiated from other local and commercial populations (Strillacci et al., 2017). Indeed, this breed appears to derive from ancient interbreeding of local Sicilian chickens with North African stock (Ceppolina, 2015). Moreover, the Siciliana is the only breed with the buttercup comb type, an incredibly rare and unusual comb type. On the other hand, there were animals from officially recognized chicken breeds that grouped together (Figure 1) and showed overlapping clusters, such as Padovana or Polverara breeds. The Val Platani population clustered in an intermediate position, but closer to the other Italian local chickens; moreover, it showed a more heterogeneous cluster (Figure 2A), which is typical of admixed populations. For Val Platani population, the results highlighted in TREEMIX might reflect gene exchanges dating back to past events, supporting the hypothesis of historical gene flow with commercial breeds.

Cendron et al. (2020) showed a specific cluster for Sicilian chicken populations that included Siciliana, Cornuta, and Val Platani and reported these 3 populations shared genetic components related to their historical local origins. From $K = 2$ to $K = 7$, admixture analysis showed the presence of shared ancestral components between Siciliana breed and Cornuta, this last one recognized as a distinct cluster since low K value ($K = 9$). The genomic components shared between these 2 populations are possibly to be found in a distant past of common origin. The Val Platani showed a more complex genetic structure, due to the clear presence of 2 subpopulations, particularly evident from $K = 7$. These 2 subpopulations match with the different sampling. The first subgroup (in dark pink from $K = 7$) is the individuals sampled and analyzed in Cendron et al. (2020), whereas the second subgroup is the individuals genotyped in this study. These 2 subpopulations behave differently; in fact the individuals here sampled exhibited an admixed patterns, whereas those from Cendron et al. (2020) clustered homogeneously. The low levels of admixture in the first subgroup indicated the few remaining genomic components of any other ancestral populations that may have interacted with them, as well as a potential typical signal of inbreeding (Tolone et al., 2022). Therefore, the genetic structure detected for Val Platani could be due to the introgression of genes from other populations (VPL) and/or to geographical isolation for a long time with genetic drift (VLP). As expected, the 2 Val Platani population samples (VPL and VLP) are genetically similar as it emerges from the phylogenetic ancestry graph generated using TREEMIX in which the 2 sampling are on the same branch.

Besides this, the results of population relationships and genomic structure showed that the genetic heritage of Sicilian populations significantly differed from the other Italian chickens.

The NJ tree based on genome-wide allele sharing, used to deepen the relationships between the 3 Sicilian populations, confirmed the differentiation among them. Moreover, for Cornuta and Val Platani populations, the individual genetic distances underlined several subclusters associated with the different farms in which the individuals have been sampled. Similar results were previously reported for the Siciliana breed, for which the subgrouping has been already highlighted (Strillacci et al., 2017).

The results obtained by the genetic differentiation survey underline the hypothesis that Cornuta is a population with a more defined genetic structure, given that no substantial differences have been identified among chickens belonging to different herds and different samplings. On the other hand, the results evidenced the substructure for Val Platani, probably as the consequence of the combined effects of genetic drift, small population size, reproductive isolation and inbreeding. The results represent a starting point for the design of monitoring and conservation plans for these 2 unrecognized animal genetic resources, in order to define a possible official recognition program as breeds, in particular for Cornuta,

which showed a more defined genetic structure. Additional analyses and a wider sampling would contribute to refine and validate these results.

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Data Availability Statement: The dataset analyzed in the current study is available from the corresponding author on reasonable request.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102692.

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